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KINETIC STUDIES OF INHIBITION OF ADENINE PHOSPHORIBOSYLTRANSFERASE BY GUANYLATE

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SUMMARY

Guanylate inhibits adenine phosphoribosyltransferase (AMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.7) and its binding to the enzyme is related to guanylate concentration in a hyperbolic manner. Double reciprocal plots of initial velocity are non-linear in the presence of guanylate, but linear in its absence. Enzyme activity is stimulated in the presence of very low concentrations of guanylate.

INTRODUCTION

Inhibition of adenine phosphoribosyltransferase (AMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.7) by purine ribo- and dexoyribonucleotides has been demonstrated by several groups[1–9]. Adenylate, 2′-deoxyadenylate and guanylate have three quite distinct types of effects on the initial velocity of this reaction[10], and 2′-deoxyadenylate and adenine 6′-deoxyallofuranoside have been shown to bind to two enzyme forms, probably at "allosteric" sites. Adenylate, adenosine diphosphate and adenosine triphosphate also bind to at least two enzyme forms, partly at the adenylate product binding site, and partly to other sites[8]. This paper concerns studies of the mechanism of inhibition of a distinct type of nucleotide inhibitor, as represented by guanylate.

METHODS

The initial velocity of the adenine phosphoribosyltransferase reaction was determined by the method of HORI AND HENDERSON[7], in which the synthesis of [^{14}C]AMP from Mg-*PP*-ribose-*P* and [^{14}C]adenine was measured. Unless specified otherwise, the Mg^{2+} concentration was 1 mM, at which 83 $\pm$ 0.5% of the *PP*-ribose-*P* present was in the form of the monomagnesium complex[11]. The enzyme preparation used was that partially purified from Ehrlich ascites tumor cells[12]; 1.0 μg of protein per ml was used.

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RESULTS

One group of purine nucleotides which inhibits adenine phosphoribosyltransferase activity is represented by guanylate and related 6-oxopurine nucleotides[10]. The data in Table I show that although several nucleotides of guanine, hypoxanthine and xanthine inhibit this enzyme reaction, guanylate was the most potent compound tested. These results are qualitatively similar to those reported by other investigators[1–9], although nucleotides appear to have quantitatively different effects on the adenine phosphoribosyltransferases from different sources.

TABLE I

INHIBITION OF ADENINE PHOSPHORIBOSYLTRANSFERASE BY PURINE NUCLEOTIDES

Enzyme activity was measured in 0.15 M Tris buffer, pH 7.4, containing 10^{-3} M Mg^{2+}, with 10^{-6} M [^{14}C]adenine, 10^{-5} M *PP*-ribose-*P*, and varying concentrations of purine nucleotides. When nucleoside di- and triphosphates were tested, an equimolar amount of Mg^{2+} was added in addition to that usually present. These results are averages of at least four determinations.

Addition	*Concentration (mM)*	*Inhibition (%)*
GMP	0.1	27
GDP	0.1	4
	2.0	62
GTP	0.1	6
	2.0	62
IMP	0.1	0
	2.0	66
IDP	0.1	0
	2.0	31
ITP	0.1	0
	2.0	60
XMP	0.1	1
	2.0	43

Kinetics of inhibition

Information concerning the mechanism of inhibition of adenine phosphoribosyltransferase by guanylate was obtained by determination of the relationship between initial velocity and the concentration of the three ligands involved, *PP*-ribose-*P*, adenine and guanylate. It should be pointed out first that double reciprocal plots of initial velocity of the adenine phosphoribosyltransferase reaction were linear over wide ranges of substrate concentrations: from $6 \cdot 10^{-7}$ to 10^{-3} M for *PP*-ribose-*P*, and from 10^{-7} to 10^{-4} M for adenine. At lower concentrations of *PP*-ribose-*P* such plots were non-linear when it was the variable substrate. This deviation can be accounted for by the near equivalence of substrate and enzyme concentrations under these conditions, where the assumptions supporting the Michaelis–Menten formulation are not valid.

Fig. 1 shows the effects of guanylate on initial rates as a function of *PP*-ribose-*P* concentrations. Double reciprocal plots were linear at low *PP*-ribose-*P*-concentrations, but curved and came to approximately a common intercept on the ordinate with the control line at infinite *PP*-ribose-*P* concentrations; such non-linear

