

The Thermodynamics of the Krebs Cycle and Related Compounds

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A survey is made of the enthalpies of formation, third law entropies and Gibbs energies available for Krebs cycle and related compounds. These include formate, acetate, succinate, fumarate, glycine, alanine, aspartate and glutamate. The potential of the NAD^+/NADH couple is recalculated based on the ethanol/acetaldehyde and isopropanol/acetone equilibria. The reported enzyme catalyzed equilibrium constants of the Krebs cycle reactions are evaluated with estimated errors. These 28 equilibria form a network of reactions that is solved by a least squares regression procedure giving Gibbs energies of formation for 21 Krebs cycle and related compounds. They appear to be accurate to $\pm 0.4 \text{ kJ}\cdot\text{mol}^{-1}$ for some compounds but $\pm 1 \text{ kJ}\cdot\text{mol}^{-1}$ in less favorable cases. This procedure indicates which third law $\Delta_f G$ and enzyme equilibria are inaccurate, and allows very accurate $\Delta_f G$ to be determined for compounds related to the Krebs cycle by measuring enzyme equilibrium constants.

Keywords: enthalpy; entropy; enzyme equilibrium constants; Gibbs energies of formation; Krebs cycle compounds; NAD^+/NADH potential; thermodynamic network.

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1. Introduction

The Krebs cycle or citric acid cycle is the major pathway in aerobic organisms for the oxidation of sugars, fatty acids and some amino acids. The Krebs cycle produces NADH and FADH₂ from these compounds, which are oxidized by O₂ through the electron transport system producing ATP.

In anaerobic organisms the Krebs cycle does not operate in this manner unless there is a terminal electron acceptor to oxidize the NADH and FADH₂. Anaerobic organisms still have the Krebs cycle enzymes, but the cycle is run in reverse and is used to synthesize alanine, aspartic acid and glutamic acid and amino acids derived from these. The cycle is believed to have originated as a synthetic cycle in primitive anaerobic bacteria [76 BRO].

In addition to amino acid synthesis, there is the glyoxylate bypass which is a modified Krebs cycle in certain microorganisms that permits the oxidation of acetate and the regeneration of the oxalacetate which is removed for amino acid synthesis and which is lost by decarboxylation.

These reactions form a network of interconnecting reactions, most of which are reversible, that can be used to determine the Gibbs energies of formation of these organic compounds. The usual method of calculating Gibbs energies of formation uses the enthalpy of formation from the enthalpy of combustion and the entropy from the third law and the measured heat capacities. This has not been particularly successful in practice to obtain accurate values of $\Delta_f G$ of organic compounds, usually because of the errors in the enthalpy of formation. A more accurate procedure would be to measure the equilibrium constants for a set of reactions, and combine these with a few third law $\Delta_f G$'s to obtain accurate values of $\Delta_f G$ for the remaining compounds. An equilibrium constant measured to an accuracy of 4% corresponds to an error of 0.1 kJ in the $\Delta_f G$. There are few $\Delta_f G$ available with an accuracy of 0.1 kJ, yet an accuracy of 4% on an equilibrium constant is not difficult to obtain in many cases. Thus a large number of very accurate $\Delta_f G$ can be obtained by determining equilibrium constants of an appropriately connected series of reactions.

This is not a new idea. Lewis and Randall [23 LEW/RAN] discussed it many years ago and used it to determine the $\Delta_f G$ of urea and related compounds. Burton [53BUR/KRE], [57BUR] employed it to a limited extent for some biochemical compounds. However, the Krebs cycle is a more favorable case on which to apply this principle.

There are several sources for compiled reference values for the formation energies of the species in the Krebs cycle, including Burton and Krebs [57BUR], [53BUR/

KRE] Wilhoit [69WIL], and Johnson [60JOH]. Thauer *et al.* [77THA/JUN] presented a review of energy conservation in chemotrophic anaerobes which included extensive tables of formation Gibbs energies and Gibbs energies for numerous reactions relevant to metabolic activity. These were largely revisions of the data presented by Burton [57BUR]. There is also a recent book *Thermodynamic Data for Biochemistry and Biotechnology* [86HIN] that has a number of compilations.

Values for some species can also be found in the NBS Tables of Chemical Thermodynamic Properties (Technical Note 270-3) [68WAG/EVA] and a revised edition [82WAG/EVA]. Other compilations include gas phase data of Stull, Westrum and Sinke [69STU/WES], heats of formation by Cox and Pilcher [70COX/PIL] and Domalski [72DOM], and heat capacities and entropies of condensed phases [84DOM/EVA][85WIL/CHA].

2. The Network and Its Analysis

The regression procedure employed is the "CATCH" system used at the National Institute of Standards and Technology. It is described by Garvin *et al.* [76GAR/PARa][76GAR/PARb] and Wagman *et al.* [77WAG/SCH], and is based on procedures developed by Pedley and coworkers [69GUE/PED]. Some complications have been discussed by Barnes *et al.* [78BAR/PED]. The procedure has been designed to provide solution sets of thermochemical formation properties (Gibbs energies, enthalpies and entropies) based on input data sets drawn from the full spectrum of thermochemical measurements. As described earlier, the present study is limited to a set of measured equilibrium constants that is solved for a set of formation free energies.

The regression process takes place in two discreet steps. The first is an equally weighted least-sums solution of the entire system of equations to assess the overall consistency. The least sums basis is less sensitive to outlying values than the least-squares. The second step is a least squares solution in which each of the input data is weighted by a factor called the "average fit". This average fit factor is simply the arithmetic average of the uncertainty assigned to the original data value and the residual (observed - calculated) obtained from the least sums. This weighting scheme has been found to achieve a reasonable balance between the intrinsic uncertainty in the data and their consistency with other data in the network.

The network of reactions treated in this study is outlined schematically in Fig. 1. The metabolites for which the network was solved are connected by arrows with the inorganic species omitted as well as NAD⁺ and NADP⁺. The transamination reactions are also omitted but are equivalent to an amino acid dehydrogenase. Some of the species e.g., acetate, appear several times to avoid crossing arrows. The direction of the arrows is not significant but is generally in the favorable direction or in the direction of reduction. More complete discussion of individual reactions will follow below.

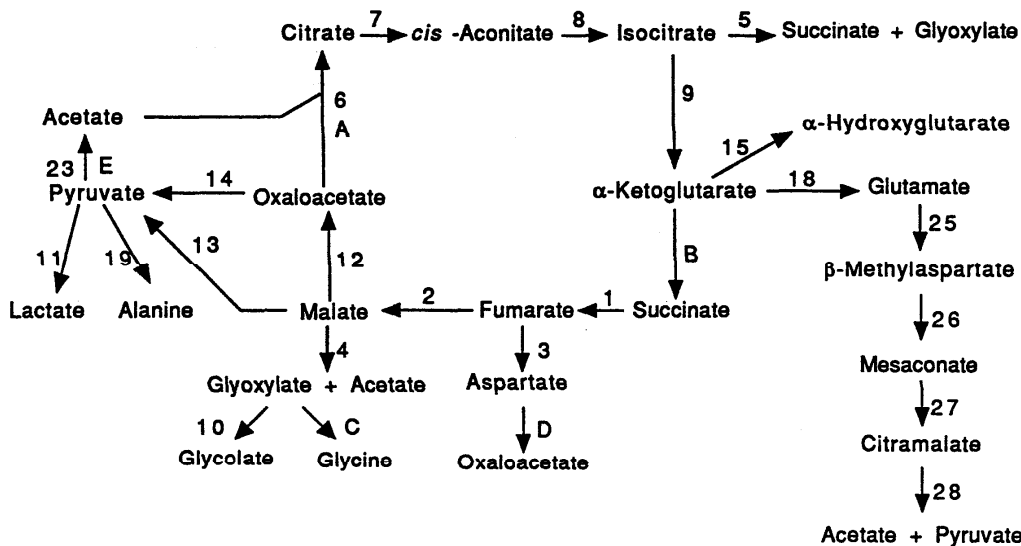


FIG. 1. Krebs cycle interconnections. NAD^+ , NADH , CO_2 , H_2O not shown. The numbers refer to the reaction as listed in Table 9.

2.1. Conventions (Standard States and Activity Coefficients)

The gas constant employed is $8.31441 \text{ J}/(\text{mol K})$ and the Faraday is taken to be $96487 \text{ C}/\text{mol}$. The Interunion Commission on Biothermodynamics [76INT] has recommended that measurements of enzyme equilibria be made at 25°C with the ionic strength brought to 0.1 M (0.1 Molar) with KCl at the lowest effective buffer concentration. The unit of concentration recommended is mol dm^{-3} ; for convenience, a solution of concentration 0.1 mol dm^{-3} (for example) may be referred to as a 0.1 M solution. This is recommended even though most mammals are at 37° and an ionic strength of 0.16 M . We follow these recommendations except for use where indicated of molal concentration (mol/kg of water). Note that a solution of molality $0.1 \text{ mol}/\text{kg}$ of water is referred to as a 0.1 m solution.

The standard state of pure water is taken as unit activity, and water will be left out of the equilibrium constant equations. The standard state of 0.1 M KCl has a $\Delta_r G$ lower by $0.01 \text{ kJ}\cdot\text{mol}^{-1}$ than pure water, but this difference will be neglected.

The Gibbs energies of formation can be corrected to zero ionic strength by using the activity coefficients in Table 6. These are single ion activity coefficients. There is a dispute as to whether these are meaningful, but the approximations used here are sufficiently accurate for a Gibbs energy table. A table of single ion activity coefficients is given by Kielland [37KIE]. Unless otherwise indicated, the values of γ_{\pm} are taken from Robinson and Stokes [59ROB/STO].

We will take $\gamma_+ = \gamma_- = \gamma_{\pm}$ for a 1:1 salt. For a 2:1 salt (e.g., Na_2 succinate) $\gamma_{\pm} = (\gamma_+^2 \gamma_-)^{1/3}$ and we approximate the single ion activity coefficients as $\gamma_+ = \gamma_{\pm}^{1/2}$ and $\gamma_- = \gamma_{\pm}^2$.

The activity coefficients for neutral molecules will be taken as 1.00. There are considerable data available on the salting out properties of various salts which are well approximated by the Sechenov equation [52LON/MCD].

$$\log f_i / f_i^\circ = km$$

where f_i is the fugacity in the salt solution, f_i° is the fugacity in pure water, m is the molal salt concentration, and k is the Sechenov constant. The ΔG of transfer from water to a 0.1 molal KCl solution would rarely amount to as much as 0.1 kJ , and so this effect will be omitted.

2.2. Optically Active and Racemic Compounds

It is evident that the Gibbs energies, enthalpies and entropies of D- and L-isomers of the same compound must be equal. The enthalpy of formation of a racemic mixture in solution at infinite dilution will also equal that of the D- or L-isomer. The enthalpy of the solid racemic compound will be the same as the isolated isomer only if it is a conglomerate (i.e., a mixture of the D- and L-crystals). Examples of conglomerates are sodium ammonium tartrate and glutamic acid. In many cases, the racemic mixture forms a DL-crystal, and this racemic crystal may have physical properties considerably different from the D- or L-crystal. Examples are proline and serine where the L-isomers are about ten times more soluble than the DL-crystals.

The entropy of the D- and L- compounds in the crystal or in solution are the same. For the DL mixture in solution the entropy differs from that for the isolated isomers by the symmetry factor:

$$S_{\text{DL}} = S_{\text{L}} + R \ln 2$$

The entropy of the DL-compound in solution is higher than that for the isolated isomers and the optically active isomer will spontaneously convert to the DL-mixture if a kinetic pathway is available. The Gibbs energy of the D- and L-compounds in the crystal and in solution are also the same. The $\Delta_f G$ of the DL mixture in solution differs from the isolated isomers by the entropy difference:

$$\Delta_f G_{DL} = \Delta_f G_L - RT \ln 2$$

The tables give the $\Delta_f G$ and S° for only the L-isomer, as this is usually the naturally occurring isomer.

3. Third Law Calculations and Ancillary Data

In solving the network for the formation Gibbs energies for individual metabolites, the data for the various equilibria have been supplemented by formation properties for inorganic species and independent estimates of formation energies for several key compounds via third law calculations. These calculations include formate and acetate which are included in the NBS Tables. The NBS evaluation for these compounds, however is over twenty years old and considerable new data exists for obtaining the formation properties for the anions in aqueous solution.

Data for the inorganic compounds needed to supplement the network are listed in Table 1. They are taken from the CODATA compilation [89COX/WAG], and are generally the same as values tabulated in the NBS Tables with a few exceptions.

The compounds for which estimates have been made via third law calculation are fumarate, succinate, glycine, alanine, aspartic acid and glutamic acid. Discussions for each of these follow.

3.1. Succinate and Fumarate

The data necessary for third law calculations for succinate and fumarate are listed in Table 2.

The enthalpy of combustion of succinic acid is known quite reliably (it is nearly a calorimetric standard) with recent determinations [72VAN/MANa] and [70ZAI/NAZ] in excellent agreement with evaluated values [70COX/PIL], [77PED/RYL] and [86PED/NAY]. The absolute entropy has been calculated from heat capacity measurements [70VAN/WES] from 5 to 328 K removing uncertainty associated with the extrapolation from 90 to 0 K [30PAR/HUF] which gave the value of S° too high by 8.4 J·K⁻¹·mol⁻¹.

The enthalpy of solution of succinic acid and the acid dissociation constants are also known quite reliably. Vanderzee *et al.* [72VAN/MANb] have made a careful study of the enthalpy of solution, while the dissociation constants have been evaluated by Martell and Smith [74MAR/SMI]. The solubility and activity coefficients are given in the footnotes of Table 2.

TABLE 1. CODATA $\Delta_f H$ and S° values for elements and inorganic compounds. $\Delta_f G$ values calculated from these $\Delta_f H$ and S° . The aqueous ions and aqueous gases refer to 1 mol dm⁻³. The gases refer to 1 atm. These values are for $I=0$ and not $I=0.1$ mol dm⁻³.

