GLUTAMINE-DEPENDENT CARBAMOYL PHOSPHATE SYNTHETASE: POLYAMINES INHIBIT THE ACTIVITY AND MODIFY THE ACTIVATING EFFECT OF 5-PHOSPHORIBOSYL 1-PYROPHOSPHATE*

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SUMMARY

Mammalian glutamine-dependent carbamoyl phosphate synthetase (EC 2.7.2.9), the first enzyme of de novo pyrimidine nucleotide biosynthesis, was strongly inhibited by polyamines at concentrations of 10^{-4} to 10^{-3} M. Spermine was the most effective, followed in order by spermidine and putrescine. The inhibition was partially reversed by increasing the concentration of Mg^{2+} or $MgATP^{2-}$, or by adding low concentrations of 5-phosphoribosyl 1-pyrophosphate, an allosteric activator of the enzyme. Polyamines increased the apparent K_a value of the enzyme for phosphoribosyl pyrophosphate. A possible physiological role of polyamines in widening the range of the effective concentrations of phosphoribosyl pyrophosphate as the activator for the enzyme is suggested.

Glutamine-dependent carbamoyl phosphate synthetase (CPSase II) catalyzes the first step of de novo pyrimidine nucleotide biosynthesis in mammals and plays a key role in the regulation of the pathway (1-4). The enzyme is subject to feedback inhibition by UTP and also to allosteric activation by PP-ribose-P (4,5).

It is well known that polyamines are present in considerable amounts in mammalian cells and their amounts increase during periods of rapid tissue growth under a variety of stimuli (6-8). There is evidence that polyamines stimulate the synthesis of ribonucleic acid in vitro (9-11) and in vivo (12). It is therefore of interest to examine the effects of polyamines on the activity of CPSase II, a key regulatory enzyme of the pyrimidine biosynthetic pathway.

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Abbreviations: CPSase II, glutamine-dependent carbamoyl phosphate synthetase; PP-ribose-P, 5-phosphoribosyl 1-pyrophosphate.

This paper reports inhibition by polyamines of CPSase II from mammalian tissues and its reversal by low concentrations of PP-ribose-P. In the presence of polyamines the apparent affinity of the enzyme for PP-ribose-P became lower and the enzyme could respond to changes in PP-ribose-P concentration over a wide range. The physiological significance of these effects is discussed.

MATERIALS AND METHODS

Materials ___ Spermine·4HCl and putrescine·2HCl were obtained from Nakarai Chemicals, Kyoto, Japan. Spermidine·3HCl was obtained from Sigma. MgPP-ribose-P was obtained from Kyowa Hakko Kogyo, Tokyo, Japan, and purified by chromatography on a DEAE-Sephadex column. Its Na salt was prepared by ion exchange on Dowex 50-X8 (Na^+ form). The purity, enzymatically determined as described previously (4), was found to be 90%. Concentrations of ATP and $MgCl_2$ were determined as described previously (13).

Enzyme Assay ___ CPSase II activity was assayed in a system containing 10% (w/v) glycerol essentially as described previously (13). One unit of the enzyme was defined as that amount of activity which produces 1 μmole of product per min under the standard conditions.

Enzyme Purification ___ CPSase II of Yoshida ascites hepatoma cells (AH 13) was purified to an essentially homogeneous state as a complex with aspartate carbamoyltransferase (EC 2.1.3.2) and dihydroorotase (EC 3.5.2.3) as described previously (14). The purified enzyme had a specific activity of 0.5 unit per mg of protein. The enzymes of hematopoietic mouse spleen and rat liver were partially purified as described previously (3,5).

RESULTS

Inhibition of CPSase II Activity by Polyamines ___ CPSase II was shown to require free Mg^{2+} in addition to $MgATP^{2-}$ for its activity (13). When homogeneous CPSase II from AH 13 cells was assayed at suboptimal concentrations of ATP (1.0 mM) and $MgCl_2$ (2.0 mM), polyamines strongly inhibited the activity (Fig. 1). Of the three polyamines tested spermine was the most effective, followed by spermidine and putrescine. Fifty percent inhibition was observed by 0.4 mM spermine, 1.0 mM spermidine, or 3.2 mM putrescine.

