ACTIVATION OF POLYADENYLATE SYNTHESIS BY BASIC POLYPEPTIDES

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Basic polypeptides are inhibitory to many enzyme systems, although a few cases of stimulation have been reported (Sela and Katchalski, 1959). The present communication deals with an apparently ADP-specific polynucleotide phosphorylase which shows complete dependence upon the presence of a basic poly-α-amino acid or high molecular weight polyamine. Extracts of the anaerobe Clostridium perfringens catalyze the synthesis of polyadenylate from ADP, a reaction which was routinely followed by determining the incorporation of C^{14}-labeled ADP into an acid insoluble precipitate (Littauer and Kornberg, 1957). The rate of this synthesis, as catalyzed by crude and partially purified enzyme, can be stimulated some 20-fold by various basic polypeptides (Dolin, et al., 1961) but not by acidic polypeptides. After a zone electrophoresis step, the enzyme shows an absolute requirement for a high molecular weight polybase. The present report summarizes the behavior of purified enzymes toward a series of cationic compounds. Two different enzyme preparations are considered, 1) a calcium phosphate gel eluate, 10-fold purified over the initial sonic extracts, and 2) a fraction, at least 100-fold purified, obtained after zone electrophoresis of the gel eluate (Dolin, in preparation).

The gel eluate fraction binds ADP poorly. In the absence of an activator, the K_s for ADP is 1.6×10^{-2} M, approximately the value reported for the phosphorylase of Azotobacter vinelandii (Grunberg-Manago, et al., 1956) and Escherichia coli (Littauer

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and Kornberg, 1957). In the presence of a saturating concentration of poly-L-lysine (9.7×10^{-6} M, M. Wt. 16,500) the K_s decreases 30-fold, to 5.8×10^{-4} M. Since routine enzyme assays are performed at ADP concentrations of 3.3×10^{-3} M, the presence of polylysine causes a large stimulation, usually about 20-fold. (The theoretical V_{max} increases 3-fold.) The polymer formed either in the presence or absence of polylysine has been identified as polyadenylate (Dolin, in preparation), and the theoretical stoichiometry for phosphate liberation is observed. In contrast to the synthetic reaction, polyadenylate phosphorolysis is inhibited 90% by polylysine.

The gel eluate fraction also catalyzes polymer formation from CDP and UDP, however the characteristics of these reactions are quite different from those of polyadenylate synthesis in that 1) with the pyrimidine compounds, substrate saturation occurs at or below 4×10^{-3} M and higher concentrations are strongly inhibitory, 2) at optimum concentration of CDP or UDP, polymer synthesis is inhibited about 90% by polylysine.

Electrophoresis of the gel eluate on starch blocks (pH 7.5, 0.05 M Tris) results in a 10-fold further purification, with a 70% yield of the enzyme units present in the gel fraction. The enzyme behaves as an acidic protein, moving to the anode with a mobility of 0.42 μ/sec/V/cm. When tested in the absence of an activator, the electrophoretically purified enzyme shows no detectable polymerase activity even at high ADP concentration, and no phosphorolysis of polyadenylate is detected either in the presence or absence of polylysine. (In the presence of polylysine, the K_s for ADP is similar to that determined for the gel fraction.) In addition, the enzyme does not catalyze polymer formation from either UDP or CDP. An enzyme with similar specificity for riboside diphosphates is present in trypsin-treated extracts of Micrococcus lysodeikticus (Olmstead and Lowe, 1959). Comparison of the activators for the clostridial enzyme preparations is given in Table I. It will be noted that polyadenylate does not activate either of these preparations.