Compound	$\Delta_f G^\circ$ kJ·mol ⁻¹	$\Delta_f H^\circ$ kJ·mol ⁻¹	S° J·mol ⁻¹ ·K ⁻¹
H ₂ (g)	0	0	130.57
H ₂ (aq)	17.73 ^a	-4.04 ^a	57.57 ^a
C(graphite)	0	0	5.74
N ₂ (g)	0	0	191.50
O ₂ (g)	0	0	205.04
O ₂ (aq)	16.52 ^b	-12.06 ^b	109.20 ^b
H ₂ O(l)	-237.19	-285.83	69.95
H ⁺ (aq)	0	0	0
OH ⁻ (aq)	-157.27	-230.02	-10.90
CO(g)	-137.15	-110.53	197.55
CO ₂ (g)	-394.37	-393.51	213.68
CO ₂ (aq)	-386.00	-413.26	119.36
HCO ₃ ⁻ (aq)	-586.93	-689.93	98.4
CO ₃ ²⁻ (aq)	-527.98	-675.23	-50.0
NH ₃ (g)	-16.49	-45.94	192.66
NH ₃ (aq)	-26.66 ^c	-81.04 ^c	109.29 ^c
NH ₄ ⁺ (aq)	-79.46	-133.26	111.17
Na ⁺ (aq)	-242.47	-240.34	58.45
K ⁺ (aq)	-263.03	-252.14	101.20

^aFrom CODATA values for H₂(g) and $\Delta_{sol}G$, $\Delta_{sol}H$, $\Delta_{sol}S$ [77WIL/BAT].

^bFrom CODATA values for O₂(g) and $\Delta_{sol}G$, $\Delta_{sol}H$, $\Delta_{sol}S$ [77WIL/BAT].

^cFrom CODATA values for NH₄⁺ and $\Delta_{ion}G$, $\Delta_{ion}H$, $\Delta_{ion}S$ [76CHR/HAN].

The data for fumaric acid on the other hand are considerably less reliable. The combustion enthalpy has been evaluated by Cox and Pilcher [70COX/PIL] and more recently by Pedley *et al.* [86PED/NAY] with the two evaluations differing by 0.7 kJ·mol⁻¹. The absolute entropy is based upon heat capacity measurements that have been extrapolated below 90 K [30PAR/HUFF], and the values for the enthalpy of solution and solubility are somewhat uncertain. The solubility and activity coefficients are discussed in the footnotes of Table 2. The acid dissociation constants have been subject to some disagreement. Robinson and Stokes [59ROB/STO] gave values of $pK_1 = 3.019$ and $pK_2 = 4.384$ while measurements by Bada and Miller [68BAD/MIL] yielded values of $pK_1 = 2.94$ and $pK_2 = 4.10$ at $I = 0.1$ mol dm⁻³. Martell and Smith [74MAR/SMI] selected values of $pK_1 = 2.94$ and $pK_2 = 4.109$ at $I = 0.1$ mol dm⁻³ in good agreement with the measurements of Bada and Miller. More accurate measurements for the properties of fumaric acid are highly desirable as it is probably the best choice for a key value upon which to base the entire network by virtue of the large number of compounds in the network to which it is connected.

3.2. Glycine, Alanine, Aspartic Acid, and Glutamic Acid

The data for these compounds are listed in Table 3 for the L- isomers. Modern values for the absolute entropies

TABLE 2. Third law calculation of thermodynamic data for succinic acid and fumaric acid. The values of ΔG and ΔH are given in $\text{kJ}\cdot\text{mol}^{-1}$ and ΔS in $\text{J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$. The molality and ionic strength (I) are given in mol kg^{-1} .

	Succinic Acid	Ref	Fumaric Acid	Ref
$\Delta_f H(\text{cr})$	-940.81 ± 0.42	a	-810.98 ± 0.63	a
$\Delta_{\text{sol}} H$	28.70 ± 0.04	b	35.8 ± 0.5	c
$\Delta_f H(\text{aq})$	-912.11 ± 0.42		-775.18 ± 0.80	
$\Delta_{\text{ion}} H$	2.73	h	-2.39	h
$\Delta_f H(\text{aq ion}^{-2})$	-909.37		-777.57	
$S^\circ(\text{cr})$	167.3 ± 0.2	d	166.1 ± 4.0	e
$\Delta_f S(\text{cr})$	-657.43		-528.08	
m_{sat}	0.699	f	0.0456	g
γ_{sat}	1.0	f	1.0	g
$\Delta_{\text{sol}} S$	+93.30		+94.40	
$S^\circ(\text{aq acid})$	+260.62		+260.45	
$\Delta_{\text{ion}} S$	-179.1	h	-152.2	h
$S^\circ(\text{aq ion}^{2-})$	+81.5		+108.3	
$\Delta_f G(\text{cr})$	-744.80		-653.54	
$\Delta_{\text{sol}} G$	+0.89		+7.66	
$\Delta_f G(\text{aq acid})$	-743.92		-643.88	
$\Delta_{\text{ion}} G$	+56.15	h	+43.93	h
$\Delta_f G(\text{ion}^{2-}, I=0)$	-687.77		-601.95	
γ_{-2}	0.40	i	0.36	i
$\Delta_f G(\text{ion}^{2-}, I=0.1)$	-690.03 ± 0.44		-604.50 ± 1.51	

[†][70COX/PIL] Later values for succinic acid are -940.35 ± 0.54 [72VAN/MANa] and -940.19 ± 0.21 [70ZAI/NAZ].

[‡][72bVAN/MANb].

[§]From [69WIL]. The International Critical Tables give 8.90 kcal. Wasserman gives 7.98 kcal [30WAS].

[¶][70VAN/WES].

^{||}[30PAR/HUF] The C_p was extrapolated from 90 K.

^{¶¶}Solubility at 25 ° is $0.706 \text{ mol dm}^{-3}$ [58LIN/SEI], which corrected for ionization of 6.94% gives $0.699 \text{ mol dm}^{-3}$ for the undissociated acid. γ taken as 1.0.

^{¶¶¶}The solubility is given only by formula in [30LAN/SIN] as $0.0521 \text{ mol dm}^{-3}$. The fumaric acid is 12.5% dissociated, so $m = 0.0456 \text{ mol dm}^{-3}$ for the undissociated acid. γ taken as 1.0. An earlier value of this solubility is $m = 0.0603 \text{ mol dm}^{-3}$ [23WEI/DOW].

^{¶¶¶¶}These are for both ionizations. See Table 6 for pK values, ΔH and ΔS of ionization.

^{¶¶¶¶¶}For succinate Kielland [37KIE] gives $\gamma_{-2} = 0.38$ at $I = 0.1 \text{ mol dm}^{-3}$ and Komar *et al.* [67KOM/MUS] give $\gamma_{-2} = 0.469$ with 0.1 mol dm^{-3} KCl and $\gamma = 0.334$ with 0.1 mol dm^{-3} NaCl. For sodium fumarate Robinson and Stokes give by interpolation $\gamma_{\pm} = 0.600$ at $I = 0.1 \text{ mol dm}^{-3}$ ($m = 0.033$), which gives $\gamma_{-2} = \gamma_{\pm}^2 = 0.360$. Komar *et al.* [72KOM/TKH] give $\gamma = 0.382$ with 0.1 mol dm^{-3} KCl and $\gamma = 0.331$ with 0.1 mol dm^{-3} NaCl.

are available for glycine and the L-isomers. The enthalpies of combustion are fairly reliable but can always be improved. The enthalpy of formation of the DL-alanine in solution confirms the value adopted for the L-isomer as it is nearly identical. The enthalpies of solution for aspartic acid and glutamic acid are weak because it is not clear whether the corrections were made for ionization at high dilution. The activity coefficients at saturation for aspartic and glutamic acids may also be in error.

3.3. Citrate

A good $\Delta_f H = -1837.6 \pm 0.4 \text{ kJ}\cdot\text{mol}^{-1}$ for citric acid monohydrate is available [70COX/PIL], and but only a poor $\Delta_f H = -1544 \pm 5 \text{ kJ}\cdot\text{mol}^{-1}$ for anhydrous citric acid has been determined. The third law entropy was determined by Evans *et al.* [62EVA/HOA] and they calculated the $\Delta_{\text{sol}} G$ based on the data of Levien [55LEV]. Evans *et al.* give

$$\Delta_f G(\text{monohydrate, cr}) = -1472.8 \pm 1.2 \text{ kJ}\cdot\text{mol}^{-1}$$

$$\Delta_f G(\text{citric acid, aq}) = -1243.4 \pm 1.2 \text{ kJ}\cdot\text{mol}^{-1}$$

$$\Delta_f G(\text{citrate}^{-3}, \text{aq}) = -1161.8 \pm 1.4 \text{ kJ}\cdot\text{mol}^{-1}$$

The single ion activity coefficients are difficult to estimate, since the γ of the Na^+ or K^+ salts have not been measured. Using Kielland's value of $\gamma_{-3} = 0.18$ [37KIE] gives

$$\Delta_f G(\text{citrate, aq}) = -1166.1 \pm 2.5 \text{ kJ}\cdot\text{mol}^{-1}$$

$$I = 0.10 \text{ mol dm}^{-3}$$

An alternative procedure is to use the apparent pK 's of citric acid to estimate the single ion activity coefficients. Using the data of Rajan and Martell [65RAJ/MAR] ($pK_1 = 2.79$, $pK_2 = 4.30$ and $pK_3 = 5.65$) we have $\gamma_{-1} = 0.346$, $\gamma_{-2} = 0.179$, $\gamma_{-3} = 0.0284$, and

$$\Delta_f G(\text{citrate, aq}) = -1170.6 \pm 2.5 \text{ kJ}\cdot\text{mol}^{-1}$$

$$I = 0.1 \text{ mol dm}^{-3} (\text{KNO}_3)$$

Using the data of Tate *et al.* [65TAT/GRZ] ($pK_1 = 2.88$, $pK_2 = 4.36$, $pK_3 = 5.85$) we have $\gamma_{-1} = 0.565$, $\gamma_{-2} = 0.224$ and $\gamma_{-3} = 0.0623$, giving

$$\Delta_f G(\text{citrate, aq}) = -1168.7 \pm 2.5 \text{ kJ}\cdot\text{mol}^{-1}$$

$$I = 0.1 \text{ mol dm}^{-3} [(\text{CH}_3)_4\text{NCl}]$$

The γ 's are in the expected direction since K^+ is known to be complexed by citrate. We will use the γ_{-3} for the KNO_3 solutions, recognizing the low activity coefficient is probably due to complexing. Since KCl is frequently used for enzyme solutions, it is simpler to include the complexing in the $\Delta_f G$ rather than correcting for it.

The difficulty in determining the γ for a -3 ion, as demonstrated by the large variation in the calculated values, suggest that citrate is a poor compound to use in the network. Therefore, its third law $\Delta_f G$ has been left out of all the network calculations.

3.4. Formate

There are several thermochemical cycles by which the Gibbs energy of formation of the formate anion can be calculated, a comparison of which will give some idea of the reliability of the estimated value.

TABLE 3. Third law calculation of thermodynamic data for several amino acids. ΔH and ΔG in $\text{kJ}\cdot\text{mol}^{-1}$ and S° in $\text{J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$. The molality and ionic strength (I) are in mol kg^{-1} .

	Glycine	Ref	L-Alanine	Ref	L-Aspartic	Ref	L-Glutamic	Ref
$\Delta_f H(\text{cr})$	-528.52 ± 0.50	a,f	-561.24 ± 0.63	b,f	-973.32 ± 0.92	a	-1005.25 ± 1.26	b,g
$\Delta H(\text{soln})$	$+14.16 \pm 0.01$	e	$+7.49 \pm 0.03$	i,h	$+25.82 \pm 0.10$	j	$+24.53 \pm 0.10$	j
$\Delta_f H(\text{aq})$	-514.36 ± 0.50		-553.75 ± 0.63		-947.50 ± 0.93		-980.72 ± 1.27	
$S^\circ(\text{cr})$	$+103.51$	c	129.21	c	$+170.12$	c	$+188.20$	c
$\Delta_f S(\text{cr})$	-535.19	c	-645.80	c	-815.67	c	-933.90	c
m_{sat}	3.33	c	1.862	c	0.0375	c	0.0586	c
γ_{sat}	0.729	c	1.045	c	1.0	c	1.0	c
$\Delta_{\text{sol}} S$	$+54.94$		$+30.59$		$+59.37$		$+58.70$	
$S^\circ(\text{aq})$	$+158.45$		$+159.80$		$+229.49$		$+246.90$	
$\Delta_f G(\text{cr})$	-368.98		-368.74		-730.19		-726.87	
$\Delta_{\text{sol}} G$	-2.22 ± 0.04	c	-1.63 ± 0.04	c	$+8.12 \pm 0.08$	c	$+7.03 \pm 0.02$	c
$\Delta_f G(I=0)$	-371.20 ± 0.60		-370.37 ± 0.70		-722.07 ± 0.93		-719.84 ± 1.27	
γ_{+-} or γ_{0+-}	0.97		0.95		0.95		0.95	
$\Delta_f G(I=0.1)$	-371.33		-370.50		-722.20		-719.97	
$\Delta_{\text{ion}} G$					$+22.26$	k	$+24.39$	k
$\Delta_f G^{+-}(I=0)$					-699.94		-695.59	
$\gamma_{+-}(I=0.1)$					0.73	k	0.73	k
$\Delta_f G^{+-}(I=0.1)$					-700.72		-696.37	
$\Delta_{\text{ion}} H$					$+4.64$	k	$+1.56$	k
$\Delta_f H^{+-}(\text{aq})$					-942.86		-979.16	

^a[70COX/PIL].

^b[70COX/PIL]. This is for D-alanine and D-glutamic acid. The values for the L-isomers seem to be in error.

^c[76HUT][84DOM/EVA].

^d[43COH/EDS], p. 198 & 219

^e[75SPI/WAD].