Reversal of Polyamine Inhibition by Mg^{2+} and $MgATP^{2-}$ ___ The effect of Mg^{2+} concentration on CPSase II activity in the presence and absence of polyamines is shown in Fig. 2A. In the absence of polyamines the maximal activity with 1 mM ATP was observed at 6 mM $MgCl_2$ or at about 5mM free Mg^{2+}. However, in the presence of polyamines a higher concentration of Mg^{2+} was required for the maximal activity. It is also notable that at higher Mg^{2+} concentrations the

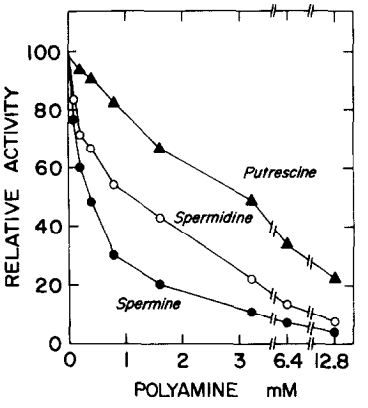

Fig. 1. Inhibition of CPSase II activity by polyamines. The reaction mixture (0.3 ml) contained the following constituents: 50 mM potassium N-2-hydroxyethylpiperazine-N'-ethanesulfonate (pH 7.0); 1.0 mM ATP; 2.0 mM $MgCl_2$; 3.3 mM L-glutamine; 16.7 mM [^{14}C]$KHCO_3$ (2,000 cpm/nmole); 0.5 mM L-ornithine; bovine liver ornithine carbamoyltransferase (EC 2.1.3.3) free from ammonia, 4 units (μmoles/min at 37°); glycerol, 10% (w/v); 1.0 mM dithiothreitol; enzyme, 0.70 munit (1.4 μg of protein). The polyamine concentration was varied as indicated. The activity in the absence of polyamines is set at 100.

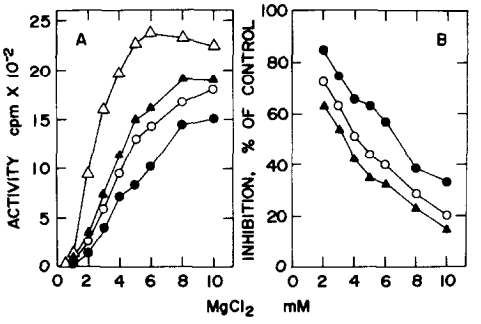

Fig. 2. Effect of polyamines on CPSase II activity at varying Mg^{2+} concentrations. The activity was assayed as described in Fig. 1, except for the addition of varying concentrations of Mg^{2+} as indicated and polyamines as follows: none (△—△); 3 mM spermine (●—●); 3 mM spermidine (○—○); 6 mM putrescine (▲—▲). Enzyme: 0.35 munit, 0.7 μg of protein. In B, the data in A are plotted in terms of inhibition by polyamines on a percent basis.

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extent of inhibition by polyamines became smaller (Fig. 2B). The inhibition was also reversed by increasing the concentration of $MgATP^{2-}$, with the free Mg^{2+} concentration kept essentially constant at 1 mM (Fig. 3).

Effect of PP-ribose-P on Mg^{2+} Kinetics — PP-ribose-P stimulates CPSase II activity by increasing the affinity of the enzyme for a substrate, $MgATP^{2-}$

