THE EFFECT OF TEMPERATURE ON THE POLYNUCLEOTIDE PHOSPHORYLASE OF MICROCOCCUS LYSODEIKTICUS

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Recently we have been interested in the effect of elevated temperature and urea on the polynucleotide phosphorylase of Micrococcus lysodeikticus. These studies were stimulated by our continuing effort to understand the peculiar properties of GDP when it is a substrate for the polymerization reaction (1,2). Two types of experiments will be reported: [1/] 1) the effect of elevated temperature on the enzyme itself, and 2) the effect of elevated temperature on the rate of the various reactions catalyzed by the enzyme. The experiments of type I indicate that the enzyme is relatively unstable above 40°. This instability is remarkably enhanced by as little as 0.1 mM GDP, and to a lesser extent by the other common nucleoside diphosphates. (It should be stressed that under the conditions used, no reaction occurs because the enzyme requires an oligonucleotide primer for the polymerization of nucleoside diphosphates (3).) The enzyme is stabilized by the presence of polyribonucleotides. Experiments of the second type lead to a different result. In this case, the presence of GDP during the polymerization reaction (made possible by the addition of primer) appears to stabilize the enzyme. Some of these results have been briefly reported (2).

[1/] Lucas and Grunberg-Manago have carried out similar experiments with polynucleotide phosphorylase purified from Escherichia coli and their results are reported in the accompanying paper.

All experiments were carried out with enzyme preparations purified as previously reported (3). The nucleoside diphosphates were purchased from Schwarz BioResearch, Inc.

Enzyme was incubated for 10 minutes at pH 8.2 and various temperatures, after which samples were removed for assay using the phosphorolysis of poly U at 37° (see assay A, reference 4). The activity was quite stable up to 36° (Table I), but 76% was destroyed at 44°. Other experiments indicated that after 10 minutes at 50°, essentially all activity is lost. The presence of 0.1 mM GDP during the first incubation lowers the temperature at which enzyme is unstable; thus, even at 23°, 60% of the activity is lost in 10 minutes (Table I). The final concentration of GDP in the assay mixture (0.01 mM) has no effect on the rate of phosphorolysis.

ADP, UDP, and CDP also potentiate the heat lability of M. lysodeikticus polynucleotide phosphorylase, but only at higher concentrations and higher temperatures than required for GDP. Thus, as indicated in Table II, 4 mM ADP, UDP, or CDP does not inactivate enzyme at 37°, while 0.1 mM GDP caused the loss of 94% of the activity at 36° (Table I). It should be mentioned further, that the extent of inactivation at 23°, or 37°, is dependent on the GDP concentration. Table II also shows that polyribonucleotides tend to stabilize the enzyme.

The effect of elevated temperature on the polymerization of nucleoside diphosphates to polyribonucleotides is shown in Figure 1. In these experiments the reaction itself was carried out at the indicated temperatures. The polymerization of UDP (at 10 mM) has an optimum at about 36°, while the polymerization of a mixture of UDP (10 mM) and GDP (4 mM), does not drop until above 45°. Thus, at 50°, the customary inhibition of the rate of UDP polymerization by GDP (1) disappears and in fact, the addition of GDP stimulates the reaction.

Table I. Heat lability of polynucleotide phosphorylase: effect of GDP

Incubation Temperature	% of initial activity remaining after 10 minutes incubation	
	-GDP	+GDP (0.1 mM)
23°	153	40
30°	80	22
36°	108	6
44°	24	8

Reaction mixtures for the initial incubations contained 0.01 M Tris, pH 8.2, 0.5 mM $MgCl_2$, GDP as indicated, and 0.25 mg enzyme per ml. Aliquots (0.01 ml) were removed at zero time and after 10 minutes of incubation and delivered into 0.09 ml of assay reaction mixture. The enzyme assays measured the phosphorolysis of poly U and were carried out as previously described (Assay A, reference 4).

Table II. Effect of nucleoside diphosphates and polyribonucleotides on the heat lability of polynucleotide phosphorylase

	Activity remaining after incubation with addition expressed as % of activity remaining after incubation with no addition		
	0 time	30 minutes	
Incubation temp.		37°	44°
Additions			
ADP, 4 mM	90	80	0
UDP, 4 mM	90	90	70
CDP, 4 mM	90	90	0
Poly U	110	90	320
Poly UG (U:G,0.5:1)	90	90	400
Poly A	80	70	300

Incubation of enzyme was as described for Table I except for additions. Polymers were 0.5 mg/ml of reaction. Aliquots (0.01 ml) were removed at zero time and after 30 minutes and assayed (see legend to Table I). The zero time values indicate that none of the additions had any significant effect on the assay itself. In various experiments enzyme without additions was stable for 30 minutes at 37° and lost about 85% of its activity in 30 minutes at 44°.