^fMore recent values are $\Delta_f H(\text{glycine}) = -528.61 \pm 0.21 \text{ kJ}\cdot\text{mol}^{-1}$ and $\Delta_f H(\text{L-alanine}) = -559.48 \pm 0.46 \text{ kJ}\cdot\text{mol}^{-1}$ [77NGA/SAB]. Values are also given by Kamaguchi *et al.* [75KAM/SAT] for $\Delta_f H(\text{L-alanine}) = -561.08 \pm 0.71 \text{ kJ}\cdot\text{mol}^{-1}$, $\Delta_f H(\text{D-alanine}) = -560.06 \pm 0.76 \text{ kJ}\cdot\text{mol}^{-1}$, $\Delta_f H(\text{DL-alanine}) = -562.35 \pm 0.65 \text{ kJ}\cdot\text{mol}^{-1}$.

^gLater values are $\Delta_f H(\text{L-glutamic}) = -1003.32 \pm 1.17 \text{ kJ}\cdot\text{mol}^{-1}$ and $\Delta_f H(\text{D-glutamic}) = -1002.47 \pm 0.97 \text{ kJ}\cdot\text{mol}^{-1}$ [75SAK/SEK].

^hAnother value can be obtained from DL-alanine. $\Delta_f H = -563.58 \pm 0.63 \text{ kJ}\cdot\text{mol}^{-1}$ [70COX/PIL] and $\Delta_{\text{sol}} H = 9.13 \pm 0.03 \text{ kJ}\cdot\text{mol}^{-1}$ [82ABA/SHE]. This gives $-554.45 \pm 0.64 \text{ kJ}\cdot\text{mol}^{-1}$ for the aqueous DL-alanine.

ⁱ82ABA/SHE.

^jL-Aspartic acid [82VAS/KOC], L-glutamic acid [80MAT/AMA]. It is not clear whether the corrections for ionization on dissolution were made.

The data of Rodante and Tocci [85ROD/TOC] are close to these values. Earlier values are given in [76HUT].

^kSee Table 6.

Standard enthalpies of formation for the pure liquid and gas phases have been evaluated by Cox and Pilcher [70COX/PIL], Pedley and Rylance [77PED/RYL], and Pedley *et al.* [86PED/NAY]. We will use the values given by Cox and Pilcher which are $-425.0 \pm 0.4 \text{ kJ}\cdot\text{mol}^{-1}$ for the liquid and $-378.9 \pm 0.6 \text{ kJ}\cdot\text{mol}^{-1}$ for the gas monomer. The enthalpy of solution has been determined [71KON/WAD] to be $-0.678 \pm 0.001 \text{ kJ}\cdot\text{mol}^{-1}$, yielding a value for $\Delta_f H(\text{HCOOH}(\text{aq}))$ of $-425.7 \pm 0.4 \text{ kJ}\cdot\text{mol}^{-1}$. The third law entropy of the liquid has been given as $129.59 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ [85WIL/CHA] from the heat capacity data of Stout and Fisher [41STO/FIS]. This gives $\Delta_f S = 211.97 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ and $\Delta_f G = -361.80 \text{ kJ}\cdot\text{mol}^{-1}$ for the liquid. The difficulty is to obtain the $\Delta_{\text{sol}} G$ at 25°C , and there seems to be no accurate data on the water-formic acid phase diagram.

An alternative is to use the gas phase data and the ΔG of solution from the gas. Chao and Zwolinski [78CHA/ZWO] give the ideal gas entropy of the formic acid monomer as $S^\circ = 248.86 \text{ J}\cdot\text{mol}^{-1} \text{ K}^{-1}$ and $\Delta_f G = -351.00 \text{ kJ}\cdot\text{mol}^{-1}$ and $\Delta_f H = -378.57 \text{ kJ}\cdot\text{mol}^{-1}$. The Gibbs energy and entropy of solution are difficult to cal-

culate due to complications from dimerization. Kaye and Parks [34KAY/PAR] reported $P_{\text{atm}}/m = 1.91 \times 10^{-4} \text{ atm (mol/kg)}^{-1}$ while Campbell and Campbell [34CAM/CAM] obtained a value of $P_{\text{atm}}/m = 1.71 \times 10^{-4} \text{ atm (mol/kg)}^{-1}$. These yield respectively, $\Delta_{\text{sol}} G = 21.21$ and $21.50 \text{ kJ}\cdot\text{mol}^{-1}$. Taking the average of these two values yields $\Delta_f G(\text{HCOOH}, \text{aq}) = -372.38 \text{ kJ}\cdot\text{mol}^{-1}$. Taking $\Delta_f H = -425.7 \text{ kJ}\cdot\text{mol}^{-1}$ for the aqueous acid gives $\Delta_f S = -178.84$ and $S^\circ = 162.72 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$.

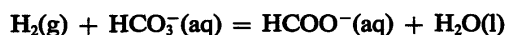
The third law entropy of NaHCO_2 ($24.80 \text{ cal K}^{-1}\cdot\text{mol}^{-1}$) has been measured [60WES/CHA]. The enthalpy of solution will be taken as $+0.74 \pm 0.03 \text{ J}\cdot\text{mol}^{-1}$ [75CHA/AHL, 82CHO/AHL]. The value of $+1.13$ [70SNE/GRE] may be too high because the final concentration was too high. Bonner has recently measured $m_{\text{sat}} = 14.7 \text{ mol kg}^{-1}$ and $\gamma_{\text{sat}} = 0.877$ [88BON].

$$\begin{aligned} \Delta_{\text{sol}} H/T + 2R \ln m_{\text{sat}} + 2R \ln \gamma_{\text{sat}} &= 45.00 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1} \\ &= S^\circ(\text{NaHCO}_2, \text{aq}) - S^\circ(\text{NaHCO}_2, \text{cr}) \end{aligned}$$

This gives $S^\circ = 148.76 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ for aqueous sodium formate, and combined with $S^\circ = 58.45 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ for aqueous Na^+ gives $S^\circ = 90.31 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ for aqueous formate.

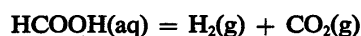
The entropy of ionization of formic acid is $71.67 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ [76CHR/HAN], so for aqueous formic acid $S^\circ = 161.98 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ and $\Delta_p S = -179.37 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$. This gives $\Delta_r G = -425.7 + 53.48 = -372.2 \text{ kJ}\cdot\text{mol}^{-1}$ for the aqueous acid using the third law entropy for sodium formate.

The Gibbs energy of formation of formate can also be calculated from the formic hydrogenlyase equilibrium:



which involves only formate and substances already adopted from the CODATA key values. The equilibrium constant was measured by Woods [36WOO] yielding the values of $\Delta_r G = -0.715 \text{ kJ}\cdot\text{mol}^{-1}$ and $\Delta_r H = -22.64 \text{ kJ}\cdot\text{mol}^{-1}$. The enthalpy is somewhat unreliable as it was estimated from equilibrium determinations at only 25 and 38 °C. The resultant value of $\Delta_r G(\text{formate, aq}) = -350.37 \text{ kJ}\cdot\text{mol}^{-1}$ and $\Delta_r G(\text{formic acid, aq}) = -371.79 \text{ kJ}\cdot\text{mol}^{-1}$. Using the $\Delta_r H = -425.7 \text{ kJ}\cdot\text{mol}^{-1}$ for the aqueous acid gives $\Delta_p S = -180.8$ and $S^\circ = 160.7 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$.

Alternatively, the non-enzymatic decomposition of formate via the equilibrium:



will also yield a formation energy for the formate anion based only on the CODATA values. This equilibrium was measured by Bredig *et al.* [29BRE/CAR] from 20 to 90 °C, but at pressures of 60 to 100 atm. The fugacity corrections were discussed by Waring [52WAR] who calculated a value for $\Delta_r G(\text{formic acid, aq}) = -371.66$ and $-370.28 \text{ kJ}\cdot\text{mol}^{-1}$ (corrected and uncorrected, respectively). This is in good agreement with the value obtained from the hydrogenlyase equilibrium and third law calculations, but the reliability of the fugacity correction is less than satisfactory for this application.

The decomposition of aqueous formic acid to carbon monoxide and water has also been examined by Branch [15BRA] at temperatures of 156 and 219 °C and can also be used to obtain the formation Gibbs energy of aqueous formic acid. This analysis has been discussed by Lewis and Randall [23LEW/RAN] and Waring [52WAR] who give values of $\Delta_r G(\text{formic acid, aq}) = -373.25 \text{ kJ}\cdot\text{mol}^{-1}$ and $-374.05 \text{ kJ}\cdot\text{mol}^{-1}$ respectively. These agree well with the estimates by other means but are less reliable because of the large temperature correction.

We select $\Delta_r G$ from the formic hydrogenlyase reaction and the entropy from the sodium formate. We also use $pK_a = 3.751$, $\Delta_{\text{ion}}H = +0.04 \pm 0.20 \text{ kJ}\cdot\text{mol}^{-1}$ and $\Delta_{\text{ion}}S = -71.67 \pm 0.71 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ and $\gamma = 0.778$ [59ROB/STO]. The lowering of $\Delta_r G$ of formate from the activity coefficient is arbitrarily put into the $\Delta_r H$. The $\Delta_r H$ for aq formic acid obtained from the formic

hydrogenlyase equilibrium and S° from sodium formate differs by only 0.4 kJ from the value selected for the liquid by Cox and Pilcher combined with $\Delta_{\text{sol}}H$.

The selected values are given in Table 4. They are close to the NBS values for aqueous formic acid of $\Delta_r G = -372.3 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$, $\Delta_r H = -425.4 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ and $S^\circ = 163 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$. The NBS values for formate are $\Delta_r G = -351.0 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$, $\Delta_r H = -425.6 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ and $S^\circ = 92 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ [68WAG/EVA][82WAG/EVA].

TABLE 4. Selected third law $\Delta_r G$, $\Delta_r H$ and S° for formic and acetic acids. Ionic strength (I) is in mol kg^{-1} .

	$\Delta_r G$ $\text{kJ}\cdot\text{mol}^{-1}$	$\Delta_r H$ $\text{kJ}\cdot\text{mol}^{-1}$	S° $\text{J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$
Formic acid (aq)	-371.79 ± 0.20	-425.3 ± 0.4	162.0
Formate (aq)	-350.37 ± 0.20	-425.2 ± 0.4	90.3
Formate (aq, $I=0.1$)	-350.99 ± 0.20	-425.8 ± 0.4	90.3
Acetic acid (aq)	-396.1 ± 0.4	-485.5 ± 0.3	177.8
Acetate (aq)	-369.0 ± 0.4	-486.0 ± 0.3	85.3
Acetate (aq, $I=0.1$)	-369.6 ± 0.4	-480.6 ± 0.3	85.3

3.5. Acetate

The thermodynamic properties of aqueous acetic acid and the acetate anion present difficulties in obtaining a reliable value for ΔS of solution because of the formation of dimers in the gas phase as in the case of formic acid.

There are two modern enthalpies of combustion given in Cox and Pilcher -115.71 ± 0.10 and $-115.79 \pm 0.09 \text{ kcal mol}^{-1}$ with a selected value of $-115.75 \pm 0.07 \text{ kcal mol}^{-1} = -484.3 \pm 0.3 \text{ kJ}\cdot\text{mol}^{-1}$ [70COX/PIL]. We take the enthalpy of solution as $-1.176 \pm 0.004 \text{ kJ}\cdot\text{mol}^{-1}$ [71KON/WAD] giving $\Delta_r H = -485.5 \pm 0.3 \text{ kJ}\cdot\text{mol}^{-1}$ for the aqueous acid.

The $pK_a = 4.756$ is reliably known. There have been a number of calorimetric determinations of the ΔH of ionization in the range -0.07 to $-0.137 \text{ kcal mol}^{-1}$ with ΔH from the temperature dependence of pK_a in the range -0.098 to -0.112 [77CHR/HAN]. We select $\Delta_{\text{ion}}H = -0.112 \text{ kcal} = -0.47 \text{ kJ}$ and $\Delta_{\text{ion}}S = -22.11 \text{ cal K}^{-1}\cdot\text{mol}^{-1} = -92.5 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ [52EVE/LAN]. This gives $\Delta_r H = -486.0 \pm 0.3 \text{ kJ}\cdot\text{mol}^{-1}$ for the aqueous ion.

There are three direct methods of obtaining the entropy of the aqueous acid and anion. They are from a) the entropy of the liquid, b) the entropy of the gaseous monomer, and c) the entropy of the sodium salt. There is a modern entropy for the liquid of $S^\circ = 37.76 \text{ cal K}^{-1}\cdot\text{mol}^{-1} = 158.0 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ [82MAR/AND] which replaces the Parks and Kelly value of $S^\circ = 38.2 \text{ cal K}^{-1}\cdot\text{mol}^{-1}$ based on extrapolation of C_p below 90 K [29PAR/KEL]. The entropy of solution (liq \rightarrow 1m aq) can be calculated from the $\gamma = 3.20$ at $x_2 = 0$ given by Hansen *et al.* [55HAN/MIL].

$$\Delta_{\text{sol}}S = \Delta_{\text{sol}}H/T - R \ln \gamma + R \ln 55.51$$

This gives $\Delta_{\text{sol}}S = 19.79 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$, $S^\circ = 177.8 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ for the aqueous acid, and $S^\circ = 85.3 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ for acetate anion.

The statistical entropy of the gas phase monomer has been calculated a number of times and reviewed by Chao *et al.* [86CHA/HAL] who give $S^\circ = 283.36 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ at 1 atm for the gaseous monomer. There are many data on the volatility of acetic acid-water solutions, which gives $\Delta_{\text{sol}}G$, but $\Delta_{\text{sol}}H$ of the monomer is difficult to estimate. This will be taken up in a subsequent paper.

