ENZYMATIC SYNTHESIS AND PROPERTIES OF 5-PHOSPHORIBOSYLPYROPHOSPHATE*

By ARTHUR KORNBERG, IRVING LIEBERMAN, and ERNEST S. SIMMS

(From the Department of Microbiology, Washington University School of Medicine, St. Louis, Missouri)

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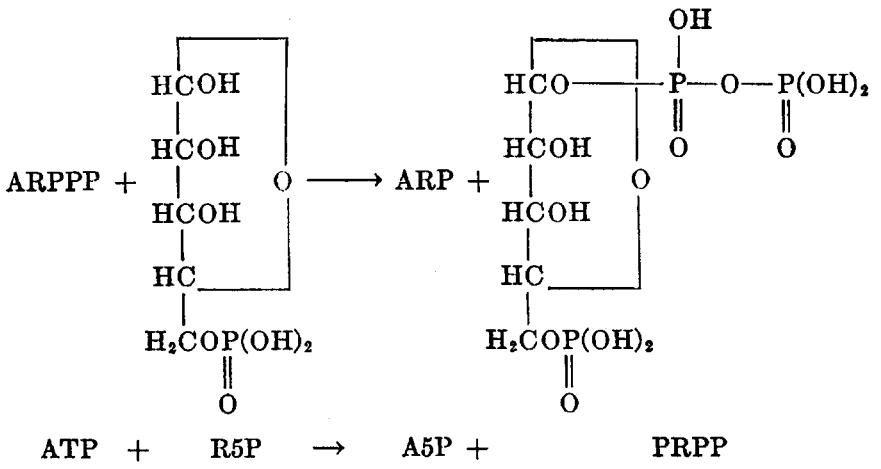
Investigations of the mechanism of pyrimidine nucleotide formation from orotic acid led to the isolation and characterization of an activated ribose phosphate derivative which serves as an intermediate in the overall process. In earlier communications (1) we have described briefly the enzymatic synthesis of this new intermediate, identified as PRPP[1] (equation (1)), and the occurrence of enzymes which condense pyrimidines and purines with PRPP to form the respective nucleotides (equation (2)).

(1)



(2) Pyrimidine (or purine) + PRPP ⇌ pyrimidine (or purine) nucleotide + PP

This report deals with the purification and properties of the enzyme which catalyzes the formation of PRPP and some of the properties of PRPP. Succeeding reports will consider the enzymatic synthesis of pyrimidine nucleotides (2), purine nucleotides (3), and nucleoside di- and triphosphates (4).

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[1] The abbreviations used are 5-phosphoribosylpyrophosphate, PRPP; adenosine triphosphate, ATP or ARPPP; ribose-5-phosphate, R5P; adenosine-5′-phosphate, A5P or ARP; inorganic pyrophosphate, PP; adenosine diphosphate, ADP; orotidine-5′-phosphate, O5P; uridine-5′-phosphate, U5P; tris(hydroxymethyl)aminomethane, Tris.

Methods

Materials—The sodium salt of R5P was prepared from the barium salt (product of the Schwarz Laboratories, Inc.) by decomposition with sodium sulfate. A5P and ADP were products of the Sigma Chemical Company and ATP of the Pabst Brewing Company. Adenine and orotic acid were obtained from Dougherty Chemicals. Ribose-1-phosphate was prepared by phosphorolysis (5) of inosine (Nutritional Biochemicals Corporation) with liver nucleoside phosphorylase[2] coupled to xanthine oxidase;[2] it was purified by anion exchange chromatography (6). ATP^{32} (ATP labeled in the two terminal phosphate groups) was prepared by aerobic phosphorylation of A5P with rat liver mitochondria[3] (7) and purified by the ion exchange chromatographic procedure of Cohn and Carter (8) and Kornberg and Pricer (9).

2-Deoxyribose-5-phosphate was prepared from 2′-deoxyadenosine-5′-phosphate (California Foundation for Biochemical Research) as follows: 1.6 ml. of a 0.04 M solution of the nucleotide was acidified to pH 2, heated at 100° for 5 minutes, neutralized, adsorbed on a Dowex 1 column (2 per cent cross-linked, chloride form, 3 cm. × 1 sq. cm.), and eluted with 0.005 N HCl-0.01 M KCl. The fractions between 15 and 23 resin bed volumes contained 97 per cent of the adsorbed deoxypentose and an equivalent amount of organic phosphate. The acid lability of the compound (94 to 98 per cent hydrolysis after 30 minutes at 100° in 1 N HCl) agrees with published observations (10).