Other experiments have shown that both the inhibition by GDP at 37° (1), and the apparent stimulation at 50°, are proportional to the GDP concentration.[2/]

The polymerization of ADP (Fig. 1) is not as sensitive to elevated temperatures as that of UDP. The extent of the increased stability is partly the result of the high concentration of ADP (40 mM) used in this

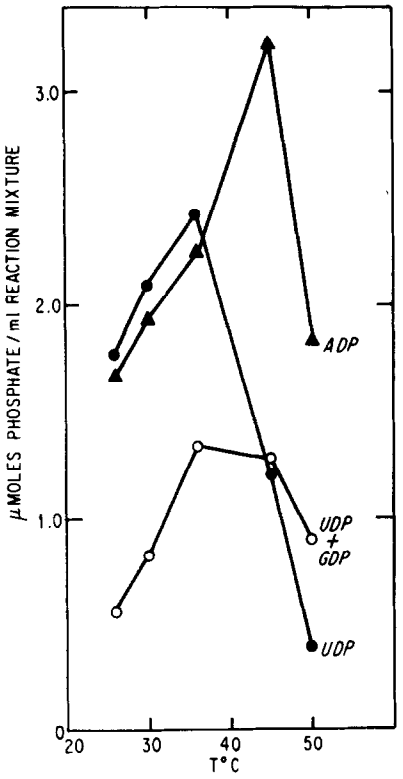

Fig. 1. The effect of temperature on polymerization. Reaction mixtures contained 0.15 M Tris, pH 9.0; 0.4 mM EDTA; 0.5 mM pApApA [this enzyme requires an oligonucleotide primer for polymerization (3)]; nucleoside diphosphate as follows, ADP, 40 mM, UDP, 10 mM, and GDP, 4 mM. $MgCl_2$ concentration was adjusted to give a ratio of 4 nucleoside diphosphate to 1 Mg^{++}. Enzyme concentration was 8.5 μgm of protein per ml of reaction. The reaction was measured by the release of inorganic P after 15 and 30 minutes at 37°(5). The data given are for the 30 minute samples.
● UDP; o UDP and GDP; ▲, ADP.

2/ F. N. Brenneman and M. F. Singer, unpublished

experiment. Thus, even with UDP, polymerization is less sensitive at elevated substrate concentrations. At comparable concentrations, however, ADP appears to be more protective than UDP. Thus, at 20 mM nucleoside diphosphate the ratio of polymerization at 50° to that at 37° is 0.43, 0.68, and 0.90, for UDP, ADP, and CDP, respectively (2). Also, as with UDP, the customary inhibition of ADP polymerization by GDP at 37° (1) disappears at 50° and an apparent stimulation is observed (2).

Figure 2 shows the influence of temperature on the phosphorolysis of poly U. By using this substrate, effects of the secondary structure of the polymer substrate on the rate of phosphorolysis (6,7) are minimized, since poly U has no secondary structure at these temperatures (8). It can be seen that phosphorolysis has an optimum at about 46°, and that GDP offers no protection to the enzyme at any temperature.

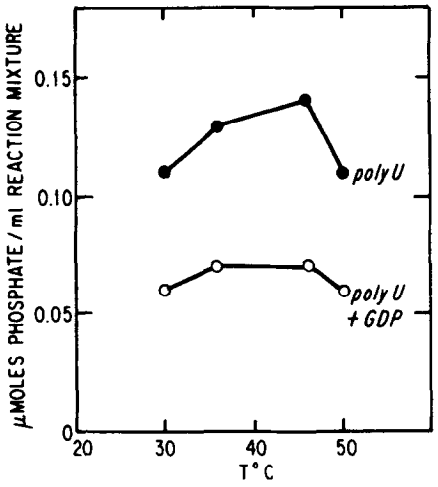

Fig. 2. The effect of temperature on phosphorolysis. Reaction mixtures contained 0.1 M Tris, pH 8.2; 0.01 M $K_2HP^{32}O_4$; 5 mM $MgCl_2$; 1 mM EDTA, and 1 mM poly U. Enzyme concentration is 17.5 µgm per ml. Mixtures were incubated for 15 minutes at 37°. The incorporation of P^{32} into charcoal-adsorbable nucleotide is measured (4) and the ordinate gives µmoles P^{32} incorporated per ml of reaction mixture.
● poly U; O, poly U plus 1 mM GDP.

The results given in Figure 1 and Figure 2 are similar to results obtained by carrying out the reactions in urea. The rate of polymerization of UDP is inversely proportional to the concentration of urea (0 to 1.5 M) while, in the presence of UDP and GDP, the reaction is unaffected by the addition of urea. On the other hand, the phosphorolysis of poly U is sensitive to these concentrations of urea both in the presence and in the absence of GDP.

Thus it appears that while GDP can potentiate the heat lability of M. lysodeikticus polynucleotide phosphorylase, it stabilizes the enzyme when polymerization conditions exist. This finding may reflect the stabilizing properties of certain polymers as they are being formed on the enzyme surface (see Table II). On the other hand, more complicated properties of the polynucleotide phosphorylase protein itself may be involved. The potentiation of the heat lability of the enzyme by its nucleoside diphosphate substrates is of special interest since most enzymes are stabilized by substrate.

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