The third law entropy of anhydrous sodium acetate has been measured [83FRA/PLA] as $138.10 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$. The enthalpies of solution include $-17.32 \pm 0.21 \text{ kJ}\cdot\text{mol}^{-1}$ [65PAR], $-16.59 \text{ kJ}\cdot\text{mol}^{-1}$ [75CHO/AHL], $-17.46 \text{ kJ}\cdot\text{mol}^{-1}$ [82CHO/AHL], and $-16.88 \text{ kJ}\cdot\text{mol}^{-1}$ from the extrapolation of the data of Snell and Greyson [70SNE/GRE] using enthalpies of dilution of Parker [65PAR]. We select an average value of $-17.03 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$. It is to be noted that the $\Delta_{\text{sol}}H$ measured is very sensitive to water in the sodium acetate. Thus if 1% of the sodium acetate is hydrated after weighing, the $\Delta_{\text{sol}}H$ measured would be -16.16 kJ instead of -17.03 kJ .

Bonner has recently measured $m_{\text{sat}} = 15.20 \text{ mol kg}^{-1}$ and $\gamma_{\text{sat}} = 2.786$ [88BON]. These are super saturated solutions since the trihydrate of sodium acetate is soluble to only 6.16 m . However, sodium acetate solutions are well known for their tendency to supersaturate. We have

$$\begin{aligned} \Delta_{\text{sol}}H/T + 2R \ln m_{\text{sat}} + 2R \ln \gamma_{\text{sat}} \\ = +15.17 = S^\circ(\text{NaAc, aq}) - S^\circ(\text{NaAc, cr}) \end{aligned}$$

This gives $S^\circ = 143.27 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ for aqueous sodium acetate, and $S^\circ = 143.27 - 58.42 = 84.85 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ for the acetate anion.

This is in good agreement with the $S^\circ = 85.3 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ from the acetic acid route, but alternative choices of the $\Delta_{\text{sol}}H$ would give less satisfactory results (86.3 for $\Delta_{\text{sol}}H = -16.59$ and 83.4 for $\Delta_{\text{sol}}H = -17.46$). We select Δ_rH from the enthalpy of combustion of the liquid, S° from the third law entropy of the liquid, and $\text{p}K = 4.756$ and $\gamma = 0.791$ from Robinson and Stokes [59ROB/STO].

The selected values are given in Table 4. These can be compared with the NBS values [68WAG/EVA] [82WAG/EVA]. For aqueous unionized acetic acid $\Delta_rG = -396.6 \text{ kJ}\cdot\text{mol}^{-1}$, $\Delta_rH = -485.8 \text{ kJ}\cdot\text{mol}^{-1}$, $S^\circ = 178.6 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$. For acetate ion $\Delta_rG = -369.4 \text{ kJ}\cdot\text{mol}^{-1}$, $\Delta_rH = -485.76 \text{ kJ}\cdot\text{mol}^{-1}$, $S_m^\circ = 86.6 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$.

3.6. Other Krebs Cycle Acids

It will be difficult to obtain third law entropy values for a number of Krebs cycle acids such as lactic acid,

malic acid, pyruvic acid, α -ketoglutaric acid, and oxaloacetic acid. Even if the S° of the crystal were known, the ΔS of solution will be difficult to measure. Oxaloacetic acid decarboxylates relatively rapidly. The others are very soluble, but more significantly the hydroxy acids form polyesters in concentrated solution and the keto acids may dimerize, thereby making osmotic coefficient measurements difficult. The alkali metal salts all seem to be very soluble.

The Δ_rH of the aqueous acid can be obtained from the enthalpy of combustion and $\Delta_{\text{sol}}H$. In some cases it may be advantageous to measure enthalpy of combustion and solution of the ammonium salt. Isocitric acid forms a lactone easily so the best approach would be to measure the enthalpy of combustion of the lactone and the $\Delta_{\text{sol}}H$ in NaOH to get Δ_rH of isocitrate.

Table 5 gives the available enthalpies of formation of the Krebs cycle acids.

TABLE 5. Δ_rH and $\Delta_{\text{sol}}H$ of some Krebs cycle acids.

Acid	Δ_rH $\text{kJ}\cdot\text{mol}^{-1}$	ΔH soln $\text{kJ}\cdot\text{mol}^{-1}$	$\Delta_rH(\text{aq acid})$ $\text{kJ}\cdot\text{mol}^{-1}$
Glycolic	-664.0 ± 4.0^a	$+15.94^a$	-651.0 ± 4.5
L-Lactic	-694.0 ± 1.0^b	$+7.82^c$	-686.2 ± 1.0
L-Malic	-1103.6^b		
DL-Malic	-1105.66 ± 0.63^b	21.85^c	-1083.81 ± 0.64
α -Hydroxyglutaric			
Glyoxylic			
Pyruvic	-584.5 ± 6.0^d	-19.10^f	-603.7
Oxaloacetic	-984.5 ± 4.0^g		
α -Ketoglutaric	-1026.2 ± 0.9^b		
Isocitric			
<i>cis</i> -Aconitic	$-1224.7 \pm 7.5^{b,h}$		
<i>trans</i> -Aconitic	-1232.7 ± 2.5^b		

^a[68WAG/EVA] error estimated.

^b[70COX/PIL]

^c[59SAV/GUN]

^d[69STU/WES] error estimated.

^e[86APE]

^f[25BLA]

^g[69WIL] error estimated

^h*cis*-Aconitic acid is the Krebs cycle intermediate, but it is unstable with respect to the *trans*-isomer.

3.7. Ionization Data

The values of $\text{p}K_a$, $\Delta_{\text{ion}}H$ and $\Delta_{\text{ion}}S$ used in this paper are listed in Table 6, together with the single ion activity coefficient data and the $\text{p}K$ at $I=0.1 \text{ mol dm}^{-3}$. This is not intended to be complete since extensive compilations are available [64SIL/MAR] [71SIL/MAR] [74MAR/SMI] [76CHR/HAN] [79PER], but the data used are stated because a considerable number of values are available in the literature. The blank spaces in the table are evident, and such data would be useful.

TABLE 6. Ionization data for some Krebs compounds. ΔH in $\text{kJ}\cdot\text{mol}^{-1}$ and ΔS in $\text{J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$.

Acid	pK_a	Ref	$\Delta_{ion}H$	Ref	$\Delta_{ion}S$	γ_0	γ_-	Ref	pK' ($I=0.1$)
Formic	3.751	b	+0.04	b	-71.7	1.00	0.778	c	3.64
Acetic	4.756	a	-0.42	b	-92.5	1.00	0.796	c	4.66
Succinic pK_1	4.207	a	+3.18	b	-69.9	1.00	0.762	c	4.09
Succinic pK_2	5.636	a	-0.45	b	-109.2	0.762	0.40	d	5.37
Fumaric pK_1	3.095	b,f	+0.46	b	-57.7	1.00	0.762	d	2.98
Fumaric pK_2	4.602	b,f	-2.85	b	-97.5	0.762	0.40	d	4.32
Glycolic	3.831	b	+0.46	b	-72.0	1.00	0.740	d	3.70
Lactic	3.858	b	-0.43	b	-75.3	1.00	0.76	d	3.74
Malic pK_1	3.458	b	+2.95	b	-56.1	1.00	0.70	d	3.30
Malic pK_2	5.097	b	-1.18	b	-101.3	0.70	0.35	d	4.80
α -Hydroxyglutaric pK_1	3.59	j				1.00	0.70	d	3.44
α -Hydroxyglutaric pK_2	5.14	j				0.70	0.35	d	4.84
Glyoxylic	3.46	b	+2.22	b	-58.8	1.00	0.60	d	3.24
Pyruvic	2.490	k	+12.13	b	-7.1	1.00	0.50	k	2.26
Oxaloacetic pK_1	2.555	k				1.00	0.57	k	2.31
Oxaloacetic pK_2	4.370	k				0.57	0.19	k	3.89
α -Ketoglutaric pK_1	2.26	l				1.00	0.70	d	2.11
α -Ketoglutaric pK_2	4.80	l				0.70	0.40	d	4.56
Citric pK_1	3.128	b	+4.17	b	-46.0	1.00	0.346	e	2.79
Citric pK_2	4.761	b	+2.44	b	-82.8	0.346	0.179	e	4.30
Citric pK_3	6.396	b	-3.36	b	-133.9	0.179	0.0284	e	5.65
Isocitric pK_1	3.287	i				1.000	0.612	d	3.07
Isocitric pK_2	4.714	i				0.612	0.246	d	4.32
Isocitric pK_3	6.396	i				0.246	0.062	d	5.80
<i>cis</i> -Aconitic pK_1	1.9	m				1.00	0.61	d	1.7
<i>cis</i> -Aconitic pK_2	4.3	m				0.61	0.25	d	3.9
<i>cis</i> -Aconitic pK_3	6.4	m				0.25	0.062	d	5.8

Acid	pK_a	Ref	$\Delta_{ion}H$	Ref	$\Delta_{ion}S$	Ref	γ_0	γ_-	Ref	pK' ($I = 0.1$)
Glycine ⁺⁺ pK_1	2.350	b	+4.01	b	-31.5	b	0.73	0.97	g	2.47
Glycine ⁺⁻ pK_2	9.780	b	+44.1	b	-39.2	b	0.97	0.73	g	9.66
Alanine ⁺⁺ pK_1	2.340	b	+3.01	b	-34.7	b	0.73	0.95	d	2.45
Alanine ⁺⁻ pK_2	9.870	b	+45.23	b	-37.2	b	0.95	0.73	d	9.76
Aspartic ⁺⁺⁺ pK_1	1.990	b	+7.46	b	-13.0	b	0.73	0.95	d	2.10
Aspartic ⁺⁺⁻ pK_2	3.900	b	+4.60	b	-59.4	b	0.95	0.73	d	3.79
Aspartic ⁺⁻ pK_3	10.002	b	+37.76	b	-64.9	b	0.73	0.40	d	9.74
Glutamic ⁺⁺⁺ pK_1	2.162	b	-0.27	b	-42.3	b	0.73	0.95	d	2.28
Glutamic ⁺⁺⁻ pK_2	4.272	b	+1.56	b	-76.6	b	0.95	0.73	d	4.16
Glutamic ⁺⁻ pK_3	9.93	h	+40.07	b	-44.8	b	0.73	0.40	d	9.67

* [64SIL/MAR] and [79PER]

† [77CHR/HAN]

‡ [59ROB/STO]

§ Estimated from γ_{\pm} of similar compounds.

¶ See discussion under citrate third law data.

‡ Bada and Miller [68BAD/MIL] give $pK_1 = 2.937$ and $pK_2 = 4.172$ at $I = 0.1 \text{ mol dm}^{-3}$ measured with a glass electrode. $pK_1 = 3.019$ and $pK_2 = 4.384$ are given in Robinson and Stokes. $pK_1 = 3.095$ and $pK_2 = 4.602$ are given by Dahlgren and Long [60DAH/LON].* Cohn and Edsall [43COH/EDS] give $\gamma = 0.970$ and $\gamma = 0.954$ for glycine in 0.1 mol dm^{-3} KCl and 0.1 mol dm^{-3} NaCl respectively.† The $pK_3 = 9.371$ given by Llapis and Ordenez [63LLA/ORD] is inconsistent with a considerable number of glass electrode values. We therefore use $pK = 9.67$ at 0.1 mol dm^{-3} KCl [53LUM/MAR] and calculate $pK = 9.93$ at $I = 0 \text{ mol dm}^{-3}$ using the activity coefficients.

‡ [58HIT]

§ Estimated

¶ [52PED]

‡ [69JEN/KNO]

* [84SCH/EMP]

3.8. Gibbs Energy and Potential for the NAD^+ / NADH and NADP^+ / NADPH Systems

The two nicotinamide redox couples, NAD^+ / NADH (nicotinamide adenine dinucleotide, formerly referred to as DPN or diphosphopyridine nucleotide) and NADP^+ / NADPH (nicotinamide adenine dinucleotide phosphate, formerly referred to as TPN or triphosphopyridine nucleotide) are centrally important energy mediators in all biological systems and have been widely studied for many years. NADH is also a principal product of the Krebs cycle.

The reduction potentials (or Gibbs energy change) for these two systems have been measured directly by potentiometry and from equilibrium constants of several reactions in which they participate. The principal difficulty with potentiometric determinations is that NAD^+ and NADH themselves do not react with the electrode, so dyes that are reversible to the electrode must be used as mediators. It is very difficult to prove that the $\text{NAD}/\text{dye}/\text{electrode}$ system is completely reversible. The major problem with calculations from equilibrium constants is the accuracy of the ancillary data needed to derive the reduction potential.

Clark [60CLA] presented an extensive evaluation of these potentials, based on the data available at the time. The best potentiometric measurements were those of Rodkey for NAD^+ / NADH [55ROD][59ROD] and NADP^+ / NADPH [59ROD/DON] who gave $E^\circ = -0.1042\text{V}$ pH 0 and $E'' = 0.3113\text{V}$ pH 7 for NAD^+ / NADH . The alcohol dehydrogenase measurements used were those of Burton and Wilson [53BUR/WIL] with isopropanol/acetone and of Bäcklin [58BAC] with ethanol/acetaldehyde. However, later measurements by Burton [74BUR] of equilibria catalyzed by yeast alcohol dehydrogenase and measurements by Happel *et al.* [74HAP/CHA] of the ethanol/acetaldehyde hydrogenation equilibrium, that have been reported in the intervening years, warrant some reconsideration of Clark's results.

The reactions and associated data from various sources that have been employed to evaluate the potentials for both couples are summarized in Table 7. The footnotes to the table give the source of the data as well as some, but not all, of the alternative values in the literature.

Burton [74BUR] measured the equilibrium constants for the reactions NAD^+ /ethanol and NAD^+ /isopropanol with yeast alcohol dehydrogenase enzyme, obtaining results that agree very well with previous determinations by Burton and Wilson [53BUR/WIL]. The potentials for NAD^+ and NADP^+ were obtained using thermodynamic data for the gas phase and aqueous phase equilibria for the dehydrogenations of ethanol and isopropanol.