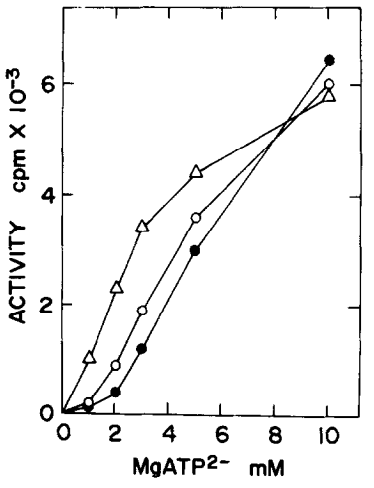
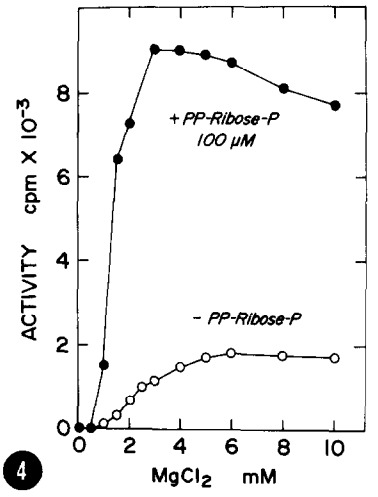


Fig. 3. Effect of polyamines on CPSase II activity at varying $MgATP^{2-}$ concentrations. The activity was assayed as described in Fig. 1, except for the addition of varying concentrations of ATP, $MgCl_2$, and polyamines as follows: none (△—△); 3 mM spermine (●—●); 3 mM spermidine (○—○). $MgCl_2$ was added in 1 mM excess over ATP. Enzyme: 0.35 munit, 0.7 μg of protein.

(4,5). It was newly found that PP-ribose-P also increased the affinity of the enzyme for free Mg^{2+} (Fig. 4). While 6 mM $MgCl_2$ or about 5 mM free Mg^{2+} was required for the maximal activity in the absence of PP-ribose-P (see also Fig. 2), in the presence of 100 μM NaPP-ribose-P the maximal activity was obtained at 3 mM $MgCl_2$ or at about 2 mM free Mg^{2+}.

The extent of activation of the enzyme by PP-ribose-P decreased with increasing Mg^{2+} concentrations; it was 10.8-fold at 2 mM $MgCl_2$ and 4.8-fold at 6 mM $MgCl_2$, respectively, when assayed in the presence of 1 mM ATP (Fig. 4).

Reversal of Polyamine Inhibition by PP-ribose-P —— It was expected on the basis of the above findings that PP-ribose-P can reverse the polyamine inhibition of the enzyme. This was proved by the results shown in Fig. 5. In the presence of 100 μM PP-ribose-P essentially no inhibition occurred at up to 0.8 mM spermine or 1.6 mM spermidine, and the concentrations of spermine and spermidine needed for 50% inhibition were approximately 4 and 11 mM, respectively (data not shown). These concentrations were more than 10 times higher than those needed in the absence of PP-ribose-P.



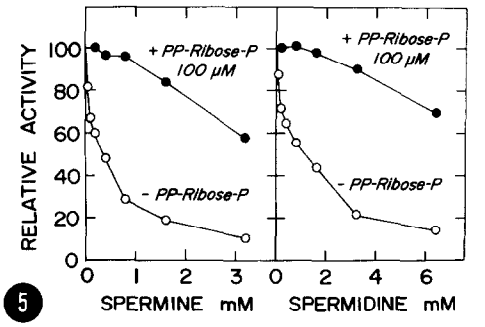


Fig. 4. Effect of varying Mg^{2+} concentrations on CPSase II activity in the presence and absence of PP-ribose-P. The activity was assayed as described in Fig. 1 except for varying Mg^{2+} concentrations in the absence (○—○) and presence (●—●) of NaPP-ribose-P (100 µM). Enzyme: 0.30 munit, 0.6 µg of protein.

Fig. 5. Reversal of polyamine inhibition by PP-ribose-P. The activity was assayed as described in Fig. 1 except for the additions indicated: none (○—○); 100 µM NaPP-ribose-P (●—●). The activities in the absence of polyamines are set at 100: the actual values ([^{14}C]citrulline formed) were 1,520 and 16,400 cpm in the absence and presence of PP-ribose-P, respectively.