Orotic acid (4,7-C^{14})[4] was prepared from 1,4-C^{14}-aspartic acid by a modification of the method of Nyc and Mitchell (11). Labeled aspartic acid was prepared as follows: Ethylene dibromide (1.7 gm.) was allowed to react with C^{14}-NaCN (10 mmoles) by a microadaptation[5] of a procedure of Vanino (12), producing 1,4-C^{14}-succinic acid, which was then partially purified by treatment with a cation exchange resin (Dowex 50 (H^+)). The percolate was evaporated to dryness and the residue was dissolved in water and neutralized. The succinate was quantitatively oxidized by succinoxidase (13) to an equilibrium mixture of fumarate and malate. The latter mixture was then converted to L-aspartate in the presence of excess ammonia by an aspartase-fumarase preparation from *Escherichia coli* (14). L-Aspartic acid was purified by chromatography on Dowex 50 (H^+), concentrated *in vacuo*, and crystallized at pH 3.0 by the addition of alcohol.

Acetone powders of liver were prepared as follows: The fresh tissue was

[2] Kindly furnished by Dr. M. Friedkin.

[3] Kindly furnished by Dr. F. E. Hunter.

[4] The numbering of the pyrimidine ring follows recent usage by "Chemical abstracts;" orotic acid is 6-carboxyuracil.

[5] We are indebted to Dr. D. Shemin for these details.

homogenized in 5 to 10 volumes of acetone (chilled to −10°). The residue, collected on a Büchner funnel, was homogenized again in cold acetone, collected, and dried in air. The powders were stored at −10°.

Determinations—ADP and ATP were determined spectrophotometrically (15, 16). A5P was determined with Schmidt's deaminase by Kalckar's method (17). PRPP was determined as described below (Assay B, Stage II). Orthophosphate was determined by the method of Fiske and Subbarow (18), acid-labile phosphate was the orthophosphate liberated after hydrolysis for 10 minutes in 1 N HCl at 100°, and total phosphate was obtained as orthophosphate after ashing with a sulfuric acid-nitric acid mixture. Reducing sugar was determined colorimetrically (19) with D-ribose as a standard. Pentose was determined by the Mejbaum procedure (20) with a 40 minute heating period and A5P as a standard. Protein was determined by the phenol method of Lowry *et al.* (21). Ion exchange chromatography was carried out at 2° with an automatic fraction collector on Dowex 1 (2 per cent cross-linked, 200 to 400 mesh, chloride form) columns according to Cohn (22). P^{32} was measured in solution in a dish under a Geiger-Müller tube. C^{14}-containing samples were plated as thin layers on metal disks and measured in a gas flow counter. Self-absorption corrections were applied where indicated.

Enzyme Assays—The activity responsible for equation (1) can be determined in a variety of ways. Three methods which have provided sensitive and reliable assays for the enzyme will be described here.

Assay A. $C^{14}O_2$ Evolution from Carboxyl-Labeled Orotate—This assay requires the addition, in excess, of O5P pyrophosphorylase and decarboxylase which carry out reactions (3) and (4), respectively. Under these

$$\text{Orotate} + \text{PRPP} \rightleftarrows \text{O5P} + \text{PP} \tag{3}$$

$$\text{O5P} \rightarrow \text{U5P} + CO_2 \tag{4}$$

conditions the amount of $C^{14}O_2$ liberated is an accurate measure of the amount of PRPP produced in the system. The incubation mixture (1.0 ml.) contained 0.02 ml. of ATP (0.04 M), 0.02 ml. of phosphopyruvate (0.1 M), 0.1 ml. of R5P (0.025 M), 0.05 ml. of 4,7-C^{14}-orotate (0.01 M, 1.4×10^5 c.p.m. per μmole), 0.05 ml. of potassium phosphate buffer (1 M, pH 7.0), 0.03 ml. of $MgCl_2$ (0.1 M), 0.01 ml. of pyruvate phosphokinase (16), 0.1 ml. of yeast autolysate (2), and less than 0.2 unit of enzyme. The mixture was incubated at 30° for 1 hour in an evacuated Thunberg tube containing 0.5 ml. of 0.2 N KOH (with a drop of 0.04 per cent thymol blue) in the bulb. To stop the reaction and acidify the reaction mixture, about 4 drops of an H_2SO_4-thymol blue solution (2 parts of 50 per cent H_2SO_4, 1 part of 0.04 per cent thymol blue) were cautiously admitted into the main space of the Thunberg tube through the side arm. The tube,