In the case of gas phase dehydrogenation of ethanol, the Gibbs free energy of reaction was calculated from enthalpies of formation and entropies tabulated by Stull, Westrum and Sinke [69STU/WES]. However, the value

calculated disagrees substantially with the measurements of the equilibrium constant reported by Happel *et al.* The disparity appears to stem from the values tabulated for the enthalpies of formation of ethanol and acetaldehyde. There are also differences in the $\Delta_{\text{sol}}G$ and $\Delta_{\text{sol}}H$ of solution between our calculation and Burton's.

The thermodynamic data for the dehydrogenation of isopropanol are less variable. Burton employed the values reported by Stull *et al.* for the gas phase formation free energies. The equilibrium in the gas phase was measured directly by Buckley and Herington [65BUC/HER] at several temperatures. Their enthalpy was in good agreement with that reported by Stull *et al.*, although the entropies adopted were slightly different. The $\Delta_{\text{sol}}G$ for acetone is significantly different, however.

There is a consistency in Table 7 in that the values of the aqueous reaction



can be obtained from the difference of aqueous dehydrogenation of ethanol and isopropanol ($\Delta G = 17.7$, $\Delta H = 5.7 \text{ kJ}\cdot\text{mol}^{-1}$) and from the NAD^+ /ethanol and NAD^+ /isopropanol reactions ($\Delta G = 17.4$, $\Delta H = 4.3 \text{ kJ}\cdot\text{mol}^{-1}$). However, it is apparent from the footnotes that a suitable choice of $\Delta_{\text{sol}}G$ and $\Delta_{\text{sol}}H$ can get the numbers to agree much better. One would have expected that the free energies and enthalpies of solution of such common compounds would be more accurately known, but this is not the case. Particularly uncertain are the $\Delta_{\text{sol}}G$ and $\Delta_{\text{sol}}H$ for acetaldehyde.

These ΔG values for NAD^+ / NADH can be converted to potentials at pH 0 and pH 7 and $I=0.1 \text{ mol dm}^{-3}$ by

$$E^\circ = -\Delta G^\circ / 2F \quad \text{pH 0}$$

$$E'' = E^\circ + \frac{RT}{2F} \log(H^+) \quad \text{pH 7}$$

The values are given in Table 8. We select the average of the ethanol, isopropanol and potentiometric values giving

$$E^\circ = -0.1042 \pm 0.0005 \text{ V} \quad \text{pH 0}$$

$$E'' = -0.3113 \pm 0.0005 \text{ V} \quad \text{pH 7}$$

The agreement of the potentiometric potential and the two equilibria are now excellent and removes the discrepancy that so troubled Clark [60CLA]. However this agreement may well be fortuitous.

The potential of the NADP^+ / NADPH has been measured at 30°C (-0.3178 V) [59ROD/DON] and can be corrected to 25°C by $dE^\circ/dT = -1.31 \times 10^{-3} \text{ V K}^{-1}$ [59ROD] to give -0.3104 V at 25°C . This is very close to the potentiometric NAD^+ / NADPH potential of -0.3113 V .

TABLE 7. Calculation of the NAD^+/NADH and $\text{NADP}^+/\text{NADPH}$ potentials from the ethanol/acetaldehyde and isopropanol/acetone equilibria. The standard state for H_2 is 1 atm. The standard state for NAD^+ , NADH , NADP^+ , NADPH and H^+ is 1 *m* aqueous at an ionic strength of 0.1 *m*. The values in parenthesis are those used by Burton [74BUR].

	ΔG° kJ·mol ⁻¹	ΔH° kJ·mol ⁻¹
$\text{CH}_3\text{CH}_2\text{OH}(\text{g}) = \text{CH}_3\text{CHO}(\text{g}) + \text{H}_2$	36.4(35.0) ^a	69.8(68.4) ^b
$\text{CH}_3\text{CH}_2\text{OH}(\text{aq}) = \text{CH}_3\text{CH}_2\text{OH}(\text{g})$	13.0(13.4) ^c	52.6(52.8) ^d
$\text{CH}_3\text{CHO}(\text{aq}) = \text{CH}_3\text{CHO}(\text{g})$	6.0(6.1) ^e	43.3(43.7) ^f
$\text{CH}_3\text{CH}_2\text{OH}(\text{aq}) = \text{CH}_3\text{CHO}(\text{aq}) + \text{H}_2$	43.4(41.6)	79.0(77.4)
$\text{NAD}^+ + \text{CH}_3\text{CH}_2\text{OH}(\text{aq}) = \text{NADH} + \text{CH}_3\text{CHO}(\text{aq}) + \text{H}^+$	63.3(63.3) ^m	46.9(46.9) ^m
$\text{NAD}^+ + \text{H}_2 = \text{NADH} + \text{H}^+$	20.0(21.8)	-32.1(-30.6)
$\text{CH}_3\text{CHOHCH}_3(\text{g}) = \text{CH}_3\text{COCH}_3(\text{g}) + \text{H}_2$	21.4(20.5) ^g	55.6(55.0) ^h
$\text{CH}_3\text{CHOHCH}_3(\text{aq}) = \text{CH}_3\text{CHOHCH}_3(\text{g})$	12.0(12.0) ⁱ	58.6(57.4) ^j
$\text{CH}_3\text{COCH}_3(\text{aq}) = \text{CH}_3\text{COCH}_3(\text{g})$	7.7(8.4) ^k	41.0(40.9) ^l
$\text{CH}_3\text{CHOHCH}_3(\text{aq}) = \text{CH}_3\text{COCH}_3(\text{aq}) + \text{H}_2$	25.7(24.4)	73.3(71.4)
$\text{NAD}^+ + \text{CH}_3\text{CHOHCH}_3(\text{aq}) = \text{NADH} + \text{CH}_3\text{COCH}_3(\text{aq}) + \text{H}^+$	45.9(45.9) ^m	42.6(42.6) ^m
$\text{NAD}^+ + \text{H}_2 = \text{NADH} + \text{H}^+$	20.2(21.7)	-30.7(-28.8)
$\text{NAD}^+ + \text{NADPH} = \text{NADH} + \text{NADP}^+$	-1.0(-3.0) ⁿ	04.0(4.0) ⁿ
$\text{NADP}^+ + \text{H}_2 = \text{NADPH} + \text{H}^+$	21.1(25.1) ⁿ	-26.7(-25.3) ⁿ

^aFrom measured equilibria by Happel *et al.* [74HAP/CHA] by our third law analysis.

^bA calorimetric value of +70.1 kJ was obtained at 82 ° by Kistiakowsky and coworkers [38DOL/GRE] and corrected to +69.1 kJ at 25 °.

^cFrom $\Delta_{\text{sol}}G(\text{g} \rightarrow \text{aq}) = \Delta_{\text{sol}}G(\text{l} \rightarrow \text{aq}) + RT \ln P^\circ$, where P° is the vapor pressure of the liquid. This $\Delta_{\text{sol}}G$ is from [70DAV/SIL]. Burton used [35BUT/RAU]. Other values include 13.2 [68WAG/EVA], 12.9 kJ·mol⁻¹ [78PEM/MAS].

^dFrom $\Delta_{\text{sol}}H(\text{liq} \rightarrow \text{aq}) = -10.2$ kJ·mol⁻¹ and $\Delta_{\text{vap}}H = 42.4$ [73WIL/ZWO]. Other values of $\Delta_{\text{sol}}H(\text{liq} \rightarrow \text{aq})$ include -10.6 [68WAG/EVA], -10.10 [69ALE/HIL], -10.18 [69ARN/KUV], and -10.4 kJ·mol⁻¹ [52ROS/WAG] used by Burton.

^eFrom Kurz [67KUR] and used by Burton. Other values include +5.9 kJ·mol⁻¹ [36WUR/FIL], +6.4 [70DAV/SIL], +6.7 kJ·mol⁻¹ [69BUT/LIN].

^fFrom $\Delta_{\text{vap}}H = 25.8$ kJ·mol⁻¹ [49COL/DEV] and $\Delta_{\text{sol}}H(\text{l} \rightarrow \text{aq}) = -17.5$ [67KUR]. Other values for $\Delta_{\text{vap}}H$ are 27.2 [50COL/POP] and 26.1 kJ·mol⁻¹ [68WAG/EVA]. Other values for $\Delta_{\text{sol}}H$ are -18.4 kJ [69WAG/EVA], -18.4 [52BEL/CLU] and -17.2 by extrapolation [83FER/MAR], -17.9 kJ·mol⁻¹ [69RAG/CAV] used by Burton.

^gAverage of $\Delta_rG = 21.5$ kJ·mol⁻¹ from a third law analysis by us of the measured equilibria of Buckley and Herington [65BUC/HER], and $\Delta_rG = 21.8$ kJ·mol⁻¹ is from the second law analysis. The data of Kolb & Burwell [45KOL/BUR] give a third law analysis of $\Delta_rG = +20.6$ and a second law analysis of $\Delta_rG = +21.3$ kJ·mol⁻¹ [69STU/WES].

^hAverage of $\Delta_rG = 56.0$ kJ from a third law analysis by us of the Buckley & Herington data [65BUC/HER] and $\Delta_rG = 55.2$ kJ·mol⁻¹ of their second law analysis. The direct calorimetric measurement of the heat of hydrogenation is +55.8 at 82 °C [38DOL/BRE] and corrected to 55.4 kJ·mol⁻¹ at 25 °.

ⁱFrom Rytting *et al.* [78RYT/HUS]. Other values include 12.0 kJ·mol⁻¹ [35BUT/RAM] used by Burton, and 12.8 [65HIN/WEI].

^jFrom $\Delta_{\text{sol}}H(\text{l} \rightarrow \text{aq}) = -13.07$ kJ·mol⁻¹ [69ARN/KOV] and $\Delta_{\text{vap}}H = 45.5$ [73WIL/ZWO]. Other values include -11.8 [51DIM/LAN] used by Burton, -13.0 [69ALE/HIL], and -13.1 kJ·mol⁻¹ [81ROU/SOM].

^kFrom [73COX/PAR]. Other values include +8.4 kJ·mol⁻¹ [30BEA/MCV] used by Burton, +8.0 [69BUT/LIN], and +8.8 kJ·mol⁻¹ [31HAR].

^lFrom $\Delta_{\text{sol}}H = -10.0$ kJ·mol⁻¹ [73COX/PAR] and $\Delta_{\text{vap}}H = 31.0$ [77CHA/WIL]. Other values of $\Delta_{\text{sol}}H$ include -10.2 and -9.9 [72ARN/BUR], -10.2 [81DEL/STR], and -9.7 [83BEN/CIL]. Burton used -9.9 kJ·mol⁻¹ without citing a source.

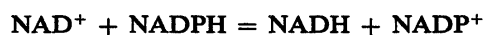
^mBurton gives $K = 6.91 \times 10^{-12}$ and $\Delta G = 63.7$ kJ·mol⁻¹ at $I = 0.013$ mol dm⁻³ for the ethanol reaction and $K = 7.71 \times 10^{-9}$ and $\Delta G = 46.5$ kJ at $I = 0.013$ mol dm⁻³ for the isopropanol reaction. These can be converted to $I = 0$ and $I = 0.1$ by Backlin's equation [58BAK], $\log K = \log K(I=0) + 0.315 \sqrt{I}$, giving $K = 6.36 \times 10^{-12}$ ($I=0$ mol dm⁻³) and 8.01×10^{-12} ($I=0.1$ mol dm⁻³), $\Delta G = 63.9$ kJ ($I=0$) and 63.3 kJ ($I=0.1$ mol dm⁻³). Using the same ionic strength dependence for the isopropanol reaction converts $K = 7.71 \times 10^{-9}$ at $I = 0.013$ to $K = 7.10 \times 10^{-9}$ ($I=0$) and 8.93×10^{-9} ($I=0.1$ mol dm⁻³). $\Delta G = 46.5$ ($I=0$) and $\Delta G = 45.9$ ($I=0.1$ mol dm⁻³).

ⁿThis is the difference of the NAD^+ reduction and the transhydrogenase equilibrium. The NAD^+ reduction values are the average of the ethyl alcohol and isopropanol equilibria. The ΔH is given in [74BUR].

TABLE 8. Potentials of the pyridine nucleotide couples at 25 ° pH 0 and 7 and $I = 0.1 \text{ mol dm}^{-3}$. The ΔG is in $\text{kJ}\cdot\text{mol}^{-1}$ at pH 0.

ΔG $\text{kJ}\cdot\text{mol}^{-1}$	$E^\circ(\text{pH } 0)$ V	$E^\circ(\text{pH } 7)$ V	
NAD ⁺ /NADH			
19.96	-0.1034	-0.3105	From ethanol/acetaldehyde (This calc)
21.75	-0.1127	-0.3198	From ethanol/acetaldehyde [74BUR]
20.25	-0.1049	-0.3120	From isopropanol/acetone (This calc)
21.54	-0.1116	-0.3187	From isopropanol/acetone [74BUR]
20.11	-0.1042	-0.3113	Potentiometric [55ROD]
20.11	-0.1042	-0.3113	Value selected
NADP ⁺ /NADPH			
19.34	-0.1033	-0.3104	Potentiometric [59ROD/DON]
21.13	-0.1095	-0.3166	Transhydrogenase $K = 1.50$
21.13	-0.1095	-0.3166	Value selected

The NADP⁺ /NADPH potential can be obtained from the NAD⁺ /NADH potential and the transhydrogenase equilibrium



This has been measured directly [53KAP/COL] as $K = 1.43$ at 37 °C but should be nearly the same at 25 °C. There are two measurements of the glutamate dehydrogenase equilibrium with both NAD⁺ and NADP⁺



the difference being the transhydrogenase reaction. Olsen and Anfinsen [53OLS/ANF] gave $K = 1.466$ and Engel and Dalziel [67ENG/DAL] gave 1.60. Burton [74BUR] uses a figure of 3.32 from Engel and Dalziel but this is apparently the value at $I=0$ instead of $I=0.1 \text{ mol dm}^{-3}$. The average of the three transhydrogenase equilibria is 1.50 and $\Delta G = -1.00 \text{ kJ}$. We take for the NADP⁺/NADPH potential for $I = 0.1 \text{ mol dm}^{-3}$

$$E^\circ = -0.1095 \pm 0.0040 \text{ V pH } 0$$

$$E^\circ = -0.3166 \pm 0.0040 \text{ V pH } 7$$

The error is higher in this case because of the differences between the potentiometric determination and the transhydrogenation equilibrium.