With the gel eluate, polylysine, polyornithine and polyvinylamine cause about the same maximum stimulation, however the low molecular weight polyamines, spermine and

Table I

Activation of Polyadenylate Synthesis in Extracts of *Clostridium perfringens*

Activator	Enzyme Activity			
	Gel Fraction		Electrophoretic Fraction	
	C^{14} ADP Incorporation	Relative Activity	C^{14} ADP Incorporation	Relative Activity
	μmoles/hr		μmoles/hr	
None	0.012	0	0	0
Poly-L-lysine·HCl (M. Wt. 16,500)	0.21	100	0.17	100
Poly-L-lysine·HBr (M. Wt. 175,000)	0.19	90	0.16	95
Poly-L-ornithine·HBr	0.21	100	0.17	100
Poly-L-glutamate (M. Wt. 80,000)	0.014	1	-	-
Protamine sulfate	0.12	53	0.0033	2
Histone	0.072	30	0	0
L-lysine·HCl	0.014	1	-	-
Polyvinylamine·HBr	0.21	100	0.069	40
Spermine·HCl, 43 μg	0.02	4	0	0
Spermidine·HCl, 69 μg	0.016	2	0	0
KCl, 0.4 M	0.088	38	0	0
KCl, 0.4 M + poly-L-lysine·HCl (M. Wt. 16,500)	0.22	103	0.0087	5
Polyadenylate, 0.15 μmoles	0.013	0	0	0

The reaction mixture contained Tris buffer, 1 M, pH 8.1, 0.025 ml; $MgCl_2$, 0.04 M, 0.02 ml; Versene, 0.01 M, 0.012 ml; 8-C^{14} ADP, 40,000 cpm per μmole, 0.83 μmole; gel eluate, 7.5 μg; electrophoretically purified enzyme, < 10 μg/ml protein, 0.06 ml. Water was added to 0.25 ml. Incubation was carried out at 30°C for 20 min. The acid insoluble precipitate was isolated, washed, dissolved in KOH and counted, essentially as described by Littauer and Kornberg (1957). Unless otherwise indicated, 60 μg of each activator was used. The values shown for the polybasic compounds represent the maximum stimulation obtainable.

spermidine are inactive. Protamine and histone are less effective than the basic polyamino acids. Polyglutamate, polyglycine (not shown) and L-lysine are inactive. High concentrations of KCl are stimulatory and do not prevent the full activation of the enzyme by polylysine. (In the presence of 0.2 M KCl, the K_s for ADP is 1.3×10^{-3} M.) A similar effect of high ionic strength has been reported for the polynucleotide phosphorylase of *M. lysodeikticus* (Beers, 1958).

As shown in the last two columns of the table, on further purification of the enzyme, there is a change in the relative effectiveness of the activators. The activity of the basic polyamino acids is about the same as for the gel fraction, however protamine and histone are inactive and the effectiveness of polyvinylamine decreases by 70%. On a weight basis, polyvinylamine·HBr is one-twentieth as active as the polylysine·HCl (Br^- is not toxic). Potassium chloride does not function as an activator and, in contrast to its effect on the gel eluate, it actually prevents the activation of the enzyme by polylysine. This suggests an ionic interaction between polylysine and enzyme. The inactivity of protamine

is interesting for two reasons: 1) it indicates that high charge density is not the sole requirement for activation, 2) it suggests that binding of polyadenylate by a polybase is not the mechanism of the activation.

At present, the change in properties of the enzyme on purification may best be explained on the assumption that the native polynucleotide phosphorylase is maintained in an active configuration by a polybase. Purification causes progressive removal of the base, with concomitant changes in configuration of the enzyme. Thus, the ability of various compounds to reactivate the enzyme would depend upon the extent of purification. The inactivity of protamine, which has internal polyarginine sequences of 4-5 residues (Felix, 1960), suggests that an uninterrupted chain length of greater than 4-5 basic residues is necessary for activation of the electrophoretically purified enzyme.

With regard to the effect of basic polypeptides on the apparent Michaelis constant, a similar effect has been described for polysaccharide phosphorylase (Krebs, 1954). Phosphorylase b (prepared with PR enzyme, but not trypsin), is stimulated by salmine, the effect being accompanied by a decrease in the K_s for AMP of about 10-fold. This resembles the phenomenon described above, for the gel eluate fraction, with the difference that the polynucleotide phosphorylase of C. perfringens can be obtained in a form which shows complete dependence upon a polybasic activator. It remains to be seen whether the type of activation described here will be found for other polynucleotide-synthesizing systems which lose large amounts of activity on purification (cf., Steiner and Beers, 1961).

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