ACTIVATION BY 5-PHOSPHORIBOSYL 1-PYROPHOSPHATE OF GLUTAMINE-DEPENDENT CARBAMYL PHOSPHATE SYNTHETASE FROM MOUSE SPLEEN

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SUMMARY

Glutamine-dependent carbamyl phosphate synthetase from hematopoietic mouse spleen, the first enzyme of the pyrimidine biosynthesis, is subject to a remarkable activation by PP-ribose-P. The effect is specific; ribose 5-phosphate, inorganic pyrophosphate, or a mixture of both can not substitute for it. The activation is reversible and there is no measurable consumption of PP-ribose-P during the reaction. PP-ribose-P stimulates the reaction by increasing the affinity of the enzyme for $MgATP^{2-}$, a substrate, without changing the maximal velocity. These results support an allosteric mechanism for this effect. A low value of the activation constant for PP-ribose-P (less than 10 μM) suggests a physiological importance of this activation in regulation of general nucleotide metabolism.

A specific property of the mammalian glutamine-dependent carbamyl phosphate synthetase is the sensitivity to feedback inhibition by UTP (1, 2), and this provided a support to the proposed key role of the enzyme in control of pyrimidine biosynthesis in mammalian tissues (1-3). Kinetic studies by us (to be published) and by Levine *et al* (4) support that UTP serves as a negative allosteric effector for this enzyme. Recently we found that a low concentration of PP-ribose-P* activates the synthetase from mouse spleen to a remarkable extent. Evidence supports that PP-ribose-P decreases the apparent *Km* of the enzyme for $MgATP^{2-}$ as a positive allosteric effector, whereas UTP increases the *Km* as a negative effector. In view of the key functions of the compound in nucleotide metabolism, the activation might be of considerable physiological

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* PP-ribose-P: 5-phosphoribosyl 1-pyrophosphate.

significance. The present paper describes the stimulatory effect of PP-ribose-P and possible physiological significance of this effect.

MATERIALS AND METHODS

Mg PP-ribose-P was obtained from Sigma and used without further purification. The purity, as determined by a modification of the enzymatic method of Henderson and Khoo (5), was found to be 67% on the basis of $C_5H_9O_{14}P_3Mg_2 \cdot 2H_2O$. The glutamine-dependent carbamyl phosphate synthetase preparation was that partially purified essentially as described previously (3). The activity was assayed by a modification of the method described (3). The reaction mixture contained 10% (w/v) glycerol, instead of 7.5% dimethyl sulfoxide plus 2.5% glycerol (3), and 1 mM dithiothreitol as enzyme stabilizers. Reaction was started by adding enzyme and incubation was for 10 min at 37°. Concentrations of $MgCl_2$ and ATP in control tubes were 8.0 and 3.0 mM, respectively, so that concentrations of free Mg^{2+} and magnesium-ATP complex ($MgATP^{2-}$) are approximately 5.0 and 3.0 mM, respectively. When phosphate compounds other than ATP were included in the mixture, an additional equimolar amount of $MgCl_2$ was supplemented. The enzyme shows a nearly absolute requirement for free Mg^{2+} as an activator (Tatibana and Shigesada, to be published).

RESULTS

Activation by PP-ribose-P —— PP-ribose-P can stimulate the enzyme activity to a remarkable extent (Table I). In the presence of 3.0 mM $MgATP^{2-}$, 2.0 mM PP-ribose-P stimulated the activity 2.6-fold. Ribose 5-phosphate, inorganic pyrophosphate, or a mixture of both could not substitute for it, as shown in the Table. Nor was observed such stimulation with a variety of nucleotides and related compounds other than PP-ribose-P, in confirmation of the previous results (1). PP-ribose-P itself did not substitute for ATP as a phosphate donor for the carbamyl phosphate synthetase reaction. Time course of the reaction in the presence of PP-ribose-P was linear.

Table I. Stimulation of mouse spleen carbamyl phosphate synthetase activity by PP-ribose-P

Addition	Enzyme Activity	
	counts/min	% of control
None	1350	100
2 mM PP-ribose-P	3520	260
2 mM ribose-5-P	1380	102
2 mM PPi	1095	81
1 mM ribose-5-P + 1 mM PPi	1175	87

The activity was assayed with ^{14}C-bicarbonate as a substrate following the formation of ^{14}C-L-citrulline in the presence of L-ornithine and ornithine transcarbamylase as described under "MATERIALS AND METHODS" except for the additions indicated. Enzyme, 0.16 unit, 11 μg of protein. Specific radioactivity of $KH^{14}CO_3$, 2000 cpm per nmole.