There is an additional source of error potentially present in all NAD⁺ equilibrium measurements. Since the time when many of the measurements cited were performed, commercial preparations of NAD⁺ have

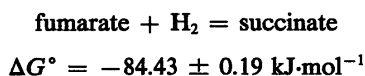
been found to contain variable amounts of the α -isomer. As the enzymes react mainly with the natural β -isomer, the presence of 5–20% α -isomer, as is found in some commercial preparations [60KAP], would lead to a commensurate error in the measured equilibrium constant. The problem should be more acute with NAD⁺ than with NADP⁺, as the latter is typically synthesized enzymatically from NAD⁺, so that the product would only consist of the β -isomer. Even then the conversion of the β to α -isomer is acid catalyzed and fast ($t_{1/2}$ is a few hours at pH 5) so that initially pure preparations may become contaminated [75OPP/KAP][87KAM/MAL].

It is evident that a more accurate value of the NAD⁺ and NADP⁺ potentials awaits attention to the α -isomer problem, as well as using a system whose potential is more accurately known than ethanol/acetaldehyde or isopropanol/acetonc. The best system would seem to be the formate/bicarbonate system, although the potential difference is rather large for an equilibrium measurement. Approximate equilibrium measurements have been made at 10 °C [76RUS/MUL] and at 55 ° [83YAM/SAI] which are in fair agreement with those calculated from the $\Delta_r G$ values, but measurements at 25 °C are needed. Even more accurate would be a potentiometric value whose reversibility could be demonstrated. The potentiometric measurement could be for NAD⁺/NADH or for some organic system that can be directly coupled to NAD⁺ (e.g., lactate/pyruvate or ethanol/acetaldehyde).

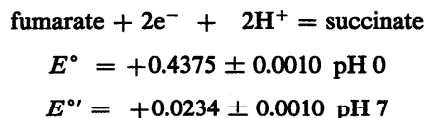
The enthalpy of reduction of NAD⁺ by H₂ and formate was measured by calorimetry [79REK/EGO] [81REK/EGO][86REK/GAL] with respective values of -27.2 ± 1.7 and $-27.6 \pm 1.7 \text{ kJ}\cdot\text{mol}^{-1}$. These are less negative by nearly 5 $\text{kJ}\cdot\text{mol}^{-1}$ than the value of $-32.1 \text{ kJ}\cdot\text{mol}^{-1}$ given in Table 7. An attempt to resolve this discrepancy will not be made here, since this study deals mainly with Gibbs energies.

4. Evaluated Equilibria

Reaction 1. Succinate Dehydrogenase

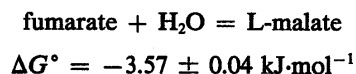


This is based on the potential measured by Borsook and Schott [31 BOR/SCHa,b] using succinate dehydrogenase, methylene blue mediator, and a platinum electrode.



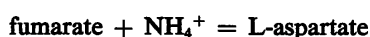
The Gibbs energy is given by $\Delta G^\circ = -nFE^\circ$. An error of 0.0010 V corresponds to 0.20 kJ·mol⁻¹ in ΔG . Since the ratio of activity coefficients should be about 1, the ionic strength dependence should be small. Potential measurements of enzyme equilibrium have always been difficult and prone to large errors. However, this determination is one of the few that seems accurate. The ΔG value is confirmed by $\Delta E'/\Delta T$ (between 18 and 30 °C) giving $\Delta H = -124.9 \text{ kJ}\cdot\text{mol}^{-1}$ that can be compared to thermal data yielding $\Delta H = -131.8 \text{ kJ}\cdot\text{mol}^{-1}$ (Table 2). Borsook and Schott calculated the ΔG for this reaction from the heats of combustion and third law entropies and obtained remarkably good agreement, $-20.14 \text{ kcal mol}^{-1}$ from the potential measurements compared to $-20.46 \text{ kcal mol}^{-1}$ from the third law calculation. This paper was widely cited as evidence that thermodynamics is applicable to biological systems [81SLA]. The excellent agreement of the two $\Delta_r G$ is apparently the result of a cancellation of errors. The error in the entropy of succinate (2.0 cal K⁻¹·mol⁻¹) by itself would result in an error in $\Delta_r G$ of 0.60 kcal mol⁻¹, and the errors in the $\Delta_r H$ of succinic and fumaric acids were comparable.

Reaction 2. Fumarase



This is based on Krebs determination, at $I=0.1 \text{ mol dm}^{-3}$, of $K=4.32$ [53KREa] and is in close agreement with Bok and Alberty's value of 4.26 [53BOK/ALB]. Recent determinations by Goldberg *et al.* [85GAJ/GOL] at several ionic strengths lead to a value of 4.20 ± 0.05 which is also in very good agreement. It is confirmed by high temperature non-enzymatic, base catalyzed reaction [59ERI/ALB]. It is in agreement with the value of 4.25 ± 0.08 given by Cook *et al.* [80COO/BLA].

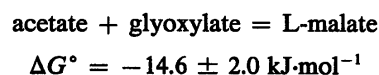
Reaction 3. Aspartase



$$\Delta G^\circ = -13.70 \pm 0.04 \text{ kJ}\cdot\text{mol}^{-1}$$

This is based on the measurements of $K = 3.98 \times 10^{-3}$ at $I=0.1 \text{ mol dm}^{-3}$ by Goldberg *et al.* [86GOL/GAJ]. The equilibrium was investigated at temperatures between 13 °C and 43 °C and ionic strengths between 0.066 and 0.366 mol kg⁻¹. There is a slight systematic difference between these results and those of Bada and Miller ($K = 4.64 \times 10^{-3}$ at $I=0.1 \text{ mol dm}^{-3}$) [68BAD/MIL], who investigated the enzyme-catalyzed reaction from 5 to 36 °C and compared the results with non-enzymatic attainment of equilibrium at temperatures of 118 °C and 136 °C. The difference may be due to the use of KCl as background electrolyte in one case [86GOL/GAJ] and NaCl in the other [68BAD/MIL].

Reaction 4. Malate Synthetase

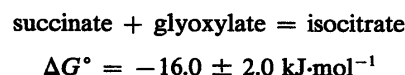


Hersh [73HER] determined the equilibrium constant for the reaction :



obtaining a value of $K = 330$ at 30 °C or $\Delta G^\circ = -14.6 \text{ kJ}\cdot\text{mol}^{-1}$. Assuming that the free energies of hydrolysis of acetyl- and maly-CoA are the same, as seems to be the case for acetyl CoA and succinyl CoA [78LYN/GUY], and neglecting the dependence on temperature, leaves this as the value for reaction 4. The approximations made should be within the stated uncertainty.

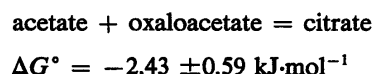
Reaction 5. Isocitrase



The active isomer is threo D₅ (+) isocitrate [62VIC] but will be referred to here as isocitrate. Williams *et al.* [71WIL/ROC] reported $K = 430$ for direct determination of the equilibrium at 27 °C, (the range was 320 and 630). They calculated $K = 630$ from the ratio of forward and reverse rate constants. These measurements were obtained at pH 7.7 and $I = 0.1 \text{ mol dm}^{-3}$, corresponding to $\Delta G = -15.0 \text{ kJ}$ and -16.0 kJ , respectively. The average is -15.5 kJ , for 27 °C. However, at 25 °C the equilibrium should be slightly more to the right, so we have assigned a value of -16.0 kJ for this reaction.

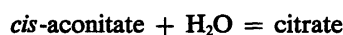
This is a difficult equilibrium to measure. For example, a previous study [57SMI/GUN] reported a value of $K = 34$, but this was in error because the reaction mixtures contained cysteine which binds to glyoxylate. This is one of the more useful equilibria in the Krebs cycle.

Reaction 6. Citrate-oxaloacetate Lyase



This is based on the value reported by Tate and Datta [65TAT/DAT] of $K = 3.08 \pm 0.72$ for 25 °C, $I = 0.1$ mol dm⁻³ and oxaloacetate in the keto form. For aqueous oxaloacetate this becomes $K = 2.65 \pm 0.72$. These measurements are complicated by complexing with magnesium ions, and the above has been corrected by extrapolation to $Mg^{+2} = 0$. Harvey and Collins [63HAR/COL] report a value of 15.7 at 30 °C but the equilibrium was measured only from the cleavage direction. Guynn *et al.* [73GUY/GEL] report a value of $K = 0.94 \pm 0.07$ at 38 °C and $I = 0.25$ mol dm⁻³; however a reliable correction to 25 °C and $I = 0.1$ mol dm⁻³ is not feasible because $\Delta_r H$ is not known and the γ corrections are large.

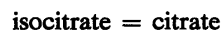
Reaction 7. Aconitase



$$\Delta G^\circ = -8.49 \pm 0.04 \text{ kJ}\cdot\text{mol}^{-1}$$

This is based on measurements of the amount of aconitate in equilibrium with citrate and isocitrate. There is an equivalent equilibrium of $cis\text{-aconitate} + H_2O = \text{isocitrate}$, but the difference between these ΔG values is that for isocitrate = citrate. There are several values for K of about 20 in the literature [43KRE/EGG] [66THO/NAN], and several around 30 [43MAR/LEO][53KREa]. We will use Krebs value of 30.7 [53KREa].

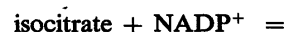
Reaction 8. Aconitase



$$\Delta G^\circ = -6.11 \pm 0.20 \text{ kJ}\cdot\text{mol}^{-1}$$

This is based on the results of Blair [69 BLA] at $I = 0.1$ mol dm⁻³ and 25 °C. The Mg^{2+} was extrapolated to zero with the ionic strength maintained at 0.1 *M* by Na^+ , giving $K = 11.73$. With tetramethylammonium as cation, the value of K was 9.31 at $I = 0.1$ mol dm⁻³. Other values include 14.7 [53KREa] and 7.8 [67ENG/DEN] The value of 11.73 is adopted here.

Reaction 9. Isocitrate Dehydrogenase



$$\Delta G^\circ = -8.0 \pm 1.2 \text{ kJ}\cdot\text{mol}^{-1}$$

This is based on the measurements of Londesborough and Dalziel [68LON/DAL] at 25 °C and $I = 0.1$ mol dm⁻³, who gave $K = 25.6$. The effect of ionic strength and Mg^{2+} was small, but there is considerable uncertainty in the potential of the NADP/NADPH redox couple as discussed above. The assigned error of 0.30

kcal is due primarily to the uncertainty in this potential. Cook *et al.* [80COO/BLA] give K_{eq} for dissolved CO_2 as 1.04 ± 0.18 *M* which becomes 30.4 for CO_2 gas.

Reaction 10. Glycolate Dehydrogenase



$$\Delta G^\circ = +84.35 \pm 0.67 \text{ kJ}\cdot\text{mol}^{-1}$$

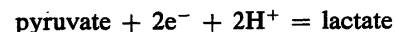
This is based on Zelitch's measurement [55ZEL] of $K = 1.65 \pm 0.43 \times 10^{-15}$. Later measurements [68KOH/JAKb][70KOH/WAR] gave values of 2.61×10^{-15} and 2.90×10^{-15} . The systematic shift towards glyoxylate may be due to the presence of Tris buffer, which can complex the glyoxylate. The value of 6×10^{-18} of Cartwright and Hullin [66CAR/HUL] seems to be too low. Other problems suggest that the equilibrium should be reinvestigated to obtain a more reliable value.

Reaction 11. Lactate Dehydrogenase



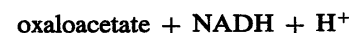
$$\Delta G^\circ = 65.98 \pm 0.42 \text{ kJ}\cdot\text{mol}^{-1}$$

We have selected $K = 2.76 \times 10^{-12}$ which is the value reported by Hakala *et al.* [56HAK/GLA]. They also measured the temperature coefficient, obtaining a value of $\Delta H = 43.1 \pm 0.8$ kJ·mol⁻¹ which is in agreement with an early calorimetric value of $\Delta H = 44.4 \pm 1.1$ kJ·mol⁻¹ [55KAT] but not with later values of $\Delta H = 61.9 \pm 1.3$ kJ·mol⁻¹ [75DON/BAR] and $\Delta H = 55.8 \pm 1.1$ kJ·mol⁻¹ [79SCH/HIN]. Cook *et al.* [80COO/BLA] reported an equilibrium constant of $K = 2.19 \pm 0.08 \times 10^{-12}$. There is a direct potentiometric measurement for the reaction Labeyrie [60LAB/NAS].



of $E^\circ = -0.188 \pm 0.003$ V at 25 °C and pH 7 (corrected from -0.190 V at 27 °). If the $NAD^+/NADH$ potential is taken as -0.320 V at 25 °C and pH 7, then $K = 3.4 \times 10^{-12}$ and $\Delta G = 65.4$ kJ·mol⁻¹, but the agreement is much less if the $NAD^+/NADH$ potential is taken as -0.311 V. Earlier measurements of Barron and Hastings [34BAR/HAS] and of Wurmser [33WUR/MAY] gave E° values of -0.158 V. These may be correctable to -0.167 V if they used DL-lactate instead of L-lactate, but -0.167 V is still in disagreement with the measurements of Labeyrie *et al.* An accurate potential for lactate/pyruvate would be most useful.