still under vacuum, was incubated in a 65° oven for 30 minutes and then cooled. 0.1 ml. of the KOH solution in the bulb was removed to a planchet and dried and the radioactivity was measured. Under these conditions (with a self-absorption of about 50 per cent), 3500 c.p.m. represented total decarboxylation of the 0.5 μmole of orotate in the reaction mixture. Proportionality of enzyme addition to $C^{14}O_2$ release was obtained when 30 per cent or less of the orotate was decarboxylated. 1 unit of enzyme is defined as the amount causing the formation of 1 μmole of PRPP (as indicated by the release of 1 μmole of $C^{14}O_2$), or the removal of 1 μmole of orotate (Assay B, below), or the removal of 1 μmole of ATP^{32} (Assay C, below), per hour, and specific activity is expressed as units per mg. of protein.

Assay B. Orotate Removal Measured Spectrophotometrically—This assay is best carried out in two stages; the first involving the formation of PRPP and the second its quantitative utilization in the removal of orotic acid as in equation (3).

Stage I—The incubation mixture (1.0 ml.) contained 0.03 ml. of ATP (0.04 M), 0.1 ml. of R5P (0.025 M), 0.02 ml. of potassium phosphate buffer (1 M, pH 7.4), 0.02 ml. of $MgCl_2$ (0.1 M), 0.02 ml. of glutathione (0.5 M), 0.05 ml. of KF (1 M), and about 1 unit of enzyme. After incubation for 20 minutes at 36°, the mixture was heated for 1 minute in a boiling water bath to inactivate the enzyme, immediately cooled, and centrifuged if necessary.

Stage II—The incubation mixture (1.0 ml.) in a quartz cuvette contained 0.02 ml. of orotate (0.01 M), 0.02 ml. of Tris buffer (1 M, pH 8.0), 0.02 ml. of $MgCl_2$ (0.1 M), 0.2 ml. of O5P pyrophosphorylase (ethanol fraction) (2), and an aliquot from Stage I containing about 0.03 μmole of PRPP. The conversion of orotic acid to O5P or U5P results in a decrease in optical density at 295 mμ; the molar absorption coefficient for the density decrease of the latter conversion is 3950. With the indicated amount of O5P pyrophosphorylase fraction, the utilization of 0.03 μmole of PRPP is usually complete in 10 minutes when measured in the Beckman DU spectrophotometer at room temperature.

Assay C. ATP^{32} Removal—This assay depends on transfer of the P^{32}-labeled terminal phosphate groups of ATP to a compound that is not adsorbable by Norit (23). The incubation mixture (0.4 ml.) contained 0.02 ml. of ATP^{32} (0.02 M, 3×10^4 c.p.m. per μmole), 0.04 ml. of R5P (0.025 M), 0.02 ml. of potassium phosphate buffer (1 M, pH 7.4), 0.01 ml. of $MgCl_2$ (0.1 M), 0.02 ml. of glutathione (0.5 M), 0.04 ml. of KF (1 M), and about 0.2 unit of enzyme. After incubation for 30 minutes at 36°, 0.8 ml. of 3.5 per cent perchloric acid and then 0.2 ml. of a 10 per cent suspension of Norit A were added to the reaction mixture. After 2 to 5 minutes the

mixture was filtered and 0.5 ml. of the filtrate was pipetted into a dish for radioactivity measurement. With crude enzyme fractions, an incubation mixture lacking R5P served as a control for interfering activities such as that of ATPase. The use of fluoride provides a means of reducing ATPase activity.

Results

Purification of Enzyme—All operations were carried out at 0–3°. 4 gm. of pigeon liver acetone powder were suspended in 40 ml. of Tris buffer (0.02 M, pH 8.0) and stirred occasionally during a 10 minute interval. The residue was removed by centrifugation in a Servall (SS-1) centrifuge at 8000 × *g* for 5 minutes. The supernatant fluid may be either clear or opalescent (acetone powder extract, Table I).

TABLE I

Purification of Enzyme

Step	Units per ml.*	Total units*	Protein	Specific activity
			mg. per ml.	*units per mg. protein*
1. Acetone powder extract	18.0	612	19.6	0.9
2. Low pH precipitation	16.4	558	3.00	5.5
3. Aluminum hydroxide gel eluate	8.1	275	0.33	24.5

* Assay B was used in this run. Essentially similar results were obtained with the other assays.