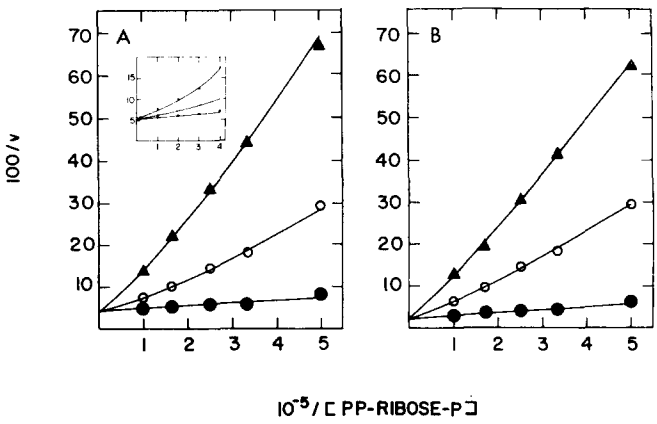

Fig. 1. Inhibition by guanylate. Double reciprocal plots of initial velocity against *PP*-ribose-*P* concentrations at adenine concentration of (A) $8 \cdot 10^{-7}$ M, and (B) $8 \cdot 10^{-6}$ M. The concentrations of guanylate were o (●), $2 \cdot 10^{-4}$ M (○), and $5 \cdot 10^{-4}$ M (▲). *v* is expressed as pmoles of AMP per min. These results are representative of those obtained in six experiments. Insert, units on abscissa $10^{-6}/[PP\text{-ribose-}P]$.

plots were always observed in the presence of guanylate. The same types of curves were obtained at low and high concentrations of the second substrate, adenine.

Fig. 2 shows the effect of guanylate on the initial velocity of the adenine phosphoribosyltransferase reaction when the concentration of adenine was varied but low. Double reciprocal plots were linear at both low and high *PP*-ribose-*P* concentrations, although the magnitude of the inhibition of guanylate was less in the latter case. In these plots, guanylate effectively caused a decrease in both the apparent maximum velocity and in the apparent Michaelis constant for adenine; as a result the extent of inhibition by guanylate increased slightly with increasing adenine concentration. Similar plots have been observed under certain conditions with adenylate as inhibitor[8].

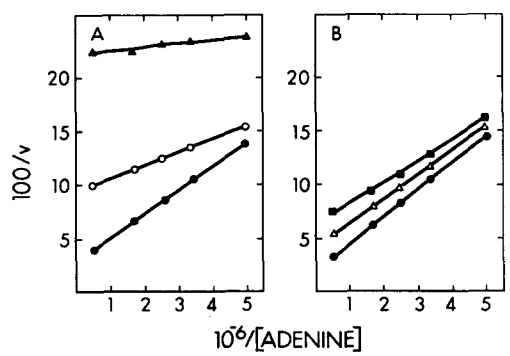

Fig. 2. Inhibition by guanylate at low adenine concentration. Double reciprocal plots of initial velocity against adenine concentration at *PP*-ribose-*P* concentration of (A) $6 \cdot 10^{-6}$ M, and (B) $6 \cdot 10^{-5}$ M. The concentrations of guanylate were o (▲), $2 \cdot 10^{-4}$ M (○), $5 \cdot 10^{-4}$ (▲), 10^{-3} M (△), and $2 \cdot 10^{-3}$ M (■). *v* is expressed as pmoles of AMP per min.

When similar experiments were done at higher concentrations of adenine (Fig. 3), double reciprocal plots were non-linear in the presence of guanylate. Inhibition by guanylate appeared to be non-competitive with respect to adenine, as in no case did

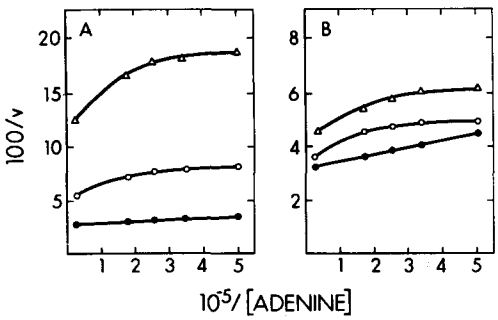

Fig. 3. Inhibition by guanylate at high adenine concentration. Double reciprocal plots of initial velocity against adenine concentration at *PP*-ribose-*P* concentrations of (A) $6 \cdot 10^{-6}$ M, and (B) $6 \cdot 10^{-5}$ M. The concentrations of guanylate were o (●), $2 \cdot 10^{-4}$ M (○), and $5 \cdot 10^{-4}$ M (▲). *v* is expressed as pmoles of AMP per min. These results are representative of those obtained in six experiments.

the curves for the inhibited reaction approach intersection on the ordinate with the control lines.

Fig. 4 shows the relationship between guanylate concentration and inhibition of the adenine phosphoribosyltransferase reaction, measured at substrate concentrations which were equal to their respective Michaelis constants. The same type of curve was also obtained when the *PP*-ribose-*P* concentration was 10-fold higher, although the effect of guanylate on velocity was correspondingly less. At very low concentrations of guanylate, stimulation of the reaction rate was observed. Although the data for inhibition in this figure follow a hyperbolic type of curve, it is not a rectangular hyperbola. Thus when the initial velocity data on which Fig. 4 was based were plotted in double reciprocal form (Fig. 5), the resultant curve was also a hyperbola.