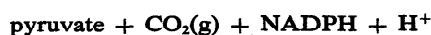
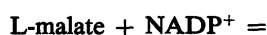
Reaction 12. Malate Dehydrogenase



$$\Delta G = +68.37 \pm 0.41 \text{ kJ}\cdot\text{mol}^{-1}$$

This is based on Burton and Wilson's [53 BUR/WIL] value of $K = 1.05 \times 10^{-12}$ for the reaction at $I = 0.1 \text{ mol dm}^{-3}$ ($K = 0.6 \times 10^{-12}$ at $I = 0$). Later results have been in very good agreement with this value. These include $K = 1.28 \times 10^{-12}$ [68KOH/JAKa], 0.79×10^{-12} [65YOS], $K = 1.03 \times 10^{-12}$ [62RAV/WOL], $K = 0.59 \times 10^{-12}$ [75SCH/RIF] and $K = 0.71 \times 10^{-12}$ [80COO/BLA]. Guynn *et al.* [73GUY/GEL] give $K = 1.02 \pm 0.03 \times 10^{-12}$ at $I = 0.25$, which corrects to $K = 0.73 \times 10^{-12}$ at $I = 0.1 \text{ mol dm}^{-3}$ using the ionic strength dependence given by Burton and Wilson [53BUR/WIL]. A calorimetric measurement gave $\Delta H = 89.5 \pm 0.8 \text{ kJ}\cdot\text{mol}^{-1}$ [76JES].

Reaction 13. Malic Enzyme



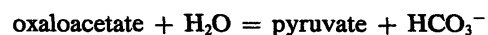
$$\Delta G = +1.00 \pm 1.20 \text{ kJ mol}^{-1}$$

Ochoa and coworkers [53HAR/KOR] measured this equilibrium at $\text{pH} = 7.4$ and $22\text{--}25^\circ\text{C}$, yielding

$$K = \frac{[\text{malate}][\text{NADP}^+]}{[\text{pyruvate}][\text{CO}_2(\text{aq})][\text{NADPH}]} = 19.6$$

Schimerlik and Cleland [77SCH/CLE] give values of $K = 83$ and 22 as determined from ratios of rate constants and a value of $K = 33.0 \pm 2.0$ determined from a directly measured equilibrium constant. Using the value of $K = 19.6 \pm 10$ for this reaction and the value given in Table 1 for $\text{CO}_2(\text{g}) = \text{CO}_2(\text{aq})$ ($\Delta G^\circ = +8.37 \text{ kJ}\cdot\text{mol}^{-1}$) yields the value given above.

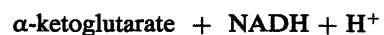
Reaction 14. Oxaloacetate Decarboxylase



$$\Delta G^\circ = -25.94 \pm 1.25 \text{ kJ}\cdot\text{mol}^{-1}$$

The value selected is that reported by Wood *et al.* [66WOO/DAV]. It is based on a complicated set of equilibria involving phosphoenol pyruvate and ATP hydrolysis. It is difficult to evaluate, but it is consistent with the malic enzyme and malic dehydrogenase equilibria. The uncertainty assigned is estimated.

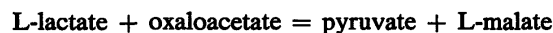
Reaction 15. α -Hydroxyglutarate Dehydrogenase



$$\Delta G = +67.53 \pm 0.21 \text{ kJ mol}^{-1}$$

This equilibrium was measured by Buckel & Miller [87BUC/MIL].

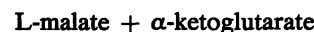
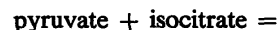
Reaction 16. Malate-lactate Transhydrogenase



$$\Delta G^\circ = -1.46 \pm 0.60 \text{ kJ}\cdot\text{mol}^{-1}$$

The equilibrium constant for this reaction is given in *Methods of Enzymology* [69ALL] as $K = 1.8 \pm 0.4$. The temperature is not specified but is assumed to be 25°C .

Reaction 17. Isocitrate-pyruvate Transcarboxylase



$$\Delta G^\circ = -6.69 \pm 0.80 \text{ kJ}\cdot\text{mol}^{-1}$$

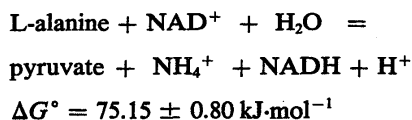
This is not a single enzyme, but rather a combination of the malic enzyme and isocitrate dehydrogenase. Ochoa [53HAR/KOR] [50OCH/VEI] gives a value of (~ 15 for the equilibrium constant. Since this is a directly measured equilibrium which does not require a potential for NADP, the uncertainty is taken to be $\pm 0.8 \text{ kJ}$.

Reaction 18. Glutamate Dehydrogenase

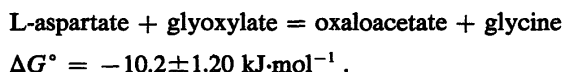


$$\Delta G^\circ = +74.80 \pm 0.80 \text{ kJ mol}^{-1}$$

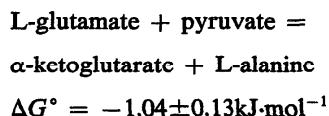
Engel and Dalziel [67ENG/DAL] measured this equilibrium using both NAD^+ and NADP^+ , obtaining values at $I = 0.1 \text{ mol dm}^{-3}$ of $K = 8.17 \times 10^{-14}$ and $K = 5.10 \times 10^{-14}$ respectively. Olsen and Anfinsen [53OLS/ANF] also measured these two systems and obtained values of $K = 14.4 \times 10^{-14}$ (NAD) and 8.9×10^{-14} (NADP). However, the ionic strength in this case was 0.47 mol dm^{-3} . Several investigators have made determinations using only NADP. Cook, *et al.* [80COO/BLA] obtained the value $K = 4.4 \pm 0.1 \times 10^{-14}$ at an ionic strength of apparently 0.1 mol dm^{-3} , Subramanian [78SUB] obtained a value of $K = 7.3 \times 10^{-14}$ at $I = 0.305 \text{ mol dm}^{-3}$ which becomes $K = 4.1 \times 10^{-14}$ at $I = 0.1 \text{ mol dm}^{-3}$ using the ionic strength dependence of Engel and Dalziel. The value for NADP relative to that for NAD, as determined by Engel and Dalziel, has been used in estimating the NADP/NADPH potential. These subsequent measurements using the NADP reaction give values that are consistent with the choice of $K = 8.17 \times 10^{-14}$ for the equilibrium with NAD. A value of $\Delta H = 73.2 \text{ kJ}\cdot\text{mol}^{-1}$ was obtained from the temperature dependence of the equilibrium constant [67ENG/DAL] and $\Delta H = 64.6 \pm 1.2 \text{ kJ}\cdot\text{mol}^{-1}$ by calorimetry [78SUB][79SUB].

Reaction 19. Alanine Dehydrogenase

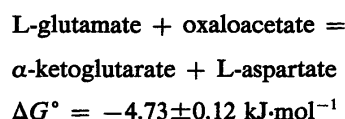
The equilibrium measured at pH 10 [60OCO/HAL] and corrected for ionization gives $K = 3.85 \times 10^{-14}$. Yoshida and Freese [65YOS/FRE] reported an equilibrium constant of $K = 3.1 \times 10^{-14}$, while Grimshaw and Cleland [81GRI/CLE] obtained a value of $K = 6.8 \times 10^{-14}$ which is selected.

Reaction 20. Aspartate-glycine Transaminase

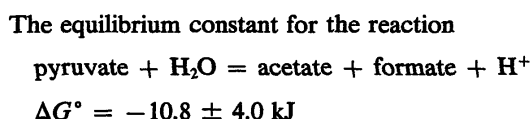
This is based on an equilibrium constant of $K = 61$ determined by Gibbs and Morris [66GIB/MOR][70GIB/MOR]. The experiment was not well described in the reference so the error may be understated. In measuring this equilibrium, care should be taken to establish that the aspartate or other amino acid has not been racemized since glyoxylate is a catalyst for this reaction.

Reaction 21. Glutamate-alanine Transaminase

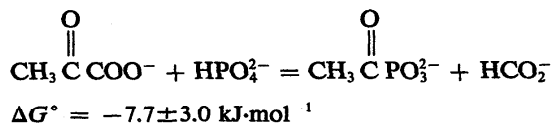
Krebs [53KREb] has reported a carefully determined value for the equilibrium constant of 1.52. The deviation between experimental values was no more than 9% and determinations using the racemic mixture yielded the same value as using the L-isomer. The experimental conditions were very close to the recommended values of 25 °C and $I = 0.1 \text{ mol dm}^{-3}$. Values of $K = 1.6$ and $K = 1.7$ at 37 °C have been reported [62SEG/BEA][65MAR/TER].

Reaction 22. Glutamate-aspartate Transaminase

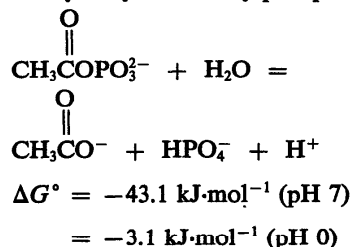
This is based on the value of 6.74 for the equilibrium constant given by Krebs [53KREb]. The deviation among reported values is only 4% (in K) and all precautions against systematic errors appear to have been taken.

Reaction 23. Pyruvate Formate Lyase

is obtained as the sum of the pyruvate-formate lyase reaction



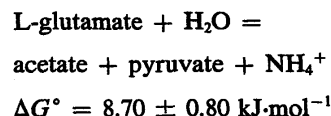
and the hydrolysis of acetylphosphate



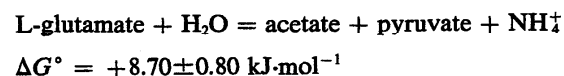
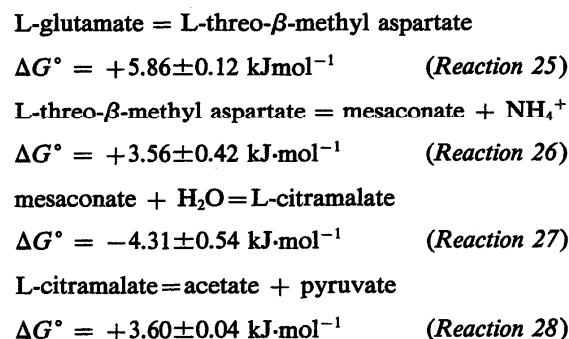
The first reaction was measured by Tanaka and Johnson [71TAN/JOH] as $K = 23$ at 37 ° with a substantial error since the acetyl phosphate is unstable. The ΔG for the hydrolysis of acetyl phosphate is Jenck's value [76JEN]

Reactions 24–28. Glutamate to Acetate and Pyruvate

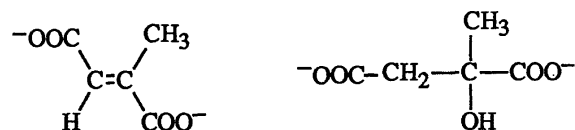
Reaction 24 is the overall reaction.



This is the sum of reactions 25–28 studied by Barker and coworkers. The sequence of reactions is:



Mesaconate is methyl fumarate and citramalate is α -methyl malate.



Barker *et al.* [64BAR/ROO][69BARa] measured

$$\frac{(\text{L-glutamate})}{(\text{L-threo-}\beta\text{-methyl aspartate})} = 10.7 \pm 0.4 \text{ at pH } 8.2, 30^\circ$$

The correction to 25° should not be large.

Barker *et al.* [59BAR/SMY] measured.

$$\frac{(\text{mesaconate})(\text{NH}_4^+)}{(\text{L-threo-}\beta\text{-methyl aspartate})} = 0.238 \text{ at pH } 7.9 \text{ and } 0.306 \text{ at pH } 9.7$$

Barker *et al.* [69WAN/BARa][69WAN/BARb] measured

$$\frac{\text{L-citramalate}}{(\text{mesaconate})} = 5.8 \pm 1.0$$

The value was 4.6 in the forward direction and 7.0 in the reverse direction.

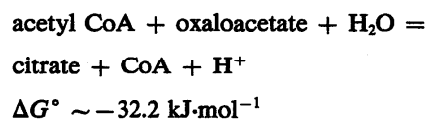
Buckel and Miller [87BUC/MIL] measured

$$\frac{(\text{acetate})(\text{pyruvate})}{(\text{L-citramalate})} = 0.232 \pm 0.008$$

This differs from an earlier value [67BAR][69BARb].

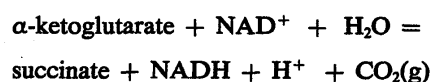
There are a number of reactions related to the Krebs cycle that have not been included because their equilibrium constants are not known. These include:

Reaction A. Citrate Synthetase (or Condensing Enzyme)

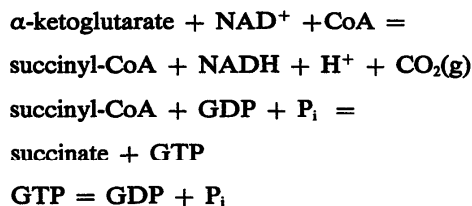


This equilibrium constant is $K = 4.65 \times 10^3$ at 22 °C and pH 7.2 (2.93×10^{-2} at pH 0) [52STE/OCH] and $K = 2.24 \times 10^6$ at 38 °C and pH 7 (0.224 at pH 0) [63GUY/GEL]. This reaction is related to Reaction 6 by the hydrolysis of acetyl-CoA, and the network could be refined if reliable measurements for this hydrolysis constant were available. The published values for the Gibbs energy of hydrolysis, variously reported as -30.8 , -36.4 and $-34.5 \text{ kJ}\cdot\text{mol}^{-1}$ [55BUR] -31.5 [64JEN/GIL] and $-35.7 \text{ kJ}\cdot\text{mol}^{-1}$ [78LYN/GUY], are too disparate to be used in the network.

Reaction B. α -Ketoglutarate Dehydrogenase

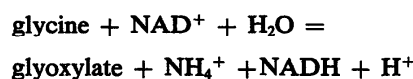


This reaction is irreversible as written ($\Delta G = -24.4 \text{ kJ}$ at pH 0 and -64.3 at pH 7) and is the sum of several reactions in the Krebs cycle, i.e.;



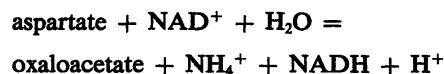
The first reaction is probably reversible, but the K_{eq} apparently has not been measured. This combined with ΔG of hydrolysis of succinyl CoA would give a value of ΔG for reaction 9.