To 34 ml. of extract were added 51 ml. of water and then, slowly, 17 ml. of potassium acetate buffer (1 M, pH 5.4). After 5 minutes, the precipitate was collected by centrifugation (as above). It was dissolved in 7.0 ml. of Tris buffer (0.1 M, pH 8.0) and diluted to 30 ml. with water. The pH was adjusted to 6.8 with 0.1 N HCl (about 1.3 ml. required). The volume was made up to 34 ml. (low pH fraction).

For the last step enough aluminum hydroxide gel Cγ was used to adsorb 85 to 90 per cent of the enzyme. 3.75 ml. of aged gel (24) (15 mg. of dry weight per ml.) were centrifuged and the supernatant fluid was discarded. 30 ml. of isoelectric fraction were thoroughly mixed with the gel, left in ice for 5 minutes, and then centrifuged. The supernatant fluid was discarded and the gel precipitate was washed with 30 ml. of potassium phosphate buffer (0.05 M, pH 6.85). The enzyme was then eluted from the gel with 30 ml. of potassium phosphate buffer (0.10 M, pH 6.85) (aluminum hydroxide gel eluate).

The acetone powders were stored at −10° and showed no significant

loss in activity over a 2 month period. The Cγ gel eluate retained 65 to 70 per cent of its activity after storage for 2 months at $-10°$ provided glutathione (0.05 M) was present; in the absence of glutathione less than 5 per cent of the activity remained.

Distribution of Enzyme—The enzyme appears to be present also in chicken and mammalian livers and in bacteria. Extracts of acetone powders of chicken, mouse, and rat livers, and of an 18 hour culture of *E. coli* B cells (ground with alumina (25)) had the following specific activities (Assay B): chicken, 0.29; mouse, 0.04; rat, 0.01; and *E. coli*, 0.34. An

TABLE II

Balance Study of PRPP Synthesis

The experimental incubation mixture (10.0 ml.) was the same as that described for Assay B, Stage I (see the text), except that ATP^{32} (2.2×10^4 c.p.m. per μmole) was used. The control mixture lacked R5P. 2.0 ml. of the aluminum hydroxide gel eluate fraction containing 0.72 mg. of protein were used. Incubation was at 35° for 60 minutes. The figures in bold-faced type were obtained by chromatography of 8.0 ml. of the reaction mixtures on Dowex 1 columns (6.5 cm. × 1.0 sq. cm.); the eluent was 0.0025 N HCl-0.1 M KCl. The other values were obtained by direct enzymatic assay of aliquots of the incubation mixtures. The results are expressed in micromoles.

	0 min.		60 min.		Δ	
	Control	Experimental	Control	Experimental	Control	Experimental
ATP	13.1	12.5	12.5	1.6	−0.6	−10.9
"			**11.1**	**1.8**		
PRPP	0.0	0.0	0.0	10.9	0.0	+10.9
"				**10.0**		
A5P	0.0	0.0	0.0	9.8	0.0	+9.8
"			**0.0**	**10.2**		

autolysate of dried brewers' yeast and a sonic extract of bakers' yeast were inactive.

Balance Study of Reaction—The stoichiometry of the reaction of ATP with R5P to yield A5P and PRPP (equation (1)) is shown in Table II. In the control sample, lacking R5P, ATP was not removed to any significant extent and the production of A5P and PRPP was not detectable. In the presence of R5P, almost all the added ATP disappeared and was matched by the accumulation of equivalent molar amounts of A5P and PRPP. Values obtained both by enzymatic and ion exchange chromatographic analyses were in good agreement. No ADP was detectable after either 30 or 60 minutes incubation when 65 and 87 per cent, respectively, of the ATP had been consumed. That orthophosphate was not released (from

the ATP^{32} in this reaction) was evident from the absence of any radioactivity in that fraction of the ion exchange chromatogram.

Isolation of PRPP—PRPP was isolated by ion exchange chromatography as a symmetrical zone, appearing after 25 to 30 resin bed volumes of effluent, and was contained within the next 10 to 15 volumes. The early fractions were contaminated by ATP. Analyses of eight fractions selected

TABLE III

Chromatography of PRPP

8.5 ml. of experimental and control incubation mixtures were chromatographed as described (see Table II).