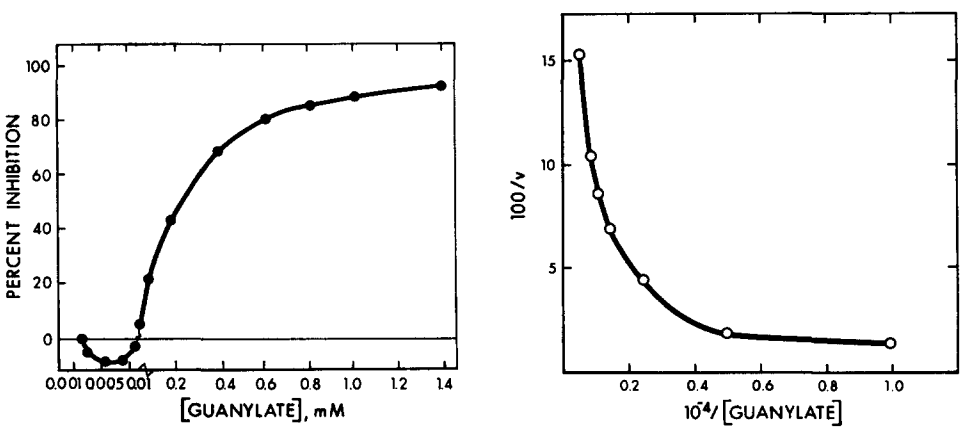

Fig. 4. Relationship between concentration of guanylate and enzyme inhibition. Enzyme activity was assayed with $6 \cdot 10^{-6}$ M *PP*-ribose-*P*, $8 \cdot 10^{-7}$ M [^{14}C]adenine and varying concentrations of guanylate. These results are representative of those obtained in eight experiments.

Fig. 5. Double reciprocal plot of initial velocity against guanylate concentration. Replot of the data on which Fig. 5 was based. *v* is expressed in pmoles of AMP per min.

Binding of guanylate

Guanylate very effectively protected adenine phosphoribosyltransferase from inactivation by trinitrobenzene sulfonate, and this property was used to measure the binding of guanylate to the enzyme. Fig. 6 shows that a hyperbolic relationship existed between the concentration of guanylate and its effect on the inactivation produced by trinitrobenzene sulfonate.

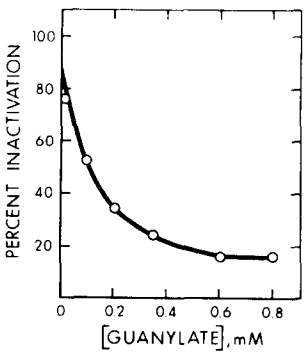

Fig. 6. Effect of guanylate on the rate of inactivation of adenine phosphoribosyltransferase by trinitrobenzenesulfonate. Enzyme (1 g) was incubated 5 min at 30° in 0.1 M Tris buffer, pH 7.4, containing 10^{-3} M Mg^{2+}, in the presence and absence of varying concentrations of guanylate. These solutions were then incubated 10 min at 30° with $4.6 \cdot 10^{-3}$ M trinitrobenzenesulfonate. Enzyme activity remaining after this treatment was assayed in the presence of $2 \cdot 10^{-3}$ M *PP*-ribose-*P* and $2 \cdot 10^{-5}$ M [^{14}C]adenine. The latter concentration of *PP*-ribose-*P* completely prevented any further inactivation by trinitrobenzenesulfonate. These results are representative of those obtained in two experiments.

Attempts were then made to determine the relationship of the site to which guanylate was bound to those which *PP*-ribose-*P* and adenylate normally occupy. Under conditions where *PP*-ribose-*P* and adenylate reduced the inactivation of adenine phosphoribosyltransferase by *p*-hydroxymercuribenzoate by 92% and 83%, respectively, $5 \cdot 10^{-4}$ M guanylate reduced this by only 14%. In other experiments, in which *PP*-ribose-*P* and AMP reduced the heat inactivation of this enzyme by 60% and 63%, respectively, $5 \cdot 10^{-4}$ M guanylate increased the inactivation by 13%. Finally, under conditions where *PP*-ribose-*P* reduced enzyme inactivation by 31% (ref. 13), $5 \cdot 10^{-4}$ M guanylate reduced this by 80% (Fig. 6).

