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ADP-polynucleotide phosphorylase

The enzyme polynucleotide phosphorylase catalyzes the reversible polymerization of various nucleoside diphosphates to form synthetic polynucleotides containing one or more bases[1]. Both the enzyme and the polymers have been studied by a number of investigators[2–6]. Evidence presented here indicates that several enzymes may be involved in this overall reaction.

In Table I the results of experiments are presented in which each diphosphate was incubated with either a crude or partially purifed enzyme preparation. In the former cases there was high polymerizing activity toward ADP and CDP but much less toward GDP and UDP. Inorganic phosphate production greatly exceeded polymer formation, indicating the presence of phosphatases.

TABLE I

Each incubation vessel contained the following in a final volume of 0.32–0.35 ml: nucleoside diphosphate, 1.0 μmole; Mg^{++}, 0.46 μmole; EDTA, 0.05μmole; glutathione, 1.84 μmoles; glycine buffer, pH 10.1, 92 μmoles; and enzyme solution, 0.20 ml. Incubations were carried out for 90 min at 37° and stopped by boiling. The values reported are averages of the number of experiments indicated in parentheses.

	Substrate	*Polymer formed (μmole) as determined by:*		
		Chromatogram origin [a]	*Polymer* [b]	P_i [c]
Crude preparations	ADP* (9)	0.33	0.31	0.53
	GDP (4)		0.01	0.09
	UDP (4)		0.04	0.13
	CDP (9)		0.26	0.54
Partially purified preparations	ADP* (4)	0.30	0.25	0.35
	GDP (2)		0.00	0.00
	UDP (2)		0.00	0.03
	CDP (4)		0.03	0.04
	CDP + poly-C [d] (1)		0.00	0.04
	CDP + RNA [e] (1)		0.00	0.07

[a] The radioactivity remaining at the origin after paper chromatography.
[b] Ultraviolet-absorbing material precipitated with $HClO_4$ and redissolved in buffer.
[c] Inorganic phosphate appearing.
[d] 0.10 μmole of poly-cytidylic acid was added to this vessel.
[e] 0.10 μmole of *M. lysodeikticus* nucleic acid was added to this vessel.

The partially purified preparations were obtained by proteolytic digestion followed by salt and alcohol fractionation; despite a 50–100-fold purification specific activities are still below those reported by others[4,7]. Using these partially purified enzymes, GDP, UDP and CDP were essentially inert, whereas ADP was rapidly polymerized. Chromatogram, polymer, and inorganic phosphate analyses agreed reasonably well.

The lack of CDP and UDP polymerization in the purified preparations was not due to ribonuclease destruction of the polymer as it was formed, since there was negligible formation of inorganic phosphate (Table I) and neither cytidine-3′-phosphate nor uridine-3′-phosphate were formed.

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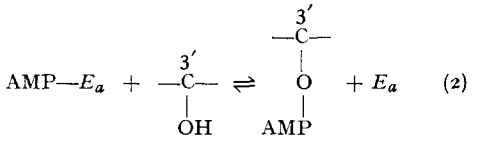
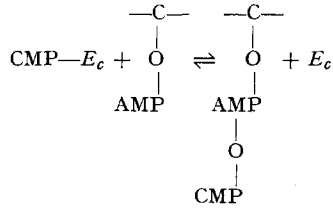
The inactivity of the purified enzyme toward CDP might also have been due to the removal of a primer, since OCHOA and co-workers (unpublished) and SINGER *et al.*[9] have reported that the initial lag period in nucleotide polymerization can be overcome by adding synthetic oligo- or polynucleotides to the reaction medium. However, the addition of poly-cytidylic acid or *M. lysodeikticus* nucleic acid did not restore CDP polymerizing activity (Table I), indicating that inactivity was not due to removal of a primer during enzyme fractionation.

It appears likely that there are several polynucleotide phosphorylases, one specific for each nucleoside diphosphate. The following mechanism of polynucleotide synthesis is presented as a working hypothesis:

$$\mathrm{ADP} + E_a \rightleftharpoons \mathrm{AMP}\text{—}E_a + \mathrm{P_i} \qquad (1)$$



(2)



(3)

where E_a and E_c are ADP- and CDP-specific polynucleotide phosphorylases respectively. In equation (1) ADP combines with its specific polymerizing enzyme, E_a, to form the enzyme-AMP complex and inorganic phosphate. In equation (2) this AMP is then transferred to an acceptor, which must be the 3-hydroxyl group of ribose containing a purine or pyrimidine at position 1 and a phosphate diester at position 5[3,8]. Other nucleotides can be added to the chain in analogous fashion, as in equation (3). This formulation is compatible with the following observations: (a) the phosphate exchange rate is greater than that of net synthesis[2]; (b) a substance has been isolated which affects only this exchange rate[4]; (c) adenosine-5′-phospho-3′-adenosine-5′-phosphate (di-AMP) can accept other nucleotides in the polymerization reaction[9]; (d) di-AMP can not be phosphorolyzed by this enzyme[3].

The differences between the *M. lysodeikticus* preparations, as reported here, and the *A. vinelandii* and *E. coli* enzymes may be due to variations in isolation and fractionation procedures or to biological differences.

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