Fraction No.*	Pentose†	Molar ratios (referred to pentose)			Specific activity§
		PRPP	Acid-labile P‡	Total P	
	μmole per ml.				*c.p.m. per μmole*
30	0.051	1.00			20,300
32	0.100	0.84	2.00	3.05	22,500
34	0.107	1.03	1.98	2.96	21,300
36	0.109	1.00	2.01	3.00	22,900
38	0.107	0.95	2.01	3.15	23,200
40	0.101	0.94	2.03	2.95	23,000
42	0.081	0.84	2.10	3.15	23,200
44	0.060		2.15	3.05	22,600
Average molar ratio	1.00	0.94	2.04	3.04	

* Approximately 5.0 ml. per fraction, corresponding to about 1 resin bed volume.

† These values are corrected for any ATP present as determined by the optical density at 260 mμ.

‡ Estimations of orthophosphate by the method of Lowry and Lopez (26) indicated 0.16 μmole or less per μmole of pentose in each fraction. With the Fiske and Subbarow procedure (18), 25 and 40 per cent of the acid-labile phosphate appeared as orthophosphate after color development (at 30°) for 10 and 30 minutes, respectively.

§ The specific activities for the ATP^{32} at zero time and that isolated from the control sample were 22,700 and 21,400, respectively.

from the middle of the PRPP zone and containing approximately 80 per cent of the PRPP contained pentose, enzymatically active PRPP (see Assay B, Stage II, equation (3)), acid-labile P, and total P in molar ratios of 1.00:0.94:2.04:3.04 (Table III). The average of the specific radioactivities of these fractions was 22.4×10^3 as compared with values of 22.7×10^3 and 21.4×10^3, respectively, for the ATP^{32} at zero time and that isolated from the control sample.

A barium salt of PRPP was prepared as follows: ATP^{32} and the components described for Assay B, Stage I on a 100 ml. scale, were incubated

for 75 minutes. After 45, 60, and 75 minutes, PRPP synthesis was estimated to be 103, 92, and 100 μmoles, respectively. The mixture was chromatographed on a 6 cm. × 3.2 sq. cm. column.[6] The fractions between 29 and 46 resin bed volumes were neutralized immediately upon their collection and pooled (366 ml., including washings). 7.5 ml. of glycine buffer (1 M, pH 8.5) and 3 ml. of saturated barium acetate were added and the pH was adjusted to 8.5. Cold ethanol (2 volumes) was added and after 30 minutes at 0° the precipitate was collected by centrifugation, washed with ethanol, and dried in a vacuum desiccator. The salt (118 mg.) was suspended in 4 ml. of water, dissolved by the addition of acid (about 0.3 ml. of 0.5 N HCl lowered the pH to about 2),[7] and decomposed with sulfate (0.3 ml. of 1 M Na_2SO_4) and the supernatant solution was neutralized. The reagents were cold and the operations were rapid. 45 μmoles of PRPP were recovered. Extraction of the precipitate with dilute acid yielded 2.8 μmoles more. The recovery of PRPP from the incubation mixture was thus approximately 50 per cent. From 20 to 30 per cent of the radioactivity in the barium salt did not appear in solution. Based on a molecular weight for the barium salt of 729 gm. and without any corrections, the purity of the salt was only 30 per cent.

Reprecipitation of the barium salt from CO_2-free solutions resulted in considerable purification. A crude barium salt (65 mg.) was dissolved in dilute acid and immediately neutralized. The precipitate which formed was washed with 50 per cent ethanol and dried. The salt (24 mg.), on decomposition with sulfate, yielded 18.3 μmoles of PRPP. Assuming that 25 per cent of the PRPP in the barium salt fails to dissolve, the purity of this salt was 75 per cent. In some purified barium salts, ATP (or an ATP-like substance) represented 10 per cent or more of the impurity. Removal of the adenine nucleotide with Norit either was incomplete or led to large losses of PRPP. The major impurities in most cases appeared to be PP and R5P, the decomposition products of PRPP. A preparation free of ultraviolet-absorbing components (<1.0 density unit at 260 mμ per 10 μmoles of PRPP) was obtained by excluding the early fractions of the chromatogram. The molar ratios of inorganic P, acid-labile P, total P, and PRPP to pentose were 0.09, 2.04, 3.02, and 0.70, respectively. Assay with inorganic pyrophosphatase indicated the presence of 5.6 μmoles of PP per 10 μmoles of PRPP. Assuming the presence of an amount of R5P equivalent to that of PP, these two impurities would adequately account for the analytic data.

