Assays of Dcd Polymerase Fractions

Priming Capacity of Morton Fractions

6/13/61

		1	2	3	4	5	6	7	8	ck
water	✓	.08								.72 ✓
glycine 1M, 9.2	✓	.02								.18 ✓
$MgCl_2$, 0.1M	✓	.02								.18 ✓
C^{14} dATP, 0.5 μM/ml	✓	.01	.20 →							.09 ✓
dCTP, "	✓	.01								.09 ✓
dGTP, "	✓	.02								.18 ✓
dTTP, "	✓	.01								.09 ✓
SH-EtOH, 0.01M	✓	.03								.27 ✓
										1.80
Pre-inc DNA		.04	.04	.04	.04	.04	—	—	—	
Morton #1, ~ 0.9 μM/ml		—	—					.02		
Morton #2, ~ 2.7 "		—	—						.01	
AS II, 3/3/60 (1:100)	✓	.01	.02							
DEAE #6, 1/4/61 (1:500)	✓			.01	.02					
P-cell #3, 12/30/60 (1:20)	✓					.01	.01	.01	.01	
water → .30	✓	.05	.04	.05	.04	.05	.09	.07	.08	
30 mins at 37° C. – Usual workup.										
Std = 895, 896		155	322	108	181	643	10	976	1956	
		151	345	94	172	663	14	976	1914	
		143	328	91	167	643	2	966	1925	
cpm corr (-10)										
mμ moles		.143	.328	.091	.667	.643	–	.97	1.93	
u/ml		143	164	460	418					

Summary:

1. Am sulf = 154 u/ml with C^{14} dATP; was 152 u/ml on 3/3/60 with C^{14} dCTP.
2. DEAE #8 fr. #6 = 439 u/ml with C^{14} dATP; was 1915 u/ml on 1/4/61 with C^{14} dGTP. This DEAE fraction has now a heavy ppt and putrid odor.
3. Pcell 19 fr. #3 assayed at too high a level – probably – is 129 u/ml; had been 859 u/ml on 12/30/60.
4. Morton DNA fractions prime!!

Remarkable that 4 of DNA attached to cytochrome is

even better than when free,

b) Unusually extensive synthesis i.e. 1.93 m μ moles incorporated of the 5 m μ moles of C^{14} dATP added; if corrected by 1.3, then this is in fact 50% utilization of the added dATP.

c) These Morton fractions are far better than the premix DNA.

Must be sure that enzyme-less controls show no trapping of the triphosphates. (See next expt).

Controls to Show Priming Capacity of Morton Fractions

#2 6/13/61

		1	2	3	4
water	✓	.16	.15	.17	.16
$MgCl_2$	✓	.02 →			
glycine	✓	.02 →			
O^{14}-dATP	✓	.01 →			
dCTP	✓	.01 →			
dGTP	✓	.02 →			
dTTP	✓	.01 →			
STEROH	✓	.03 →			
Morton #1	✓	.02	.02	–	–
Morton #2	✓	–	–	.01	.01
P-cell #3 (1:20)		–	.01	–	.01
30'-37°. Usual workup.					
		9	1020	12	1437
			1057		1446
	gpm (corr)	0	1029	0	1432

Summary:

1. No incorp. in absence of enzyme.
2. Values for Morton fractions similar to those found in Expt. 1 although discrepancy between Fract. #1 and #2 is less striking.

6/13/61

Deoxyribonucleic Acid from Crystalline Cytochrome b_2

SAMPLE No.1

Absorption Spectrum : E_{max} 260 mμ = 6.9

E_{min} 232 mμ = 3.63

in Volume 3 mls.

Estimated weight 810 μg

Sample in 0.1M NaCl, 0.01M triethanolamine - HCl pH 7.0

Frozen in dry ice at Adelaide on 9/6/61 and shipped air freight to Stanford University

Details of preparation

1. Three times crystalline cytochrome b_2 dissolved in 0.5 M sodium lactate, 0.04 M sodium pyrophosphate, 0.1 mM EDTA pH 6.8 and dialysed against re-crystallised metal-free ammonium sulphate (0.5 saturated) at 2°C for 21 hours, then dialysed against 0.7 saturated ammonium sulphate at 2°C for 18 hours.

2. Precipitate of DNA-free cytochrome b_2 collected by centrifuging (16220 g. for 20 minutes). Colourless supernatant containing approx. 4.7 mg. was dialysed, concentrated, loaded onto an "Ecteola" column, and chromatographed with a NaCl gradient (Montague and Morton: Nature 187, 916-917, 1960). The tubes containing the DNA were pooled, dialysed against dist distilled water and freeze dried. The residue was taken up in 1.0M NaCl, insoluble (aggregated) matter was removed by centrifuging. The supernatant was dialysed against 0.1M triethanolamine - HCl pH 7.0 for 24 hours to give sample No.1

SAMPLE No.2

Intact cytochrome b_2 - 2 times crystalline

Absorption spectrum : $E_{u.v.}$ 265 mμ = 52.5

E_{soret} 423.5 mμ = 59.5

in Volume 0.5 ml.

Estimated weight 10 mg.

Sample in 0.5M sodium lactate, 0.04M sodium pyrophosphate

0.1mM EDTA pH6.8

Frozen in dry ice at Adelaide on 9/6/61 and shipped air freight to Stanford University.

I have also included 10 mg. of three times crystalline cytochrome b_2. This has about 4% dry weight of DNA. This may be enough for your experiments. This should be in the undenatured form. I think it would be interesting if you could try your technique with the DNA attached to the enzyme. If this does not work you could split the DNA from the cytochrome as in the attached sheet.

We have recently crystallised cytochrome b_2 free of DNA. This material is an entirely different crystal. Whereas DNA-containing cytochrome b_2 consistently forms small crystals belonging to a tetragonal system - the crystals are very flat pyramids - the DNA-free cytochrome b_2 crystallises as large bi-pyramids belonging to a hexagonal system. At present therefore, we know that the DNA causes the following effects:-

(a) Stabilisation of the enzyme, but no effect on lactate dehydrogenase activity.
(b) Slight shift of the sedimentation coefficient and marked effect on the concentration dependence of the sedimentation coefficient
(c) Rapid crystallisation when enzyme is dialysed against 0.05M-lactate, pH6.8; DNA-free enzyme fails to crystallise in the same period.
(d) Modification of crystalline form of the enzyme which may be very slowly crystallised under the same conditions, even when free of DNA.

I now have a much improved procedure for purification and crystallisation. It cuts out one of the steps, making the procedure a practical one for relatively untrained assistants. Thus I should be able to give you more DNA in the next few months if you require it.