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EFFECT OF SPERMINE ON THE REACTION CATALYZED BY THREONYL-tRNA SYNTHETASE FROM RAT LIVER

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SUMMARY

The effect of spermine on the rates of ATP–pyrophosphate exchange and of formation of threonyl-tRNA by threonyl-tRNA synthetase has been studied. It is shown that spermine alone has no effect on the overall reaction as well as on the rate of ATP–pyrophosphate isotope exchange. At low Mg^{2+} concentration spermine activates 8-fold the ATP–pyrophosphate isotope exchange and 23-fold the threonyl-tRNA synthesis. The possible role of spermine in this activation is discussed.

INTRODUCTION

It has generally been accepted that the formation of aminoacyl-tRNA, catalyzed by aminoacyl-tRNA synthetase (EC 6.1.1.) proceeds by a two-step reaction pathway:

$$E + \text{amino acid} + \text{ATP} \rightleftharpoons E\text{–aminoacyl-AMP} + PP_i \quad (1)$$

$$E\text{–aminoacyl-AMP} + \text{tRNA} \rightleftharpoons \text{aminoacyl-tRNA} + \text{AMP} + E \quad (2)$$

$$\text{Amino acid} + \text{ATP} + \text{tRNA} \rightleftharpoons PP_i + \text{AMP} + \text{aminoacyl-tRNA} \quad (3)$$

The intermediate aminoacyl-AMP–enzyme complex has been isolated from various systems[1–4] and it has been demonstrated that it can effectively transfer the aminoacyl moiety to tRNA. Recently, it has been shown that the rate of aminoacyl transfer from this complex to tRNA is sufficient to account for the overall rate of the reaction by isoleucyl-tRNA synthetase[4]. This fact was a necessary evidence for establishing the central and functional position of the *E*–aminoacyl-AMP complex in the main reaction pathway.

Reactions 1 and 2 occur in the presence of Mg^{2+}. It has been demonstrated

Abbreviation: *E*–aminoacyl-AMP, aminoacyl-AMP–enzyme complex.

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though, that in Reaction 2, Mg^{2+} can be replaced by polyamines[5] and that a variety of metals can replace Mg^{2+} in Reaction 3 (ref. 6).

In studying the effect of polyamines in Reactions 1, 2 and 3, it has been shown that in various systems [7–10], polyamines can be used as cofactors in Reaction 3 but that Reaction 1 does not occur when Mg^{2+} is replaced by polyamines.

Based on these and other facts, a concerted reaction pathway has been proposed[8] that would not involve the formation of *E*–aminoacyl-AMP for the synthesis of aminoacyl-tRNA.

This report describes the effect of spermine on the rate of formation of threonyl-tRNA and the rate of PP_i–ATP exchange using threonyl-tRNA synthetase from rat liver.

MATERIALS AND METHODS

ATP, glutathione and yeast tRNA were purchased from Sigma. Before use the yeast tRNA was extensively dialyzed against 0.025 M EDTA and then against twice distilled water, precipitated with ethanol, and dried. L-Threonine and spermine were purchased from Calbiochem (Los Angeles, Calif.). L-[^{14}C]Threonine (166 Ci/mole) was obtained from New England Nuclear (Boston, Mass.). $^{32}PP_i$ was prepared by the method of Bergman[11] from radiochemically pure $^{32}P_i$ obtained from the Instituto de Energia Atômica (São Paulo). Threonyl-tRNA synthetase from rat liver was prepared according to Allende *et al.*[12].

Enzyme assays

$^{32}PP_i$–ATP exchange. The method described by Papas and Mehler[13] was used with slight modifications. The reaction mixture contained in a total volume of 1.0 ml: 100 mM Tris–HCl buffer (pH 7.8), 2 mM ATP, 5 mM L-threonine, 5 mM $MgCl_2$, 2 mM $^{32}PP_i$ ($5 \cdot 10^4$ to 10^5 cpm). The reaction was initiated by the addition of the enzyme and the mixture was incubated for 10 min at 37 °C. The reaction was then stopped by the addition of 0.5 ml of cold 7% $HClO_4$. After addition of 0.5 ml of 0.1 M PP_i and 0.5 ml of 10% Norit A, the mixture was vigorously agitated for 30 s and filtered through Whatman 3 MM filter discs. The radioactivity in the filter discs was measured using a Nuclear Chicago Model 2142 automatic counter. Units of enzymatic activity are defined as nmoles of $^{32}PP_i$ incorporated into ATP per min. Specific activity is defined as units/mg of enzyme.