[6] As compared with the smaller scale run (Table II), 10 times as much reaction mixture was chromatographed on a column only 3 times as large; the separation of PRPP and adenine nucleotides appeared to be less effective.

[7] A large evolution of gas indicated the presence of $BaCO_3$ as an impurity.

Neutral solutions of PRPP have been stored at −15° for 3 months without detectable destruction. At low pH or at elevated temperatures (see below), PRPP decomposition is rapid.

TABLE IV

Decomposition of PRPP

A solution containing 1.45 μmoles of PRPP (P^{32}-labeled, see Table II) in 0.1 M acetate buffer, pH 4.0, was heated at 65°. A solution immediately neutralized without heating served as a control.

Experiment No.	Time of heating	−Δ PRPP	+Δ reducing sugar	+Δ PP
	min.	*μmoles*	*μmoles*	*μmole*
1	10	0.94	0.96	
	40	1.45	1.73	
2	30	1.13	1.07	0.99*

* Determined by ion exchange chromatographic analysis; 0.30 μmole of orthophosphate was also produced.

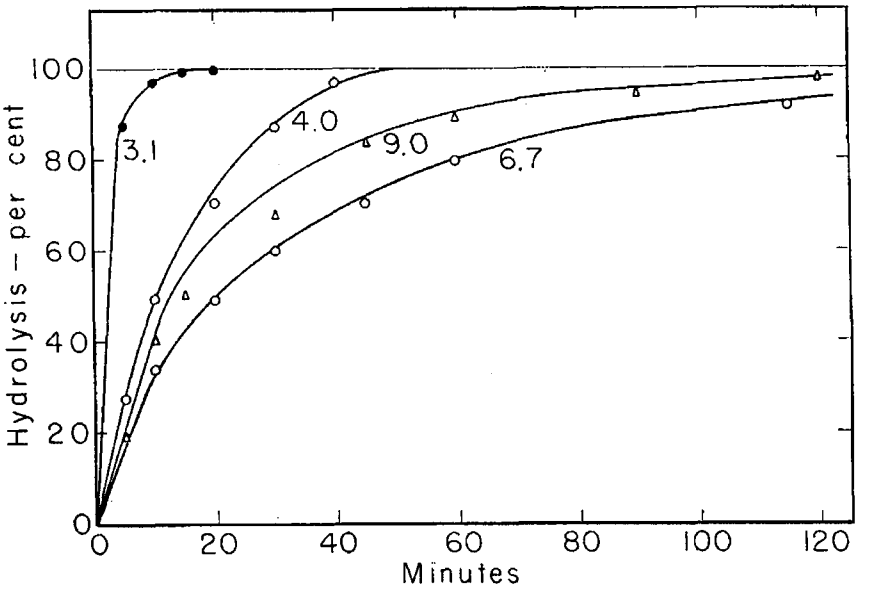

FIG. 1. Decomposition of PRPP at several pH levels. PRPP at a concentration of 0.4 μmole per ml. was incubated in 0.02 M buffer at 65°. The buffers were as follows: formate, pH 3.1, acetate, pH 4.0, glycylglycine, pH 6.7, glycine, pH 9.0.

Structure and Decomposition of PRPP—Heating of PRPP at 65° at pH 4.0 results in a loss of enzymatically active compound (see Assay B, Stage II, equation (3)) and the appearance in equivalent amounts of reducing sugar and inorganic pyrophosphate (Table IV).

The hydrolysis of PRPP at 65° at several pH levels is shown in Fig. 1. At pH 3.1, hydrolysis was virtually complete in 10 minutes, while at neu-

tral pH only 50 per cent was destroyed after 20 minutes. The rate of PRPP decomposition was catalyzed by Mg^{++} and this effect was overcome by agents which bind Mg^{++} (Table V).

The enzymatic decomposition of PRPP has been demonstrated in the reversible pyrophosphorolysis of pyrimidine and purine nucleotides (equation (2)) (2, 3). Nucleotide pyrophosphatase (27) and inorganic pyrophosphatase (28) failed to split (less than 0.005 μmole) PRPP; under comparable conditions, over 25 and 100 μmoles of diphosphopyridine

TABLE V

Mg^{++} Activation of PRPP Decomposition

1 ml. of a 1×10^{-4} M neutral solution of PRPP (in a 12 × 100 mm. glass tube) was heated for 1 minute in a briskly boiling water bath and then immediately placed in ice water. PRPP was assayed as described under "Methods." The concentrations of the indicated additions were $MgCl_2$ 0.002 M, potassium phosphate buffer, pH 7.4, 0.02 M, sodium pyrophosphate, pH 8.0, 0.02 M, Versenate, pH 7.0, 0.005 M. Experiment II differed from Experiment I only in that Tris-maleate buffer, pH 7.4, 0.02 M, was included.