Reaction C. Glycine Dehydrogenase



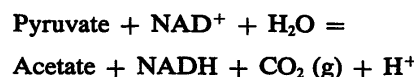
A value of the equilibrium constant of 2.3×10^{-11} has been reported for this reaction [62GOL/WAG], but this value is inconsistent with the transamination equilibria, so this reaction has not been included in the analysis.

Reaction D. Aspartate Dehydrogenase



There is no direct measurement of this equilibrium ($\Delta G \sim 80 \text{ kJ}\cdot\text{mol}^{-1}$). However several transamination reactions can be manipulated to yield an equivalent reaction.

Reaction E. Pyruvate Decarboxylase



This reaction is considered irreversible ($\Delta G = -32 \text{ kJ}\cdot\text{mol}^{-1}$ at pH 0 and $-72 \text{ kJ}\cdot\text{mol}^{-1}$ at pH 7). However, the reaction is just measurable if the reaction is pulled to the left by reduction of the pyruvate to lactate.

5. Results and Discussion

The results of the CATCH analysis of this Krebs cycle network are shown in Table 9. The differences between the experimental and calculated ΔG of reaction are mostly less than the uncertainty of the experimental data. The "succinate + acetate" results appear to be superior to the "all 3rd law" calculation, even though one would expect superior results when the system is anchored with more third law data. However, it takes only one bad third law value to distort the results. The most probable compound with bad third law $\Delta_f G$ in this system is fumarate, where the uncertain entropy could result in a third law $\Delta_f G$ too low by $1 \text{ kJ}\cdot\text{mol}^{-1}$ or more. The 3rd law aspartic acid and glutamic acid values may also be in error, in this case from the enthalpy of combustion.

TABLE 9. CATCH analysis of the Krebs cycle network. The succinate + acetate calculation used only these third law Δ_rG . The All 3rd law calculation used the Δ_rG of fumarate, glycine, alanine, aspartic acid and glutamic acid in addition.

Reaction	$\Delta_rG/\text{kJ}\cdot\text{mol}^{-1}$		
	Expt	Succinate + acetate	All 3rd law
01. Fumarate + H ₂ = Succinate	-84.43	-84.46	-84.49
02. Fumarate + H ₂ O = Malate	-3.57	-3.34	-3.27
03. Fumarate + NH ₄ ⁺ = Aspartate	-13.72	-13.77	-13.80
04. Acetate + Glyoxylate = Malate	-14.39	-14.33	-14.30
05. Succinate + Glyoxylate = Isocitrate	-15.98	-16.11	-15.93
06. Acetate + Oxaloacetate = Citrate	-2.43	-2.32	-2.36
07. Aconitate + H ₂ O = Citrate	-8.49	-8.49	-8.49
08. Isocitrate = Citrate	-6.11	-6.15	-6.13
09. Isocitrate + NADP ⁺ = α -Ketoglutarate + CO ₂ (g) + NADPH	-8.03	-7.86	-7.75
10. Glycolate + NAD ⁺ = Glyoxylate + NADH + H ⁺	84.35	84.35	84.35
11. Lactate + NAD ⁺ = Pyruvate + NADH + H ⁺	65.98	66.35	66.35
12. Malate + NAD ⁺ = Oxaloacetate + NADH + H ⁺	68.37	68.35	68.34
13. Malate + NADP ⁺ = Pyruvate + CO ₂ (g) + NADPH + H ⁺	1.00	1.07	-0.24
14. Oxaloacetate + H ₂ O = Pyruvate + HCO ₃ ⁻	-25.94	-25.89	-25.68
15. α -Hydroxyglutarate + NAD ⁺ = α -Ketoglutarate + NADH + H ⁺	67.53	67.53	67.53
16. Lactate + Oxaloacetate = Pyruvate + Malate	-1.46	-1.99	-1.99
17. Pyruvate + Isocitrate = Malate + α -Ketoglutarate	-6.69	-6.79	-6.70
18. Glutamate + NAD ⁺ + H ₂ O = α -Ketoglutarate + NH ₄ ⁺ + NADH + H ⁺	74.70	74.12	74.16
19. Alanine + NAD ⁺ + H ₂ O = Pyruvate + NH ₄ ⁺ + NADH + H ⁺	75.15	75.16	75.17
20. Aspartate + Glyoxylate = Oxaloacetate + Glycine	-10.21	-10.21	-10.38
21. Glutamate + Pyruvate = α -Ketoglutarate + Alanine	-1.05	-1.04	-1.01
22. Glutamate + Oxaloacetate = α -Ketoglutarate + Aspartate	-4.73	-4.66	-4.72
23. Pyruvate + H ₂ O = Acetate + Formate + H ⁺	-10.92	-10.64	-10.85
24. Glutamate + H ₂ O = Acetate + Pyruvate + NH ₄ ⁺	8.70	8.74	8.74
25. Glutamate = β -Methylaspartate	5.86	5.86	5.86
26. β -Methylaspartate = Mesaconate + NH ₄ ⁺	3.56	3.57	3.57
27. Mesaconate + H ₂ O = Citramalate	-4.31	-4.30	-4.30
28. Citramalate = Acetate + Pyruvate	3.60	3.61	3.61
29. NAD + H ₂ = NADH + H ⁺	20.12	19.92	20.00
30. NADP + H ₂ = NADPH + H ⁺	21.02	21.04	20.93
31. Elements = Formate	-351.16	-351.17	-351.17
32. Elements = Succinate	-690.07	-690.08	-689.84
33. Elements = Acetate	-372.21	-371.55	-371.29
34. Elements = Fumarate	-604.25	-605.63	-605.35
35. Elements = Glycine	-371.29	-370.61	-370.74
36. Elements = Alanine	-370.49	-371.40	-370.84
37. Elements = Aspartate	-700.74	-698.87	-698.62
38. Elements = Glutamate	-696.34	-697.45	-696.71

The Δ_rG of the compounds are given in Table 10. The results of the two calculations are mostly within 0.5 kJ·mol⁻¹ and none differ by more than 1.0 kJ·mol⁻¹. The differences with the third law Δ_rG is somewhat more but still satisfactory.

The errors given for Δ_rG in Table 10 may be too small, since they are dependent on the input data and the rather arbitrary errors assigned to the enzyme equilibria. In cases where the compounds are involved in a number of reactions, (e.g., fumarate and alanine), the error estimates are probably correct. The error estimates may be too low for compounds connected to the network by only one reaction. Examples are glycolate, glycine, and α -hydroxyglutarate. The Δ_rG for glyoxylate may be in error by an amount greater than indicated because all the

equilibrium constants involving this compound are relatively uncertain.

The Δ_rG 's in Table 10 are self-constant. Care should be taken in combining these values with those in other tables, since the combined errors in the Δ_rG could be quite large.

6. Extensions and Improvements

There are a number of branches to the Krebs cycle which can be put into this network. These include oxalic acid, fatty acids, alcohols, amines, other amino acids and even sugars. Equilibrium constants are not available for all the steps in these branches, so some experimental work will be required.

TABLE 10. Gibbs energies of formation of Krebs cycle compounds. The "succinate + acetate" and "all third law" columns are as described in Table 9. The $\Delta_f G$ of NAD^+ and NADP^+ have been set at -418.40 kJ/mol and are not the actual values.

	$\Delta_f G/\text{kJ}\cdot\text{mol}^{-1}$	
	succinate + acetate	all 3rd law
Formate	-350.99 ± 0.20	-350.99 ± 0.20
Acetate	-371.57 ± 0.33	-371.33 ± 0.37
Succinate	-690.08 ± 0.29	-689.74 ± 0.35
Fumarate	-605.61 ± 0.35	-605.24 ± 0.34
Mesaconate	-608.54 ± 0.68	-607.83 ± 0.61
Glycolate	-523.68 ± 0.82	-523.75 ± 0.91
L-Lactate	-520.29 ± 0.52	-519.92 ± 0.48
L-Malate	-846.14 ± 0.41	-845.91 ± 0.36
L-Citramalate	-850.03 ± 0.67	-849.35 ± 0.61
L- α -Hydroxyglutarate	-848.58 ± 0.83	-847.90 ± 0.83
Glyoxylate	-460.25 ± 0.44	-460.30 ± 0.44
Pyruvate	-474.85 ± 0.46	-474.44 ± 0.44
Oxaloacetate	-798.71 ± 0.46	-798.80 ± 0.50
α -Ketoglutarate	-801.97 ± 0.75	-801.26 ± 0.72
Glycine	-370.60 ± 0.69	-370.77 ± 0.51
L-Alanine	-371.38 ± 0.49	-370.88 ± 0.39
L-Aspartate	-698.85 ± 0.37	-698.52 ± 0.35
L-Threo- β -methylaspartate	-691.57 ± 0.67	-690.83 ± 0.57
L-Glutamate	-697.43 ± 0.67	-696.90 ± 0.55
Citrate	-1172.59 ± 0.62	-1172.11 ± 0.62
D-Isocitrate	-1166.44 ± 0.61	-1165.98 ± 0.61
cis-Aconitate	-926.91 ± 0.75	-926.43 ± 0.80
NAD^+	-418.40 ± 0.00	-418.40 ± 0.00
NADH	-397.48 ± 0.31	-397.51 ± 0.36
NADP^+	-418.40 ± 0.00	-418.40 ± 0.00
NADPH	-396.38 ± 0.36	-396.53 ± 0.41

The present network can be improved in a number of ways. Enthalpies of combustion can always be improved. It is recognized how difficult these measurements are, but the data are badly needed. It is suggested that the enthalpies of combustion be measured on different compounds of the same substance. Thus, to obtain better data on the acetate ion, it would be better to measure five samples of acetic acid and five of ammonium acetate instead of ten samples of acetic acid, which is difficult to obtain water free. It is necessary to have the enthalpy of solution to compare the results, but $\Delta_{\text{sol}}H$ are relatively easy to obtain accurately. Similarly it is better to split the samples among D, L and DL-alanine instead of using only L-alanine.

There are a number of third law entropies that would be useful to have, with fumarate being the prime candidate since it is more central to the Krebs cycle than succinate. There seems little point in obtaining third law entropies of compounds for which the entropy of solution is difficult to obtain. Examples are lactic acid, pyruvic acid and isocitric acid, which are very soluble and which form polyesters, aldol products and a lactone. The heat capacity should be measured on the compound which is in equilibrium at saturation. It was fortunate that the S° of anhydrous sodium acetate was usable

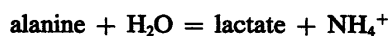
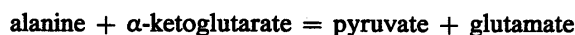
because of the metastability of the supersaturated solutions, even though the stable phase is the trihydrate. Third law entropies have been obtained for L-serine and L-proline, but the solid phases at saturation are hydrates. In addition the DL-amino acids are very much less soluble in these two cases, and the activity coefficients have been measured nearly to saturation for these racemic amino acids.

Modern enthalpies of solution for a number of amino acids are needed with aspartic acid, glutamic acid, asparagine and glutamine being prime candidates. Those planning to make measurements of $\Delta_{\text{sol}}H$ should select compounds with modern third law entropies and enthalpies of combustion.

The problems of the NAD^+ and NADP^+ potentials have been reviewed along with the problem of the α -isomer. An accurate potentiometric measurement would be ideal. Although the potential at $I=0$ mol dm⁻³ is good to have, the real need is at $I=0.1$ mol dm⁻³. The next most accurate way to obtain this potential is to measure the equilibrium constant for reaction with formate/bicarbonate at 25 °C. This is an accurately known potential although the equilibrium lies far to the side of reduction of NAD^+ . Even better would be the equilibrium using H_2 .

The lyase reactions tie the Krebs cycle network tightly together, so that really accurate $\Delta_f G$ for the citrate, isocitrate and malate lyase reactions would be very valuable. Several carboxylation reactions would be of interest, examples being acetate to pyruvate and succinate to α -ketoglutarate. These reactions are very unfavorable at the level of the keto acid, but reduction to the amino acid or hydroxy acid would be measurable with a strongly reducing couple such as formate/bicarbonate.

Accurate equilibrium constants are usually easier to measure with pH independent reactions than with those involving NAD^+/NADH . Examples are transamination and amination equilibria, e.g.



These reactions are various sums of the alanine, glutamate and lactate dehydrogenases, but more accurate data for the dehydrogenases should be obtainable using the transamination and amination equilibria combined with one good dehydrogenase equilibrium, e.g., alanine dehydrogenase.

7. Glossary of Symbols and Terminology

English

m	concentration of solution expressed as molality (amount of solute per kg of water)
n	moles of substance
x	mole fraction

E°	Emf at pH 0
$E^{\circ'}$	Emf at pH 7
F	Faraday (96487 C mol ⁻¹)
G	Gibbs energy
H	enthalpy
I	ionic strength
K	equilibrium constant
M	concentration of solution expressed as molarity (amount of solute per 1000 cm ³ of solution)
P	pressure (atm)
R	gas constant (8.31441 J·mol ⁻¹ ·K ⁻¹)
S	entropy
T	thermodynamic temperature
V	volts

NAD ⁺	Nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
NADP ⁺	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced nicotinamide adenine dinucleotide phosphate

CoA	Coenzyme A
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
GTP	Guanosine triphosphate
GDP	Guanosine diphosphate
P _i	Inorganic orthophosphate

Greek:

γ	activity coefficient
Δ	change in a property

Superscripts:

°	standard state quantity
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Subscripts:

f	formation property
r	reaction property
sol	solution property
ion	ionization property

Physical States:

ao	aqueous, standard state of the indicated species
aq	aqueous solution, concentration not specified
cr	crystalline solid
g	gas
l	liquid

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9. References

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