Stimulation of the synthetase activity by increasing concentrations of PP-ribose-P is shown in Fig. 1. The concentration of PP-ribose-P required to give a half maximal activation was found to be in the range of 4 to 9 μM under the conditions. At PP-ribose-P concentrations of 4 to 8 μM, there are pronounced deviations of data points from a hyperbolic kinetics. A similar curve was obtained in another set of experiments. The reason for the unusual kinetics is not clear at the present.

The velocity of the synthetase reaction plotted against varying concentrations of $MgATP^{2-}$ in the presence of 50 μM PP-ribose-P gave a rectangular hyperbola (Curve A, Fig. 2). In the absence of effectors, a slight sigmoidicity was observed at low concentrations of $MgATP^{2-}$ (Curve B). The apparent Km for $MgATP^{2-}$ (0.72 mM) in the presence of PP-ribose-P was much smaller than the constant in its absence (5.2 mM), whereas the maximal velocities were the same. The effect of PP-ribose-P, which lowers the Km for $MgATP^{2-}$, makes a clear contrast to the effect of $MgUTP^{2-}$ (2.0 mM) as shown by Curve C; the apparent Km for $MgATP^{2-}$ is much greater and there is a marked sigmoidicity of the curve (6). The observations on UTP are consistent with the recent report by Levine et al (4).

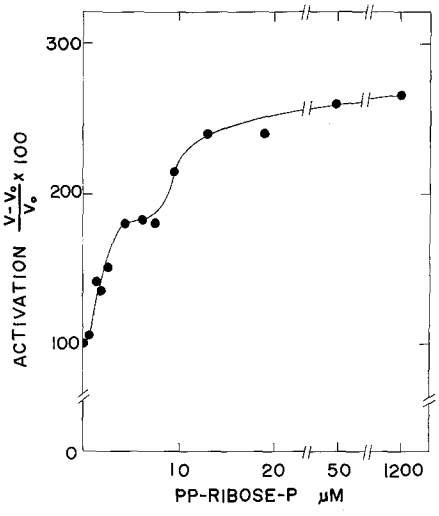

Fig. 1. Activation of the synthetase reaction by varying concentrations of PP-ribose-P, with $MgATP^{2-}$ and free Mg^{2+} fixed at 3.0 and 5.0 mM, respectively. The PP-ribose-P concentration was varied as indicated. Except for those variations, conditions and enzyme were as in Table I.

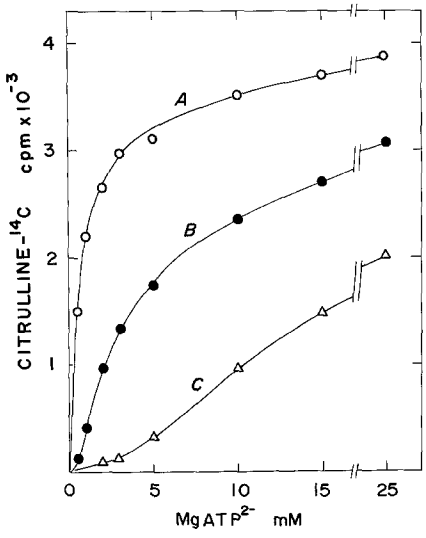

Fig. 2. Effect of varying $MgATP^{2-}$ concentrations on the reaction velocity, with free Mg^{2+} fixed at 5.0 mM; activation by PP-ribose-P and inhibition by $MgUTP^{2-}$. Curve A (O—O), in the presence of 50 μM PP-ribose-P; Curve B (●—●), no effectors present; Curve C (△—△), in the presence of 2.0 mM $MgUTP^{2-}$. Except for those variations, conditions and enzyme were as in Table I.

The stimulation is apparently reversible; when the reaction mixture was diluted so as to lower only the PP-ribose-P concentration and not to change

other conditions, the stimulation was promptly reversed. When the content of PP-ribose-P was assayed before and after the reaction, there was no measurable consumption of PP-ribose-P associated with the production of carbamyl phosphate. Although the experiments did not give a definite answer as to whether the enzyme protein can be modified by a reaction involving PP-ribose-P such as "phosphoribosylation" (7), such a possibility may not be compatible with the observations described above. The results are consistent with an allosteric mechanism for the effect of PP-ribose-P.