Attempts were made to desensitize adenine phosphoribosyltransferase to inhibition by guanylate without at the same time destroying its catalytic activity. Enzyme preparations were treated with several concentrations each of Hg^{2+}, *p*-chloromercuribenzoate, urea and ethylene glycol, and by heating both in the presence and absence of ammonium sulfate. In no case could a clear distinction be made between the effects of these treatments on inhibition by adenylate, a product, and that by guanylate. Guanylate was as effective an inhibitor when tested at pH 6.0 as at pH 7.4 (as was adenylate), and it had the same degree of effectiveness at each stage of enzyme purification[12]. The extent of inhibition by guanylate did, however, increase with the length of time a lyophilized enzyme preparation had been in solution at 4° in 0.15 M Tris buffer, pH 7.4, containing 0.34 M $(NH_4)_2SO_4$, whereas the extent of inhibition by adenylate remained constant.

DISCUSSION

The kinetics of inhibition of adenine phosphoribosyltransferase by guanylate are unusual in several respects. The observed transition from double reciprocal plots which were linear in the absence of the inhibitor, to plots which were nonlinear in its presence, has been observed for only a few enzymes, and differentiates these enzymes from other cases of "allosteric" phenomena[14–18]. The non-linear plots observed when *PP*-ribose-*P* was the variable substrate also were not produced by other adenine nucleotide and nucleoside inhibitors of this enzyme. Finally, although both guanylate and adenine nucleotides produced non-intersecting plots when adenine was the variable substrate, these were linear when the latter class of inhibitor was used, but nonlinear in the former case.

The only data known to us which resemble the present results closely are those obtained by MELO AND GLASER[19] in their study of the inhibition of thymidine diphosphate D-glucose pyrophosphorylase by TDP-L-rhamnose. TDP-glucose, pyrophosphate, glucose 1-phosphate and TTP appear to be first and second substrates, and first and second products, respectively, in this reaction. The inhibitor, TDP-rhamnose, induces changes in otherwise linear double reciprocal plots which very closely resemble those in our Fig. 1–3. FRIEDEN[18] has interpreted the data of Melo and Glaser which are similar to our Fig. 1 in terms of a model of MONOD *et al.*[14] which has four binding sites for each ligand. However, there is as yet no evidence for multiple substrate or inhibitor binding sites on adenine phosphoribosyltransferase.

Although there is ample suggestive evidence that guanylate binds to a site (or sites) different from those to which *PP*-ribose-*P* and adenylate bind, this point cannot be considered proven. Thus it remains possible that the binding of guanylate to one of these substrate or product binding sites produces different conformational changes than do the binding of *PP*-ribose-*P* or adenylate at the same sites. Direct binding studies are required to settle this point.

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REFERENCES

1 R. D. BERLIN AND E. R. STADTMAN, *J .Biol. Chem.*, 241 (1966) 2679.
2 A. W. MURRAY, *Biochem. J.*, 100 (1966) 671.
3 E. R. STADTMAN, *Adv. Enzymol.*, 28 (1966) 41.
4 A. W. MURRAY, *Biochem. J.*, 103 (1967) 271.
5 A. W. MURRAY AND P. C. L. WONG, *Biochem. J.*, 104 (1967) 669.
6 A. W. MURRAY, *Biochem. J.*, 104 (1967) 675.
7 M. HORI AND J. F. HENDERSON, *J. Biol. Chem.*, 241 (1966) 3404.
8 J. F. HENDERSON, R. E. A. GADD, H. M. PALSER AND M. HORI, *Can. J. Biochem.*, 48 (1970) 573.
9 D. G. R. BLAIR, S. J. PEESKER AND M. DOMMASCH, *Gann*, 61 (1970) 495.
10 M. HORI, R. E. A. GADD AND J. F. HENDERSON, *Biochem. Biophys. Res. Commun.*, 28 (1967) 616.
11 R. E. A. GADD AND J. F. HENDERSON, *Can. J. Biochem.*, 48 (1970) 302.
12 M. HORI AND J. F. HENDERSON, *J. Biol. Chem.*, 241 (1966) 1406.
13 R. E. A. GADD AND J. F. HENDERSON, *J. Biol. Chem.*, 245 (1970) 2979.

14 J. MONOD, J. WYMAN AND J.-P. CHANGEUX, *J. Mol. Biol.*, 12 (1965) 88.
15 P. MAEBA AND B. D. SANWAL, *Biochemistry*, 5 (1966) 525.
16 C. FRIEDEN, *J. Biol. Chem.*, 239 (1964) 3522.
17 J. F. HENDERSON, *Can. J. Biochem.*, 46 (1968) 1381.
18 C. FRIEDEN, *J. Biol. Chem.*, 242 (1967) 4045.
19 A. MELO AND L. GLASER, *J. Biol. Chem.*, 240 (1965) 398.

Biochim. Biophys. Acta, 268 (1972) 70–76