The reaction of threonyl-tRNA formation was followed as described by Allende *et al.*[14]. The incubation mixture contained: 30 mM Tris–HCl buffer (pH 8.0), 25 mM KCl, 2 mM glutathione, 5 mM $MgCl_2$, 0.5 mM ATP, 0.012 mM threonine (166 Ci/mole) and 0.1 mg of tRNA in a total volume of 0.05 ml. The reaction was initiated by the addition of enzyme, incubated for 15 min at 37 °C and stopped with 3 ml of cold 5% trichloroacetic acid. The mixture was then filtered through Millipore filters HAWP (HA 0.45 nm) and the filters were washed with 15 ml of cold 5% trichloroacetic acid containing 1 mM threonine. Radioactivity was measured in the filters in a Beckman LS 250 scintillation spectrometer. Units of enzyme activity are expressed as nmoles of [^{14}C]threonyl-tRNA formed per min. Specific activity is defined as units/mg of enzyme.

RESULTS

Effect of spermine on the PP_i–ATP exchange

When spermine is used instead of Mg^{2+} in the exchange reaction there is no detectable $^{32}PP_i$–ATP exchange. However, as can be seen in Table I, under the conditions used, there is a small but significant increase of reaction rate in the presence of both Mg^{2+} and spermine. The small activation by spermine alone can be eliminated by 10 mM EDTA. This can be interpreted as a small contamination by Mg^{2+} in spermine. When the spermine concentration is doubled (2 mM) there is no change in the rate of exchange (Table II).

TABLE I

EFFECT OF SPERMINE ON THE $^{32}PP_i$–ATP EXCHANGE

Assay conditions as indicated in Materials and Methods except that the "complete" system does not contain Mg^{2+}. 30 μg of enzyme were used per assay.

Additions to "complete" system	*[^{32}P]ATP formed (nmoles/min per mg)*
None	13.6
+ 5 mM Mg^{2+}	250
+ 1 mM spermine	18.0
+ 1 mM spermine + 10 mM EDTA	13.4
+ 5 mM Mg^{2+} + 10 mM EDTA	13.4
+ 5 mM Mg^{2+} + 1 mM spermine	310

TABLE II

EFFECT OF tRNA ON $^{32}PP_i$–ATP EXCHANGE

Assay conditions as indicated in Materials and Methods. 30 μg of enzyme were added per assay.

Omissions or additions to complete system	*[^{32}P]ATP formed (nmoles/min per mg)*
None	280
− Mg^{2+}	19.5
− Mg^{2+} + 1 mM spermine	21.5
− Mg^{2+} + 2 mM spermine	18.8
+ 500 μg tRNA	260
+ 500 μg tRNA + 1 mM spermine	280
− Mg^{2+} + 500 μg tRNA + 1 mM spermine	21.8

Effect of tRNA on the PP_i–ATP exchange

In different systems, the effect of tRNA on the $^{32}PP_i$–ATP exchange reaction, catalyzed by aminoacyl-tRNA synthetases varies from absolute dependance on tRNA for exchange to no effect, activation or inhibition[15]. Table II shows that the addition of tRNA has no effect on the $^{32}PP_i$–ATP exchange reaction catalyzed by threonyl-tRNA synthetase. Moreover, the addition of tRNA to the system does not change the effect of spermine.

Effect of spermine on the formation of threonyl-tRNA

It can be seen in Table III that the addition of Mg^{2+} is necessary for aminoacyl-tRNA formation. Spermine, in this system, cannot replace Mg^{2+} in the formation of aminoacyl-tRNA. The small activating effect of spermine can be completely eliminated by EDTA at concentrations as low as 0.4 mM (not shown in the Table).

TABLE III

EFFECT OF SPERMINE ON THREONYL-tRNA FORMATION

Conditions are described under Materials and Methods. 10 μg of enzyme were used. The complete system does not contain Mg^{2+}.