Antagonistic Effect of PP-ribose-P and of $MgUTP^{2-}$ —— The effect of varying concentrations of PP-ribose-P, a positive effector, was examined in the presence of varied levels of $MgUTP^{2-}$, a negative effector (Fig. 3). A high concentration of PP-ribose-P (0.60 mM) could almost completely overcome the inhibitory effect of $MgUTP^{2-}$. The antagonistic effect of PP-ribose-P was remarkable also at lower concentrations; the apparent activation by a low concentration of PP-ribose-P was more pronounced in the presence of $MgUTP^{2-}$ than in its absence. The effects of PP-ribose-P and of $MgUTP^{2-}$ are qualitatively competitive. However, in view of the structural difference of the two com-

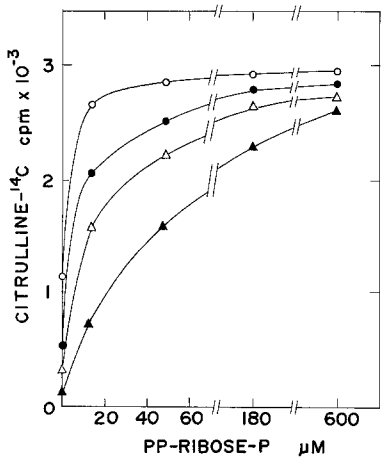

Fig. 3. Antagonistic effect of PP-ribose-P and of $MgUTP^{2-}$. The concentrations of $MgATP^{2-}$ and free Mg^{2+} were fixed at 3.0 and 5.0 mM, respectively. The PP-ribose-P concentration was varied as indicated. O—O, no $MgUTP^{2-}$ present; ●—●, in the presence of 0.5 mM $MgUTP^{2-}$; △—△, in the presence of 1.0 mM $MgUTP^{2-}$; ▲—▲, in the presence of 2.0 mM $MgUTP^{2-}$. Except for those variations, conditions and enzyme were as in Table I.

pounds, simple competition for the same site may not be possible. An allosteric interaction between the two effectors is likely. The problem remains to be elucidated.

DISCUSSION

The observations described in this communication show that a low concentration of PP-ribose-P can markedly stimulate the activity of glutamine-dependent carbamyl phosphate synthetase from mouse spleen, possibly through an allosteric effect. Although the PP-ribose-P content in the spleen is not known, the levels in Ehrlich ascites tumor cells in vivo were 0.28 to 0.66 μmoles per g wet weight (5), and those in cultured human fibroblasts were 0.85 to 3.6 nmoles (8). The data indicate that the concentration may vary over a wide range possibly with cell types as well as with other miscellaneous conditions (e.g., glucose can enhance the level in Ehrlich cells (5)). In view of these data and the results reported in this communication, PP-ribose-P could be considered a physiological regulator of pyrimidine biosynthesis.

In association with the known important functions of PP-ribose-P, the newly revealed role of the compound would be of prime importance in regulation of general nucleotide metabolism. First, PP-ribose-P can control the two regulatory sites of pyrimidine biosynthesis, the production of carbamyl phosphate as an effector for the synthetase, and the conversion of orotate to orotidine 5'-monophosphate as a substrate. The latter reaction, coupled with the conversion of orotidine monophosphate to UMP, seems to be the second limiting step of the orotic acid pathway (9-13). The control of the two steps through the level of PP-ribose-P may provide an important means for matching the rates of both the early and later steps of the pathway. Second, PP-ribose-P can correlate the first steps of both purine and pyrimidine synthesis de novo. The first reaction of the purine pathway, the formation of 5-phosphoribosyl 1-amine from PP-ribose-P and glutamine, is considered a key regulatory site of the synthesis (8, 14), and can be limited by supply of PP-ribose-P (8, 15).

Thus PP-ribose-P may function as a regulator for maintaining a balance between the rates of purine and pyrimidine biosynthesis. Third, the initial step of the pyrimidine pathway might be related in some manner to "salvage" reactions of purines as well as to biosynthesis of pyridine nucleotides and other reactions involving PP-ribose-P.

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