Addition	1 min. hydrolysis (100°)	
	Experiment I	Experiment II
	per cent	*per cent*
None	55	50
Mg^{++}*	78	80
Phosphate	29	
Mg^{++} + phosphate†	18	22
" + pyrophosphate		10
" + Versenate		17

* Heating for 60 minutes at 36° results in a 51 per cent loss of PRPP.

† Heating for 2 minutes at 100° results in a 46 per cent loss of PRPP.

nucleotide and PP, respectively, were hydrolyzed by these enzyme preparations.

Influence of ATP and R5P Concentration on Reaction Rate and Specificity of ATP and R5P for Enzyme—The influence of the concentration of ATP and R5P on the rate of reaction is shown in Fig. 2. No reaction was detectable in the absence of ATP and R5P; the levels at which the rates were half maximal were 4×10^{-4} M and 6×10^{-4} M for ATP and R5P, respectively. ATP at higher concentrations (2×10^{-3} M) appeared to be inhibitory.

ADP (1×10^{-3} M), inosine triphosphate (0.8×10^{-3} M), and uridine triphosphate (0.4×10^{-3} M) either failed to replace ATP or showed less than 2 per cent of the activity of ATP. Ribose-1-phosphate (0.6×10^{-3} M),

2-deoxyribose-5-phosphate (2.5×10^{-3} M), and glucose-6-phosphate (2.5×10^{-3} M) appeared totally inactive in replacing R5P as determined by Assays

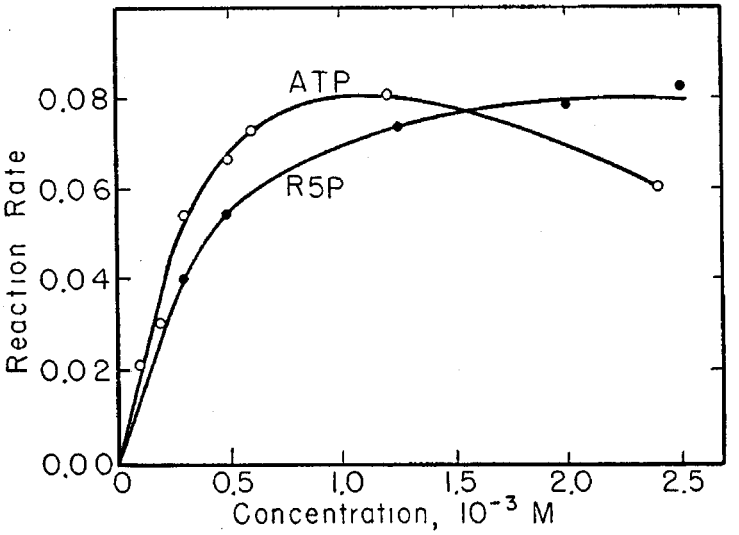

FIG. 2. Influence of ATP and R5P concentration on the rate of PRPP synthesis. The incubation conditions were as described in Assay B, Stage I, except for the varying concentrations of ATP and R5P indicated in the graph. 0.04 ml. of the alumina gel eluate fraction containing about 0.3 unit of enzyme was used. The reaction rate is expressed as units of enzyme activity.

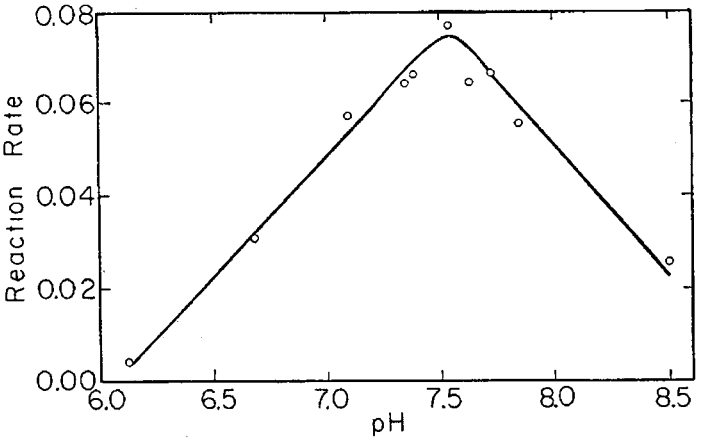

FIG. 3. Influence of the pH on the rate of PRPP synthesis. The incubation conditions were as described in Assay B, Stage I, except for adjustment of the phosphate buffer to the values indicated in the graph. 0.05 ml. of the alumina gel eluate fraction containing about 0.4 unit of enzyme was used. The reaction rate is expressed as units of enzyme activity.