Effect of Polyamines on PP-ribose-P Kinetics — The effect of PP-ribose-P concentrations on the enzyme activity in the absence and presence of polyamines is shown in Fig. 6. In the absence of polyamines the maximal activation (11-fold) was observed at about 40 µM PP-ribose-P. Polyamines increased the apparent K_a value of the enzyme for PP-ribose-P thus widening the range of its effective concentrations and also augmented the degree of amplification of the enzyme activity by PP-ribose-P (the extent of activation was 40-, 28-, and 27-fold at 100 µM PP-ribose-P in the presence of 3 mM spermine, 3 mM spermidine, and 6 mM putrescine, respectively).

Studies on the CPSases II from Rat Liver and Mouse Spleen — Partially purified CPSases II from rat liver and mouse spleen were also inhibited by polyamines and the inhibition was reversed by PP-ribose-P in a similar manner as the AH 13 enzyme.

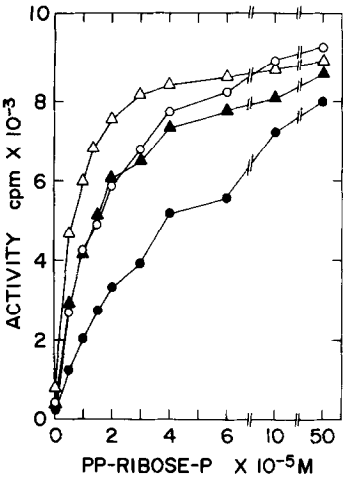


Fig. 6. Activation of AH 13 CPSase II by varying concentrations of PP-ribose-P in the presence and absence of polyamines. The activity was assayed as described in Fig. 1 except for the addition of varying concentrations of NaPP-ribose-P as indicated and polyamines as follows: none (△—△); 3 mM spermine (●—●); 3 mM spermidine (○—○); 6 mM putrescine (▲—▲). Enzyme: 0.30 munit, 0.6 μg of protein.

DISCUSSION

Spermine and spermidine are present in the order of 10^{-4} to 10^{-3} M in mammalian tissues (15). The present study shows that physiological concentrations of polyamines inhibited the CPSase II activity significantly. The inhibition could be reversed by Mg^{2+} and also by PP-ribose-P which was shown to increase the apparent affinity of the enzyme for free Mg^{2+} (Fig. 3). These results suggest that the inhibition is due to competition of polyamines with Mg^{2+} for free Mg^{2+} sites (13) of the enzyme. It is unlikely that polyamines inhibit the enzyme by forming polyamine-ATP complex because polyamines did not significantly affect the activity of the following enzymes that require $MgATP^{2-}$ as a substrate but not free Mg^{2+} (or its requirement is not clear): ammonia-dependent carbamoyl phosphate synthetase (EC 2.7.2.5) of rat liver, uridine kinase (EC 2.7.1.48), thymidine kinase (EC 2.7.1.75), or PP-ribose-P synthetase (EC 2.7.6.1) of AH 13 cells (M. Mori and M. Tatibana, unpublished results). Polyamines are known to replace Mg^{2+} in several biochemical reactions and the

stimulation by polyamines and Mg^{2+} of aminoacyl transfer RNA formation was studied in detail (16).

The intracellular level of PP-ribose-P may vary over a wide range with cell types as well as other miscellaneous conditions (17-19). In ascites tumor cells it was generally high (0.07 to 0.5 μmoles per g), and reached 2 to 3 μmoles per g when the cells were incubated with glucose (17). These values were far higher than the apparent K_a value of CPSase II for PP-ribose-P in the absence of polyamines and a change in its concentration in the higher range would have no effect on the enzyme activity (Fig. 6). However, in the presence of polyamines the apparent K_a value became higher and the enzyme activity would vary in response to a wider range of variation in the PP-ribose-P concentrations and, besides, the degree of amplification of the activity by PP-ribose-P could be increased. Thus, polyamines, in collaboration with PP-ribose-P, may play a role in the regulation of pyrimidine biosynthesis.

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