B and C; the action of R5P (2.5×10^{-3} M) was not inhibited by the presence of an equimolar concentration of 2-deoxyribose-5-phosphate.

Effects of Mg^{++} and pH on Reaction—Mg^{++} is essential for the enzyme

activity. In its absence no reaction was observed. The rate was half maximal at a concentration of 0.8×10^{-3} M and maximal at 3×10^{-3} M. The optimal pH for the reaction under standard assay conditions was found to be near 7.5 (Fig. 3). Fluoride did not inhibit the enzyme even at a concentration of 0.1 M.

DISCUSSION

The proposed structure of PRPP is derived from several lines of evidence: (1) utilization of 1 mole of ATP in its synthesis, with the appearance of 1 mole of A5P, but without liberation of ortho P, (2) specificity of R5P and ATP in its synthesis, (3) isolation of a compound, eluted as a symmetrical peak in an ion exchange chromatogram, containing 1 mole of pentose and 3 moles of P of which 2 are acid-labile, (4) decomposition of the compound by very weak acid hydrolysis to liberate one reducing group and 1 mole of inorganic pyrophosphate, and (5) the participation of this compound in the reversible pyrophosphorolysis of pyrimidine and purine nucleoside-5′-phosphates.

In studies on the mechanism of incorporation of adenine into A5P, a 5-phosphoribokinase (29) was described in pigeon liver extracts which catalyzes the reaction

$$\text{ATP} + 2\text{R5P} \rightarrow \text{A5P} + 2 \text{ ribose-1,5-diphosphate}$$

The report contained no evidence for the stoichiometry of the reaction or for the chemical structure of the ribose diphosphate. The similarity of the substrate, product, and enzyme source to those used in our studies suggests that a more thorough inquiry into the ribose diphosphate-forming system is indicated.

The enzymatic synthesis of PRPP may represent a two-stage transfer of phosphate from ATP to R5P or, what appears more likely, a direct pyrophosphorylation of the substrate. The latter mechanism, if correct, would be unique, since previously described reactions such as the pyrophosphorolysis of coenzymes (30) are reversible systems in which the adenyl rather than the phosphate group is transferred. Maas and Lipmann (31) recently proposed, on the basis of isotopic evidence, that a direct pyrophosphorylation of the enzyme involved in pantothenate formation is a step in the over-all scheme. A more direct demonstration of this mechanism is needed.

The metabolic functions of PRPP form the basis for the succeeding reports on a pathway of synthesis of pyrimidine and purine ribonucleotides. It is possible that reaction schemes leading to the formation of other *N*-ribosyl compounds, such as the ribotide of acyclic purine precursors, and

of nicotinamide may also utilize PRPP as an intermediate.[8] The question as to whether a 2-deoxy analogue of PRPP may provide a means for condensing with pyrimidine and purine bases to form deoxyribonucleotides is also worthy of investigation.

SUMMARY

1. Enzymatic studies of pyrimidine and purine nucleotide synthesis have led to the discovery of a new intermediate, 5-phosphoribosylpyrophosphate (PRPP).

2. An enzyme has been partially purified from pigeon liver which catalyzes the formation of PRPP from ribose-5-phosphate (R5P) and ATP.

$$\text{ATP} + \text{R5P} \rightarrow \text{adenosine-5'-phosphate} + \text{PRPP}$$

This enzyme appears to be present also in mammalian liver and in bacteria.

3. ATP and R5P are specific in this reaction; Mg^{++} is also required.

4. PRPP has been isolated and characterized, and some of its properties have been described.

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[8] A sample of PRPP has been tested by Buchanan and coworkers and found to be active in the formation of ribotides of acyclic purine precursors. These workers have also isolated a pentose phosphate ester which is involved in purine nucleotide synthesis and has been identified as PRPP (personal communication from Dr. J. M. Buchanan).

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