Additions to complete system	*Formation of [^{14}C]threonyl-tRNA (nmoles/min per mg)*
None	0.0016
+ 5 mM Mg^{2+}	0.47
+ 1 mM spermine	0.0038
+ 5 mM Mg^{2+} + 1 mM spermine	0.58

Effect of spermine on the PP_i–ATP exchange at different Mg^{2+} concentrations

The effect of spermine is negligible as a cofactor in the $^{32}PP_i$–ATP exchange catalyzed by threonyl-tRNA synthetase. Fig. 1 shows that spermine has an effect on the Mg^{2+}-promoted $^{32}PP_i$–ATP exchange. The activation of the exchange reaction is more significant at low Mg^{2+} concentration. At higher Mg^{2+} concentrations, the activation effect tends to disappear. This activation can also be obtained at higher ATP concentrations (not shown).

Effect of spermine on threonyl-tRNA formation at different Mg^{2+} concentrations

Spermine alone, in this system, is not a cofactor in the formation of threonyl-

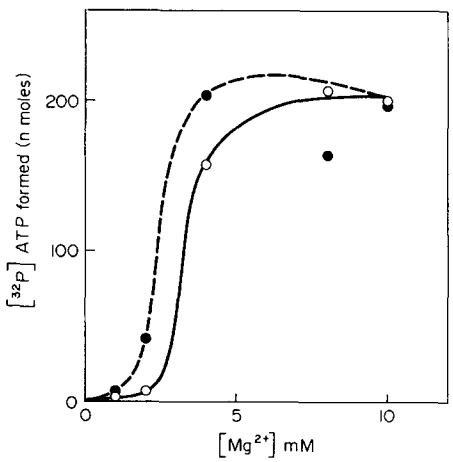

Fig. 1. Effect of spermine on $^{32}PP_i$–ATP exchange at different Mg^{2+} concentrations. Conditions described in Materials and Methods, except that ATP concentration was 0.5 mM, and Mg^{2+} varied as indicated. 30 μg of enzyme were used in these assays. ●—●, 1 mM spermine; ○—○, without spermine.

tRNA. However, a small but significant activation occurs at 1 mM spermine under the conditions of the standard assay procedure. When the Mg^{2+} concentration is varied, spermine activates the Mg^{2+} promoted threonyl-tRNA formation. These results are shown in Fig. 2.

The activation was up to 23 times, at 1 mM Mg^{2+}, 1 mM spermine.

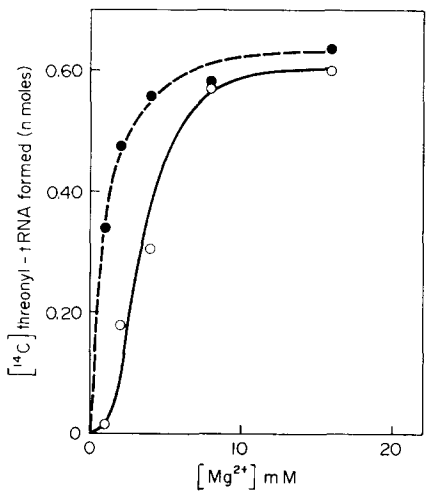

Fig. 2. Effect of spermine on the formation of threonyl-tRNA at different Mg^{2+} concentrations. Conditions of assay are described in Materials and Methods except that ATP concentration was 0.5 mM, and Mg^{2+} varied as indicated. The total enzyme concentration was 10 μg. ●—●, 1 mM spermine; ○—○, without spermine.

DISCUSSION

The failure to obtain significant $^{32}PP_i$–ATP exchange in the presence of spermine, using threonyl-tRNA synthetase, is in agreement with the finding of other authors in different amino acid activating systems[7–10]. Nevertheless the results presented in this paper indicate that spermine activates the exchange promoted by Mg^{2+}, when the latter is present at low concentrations.

In the absence of EDTA and Mg^{2+} in the reaction mixture, both the PP_i–ATP exchange and threonyl-tRNA formation are activated to a maximum of 1% when 1 mM spermine is added. However, this activation can be ascribed to the presence of traces of Mg^{2+} in the reaction, as EDTA completely inhibits the activation. The effect of EDTA on the spermine-activated aminoacyl-tRNA formation has been ascribed to a direct effect on tRNA[6]. This may be the case in our system, but the fact that EDTA has the same effect on the exchange reaction (Table I) rends this possibility unlikely.

It is worth stressing the high stimulatory effect of spermine in the presence of small concentrations of Mg^{2+}. One could suppose that the activation effect is due exclusively to an ATP–spermine complex formation, which in turn would set the Mg^{2+} free. This free ion would then be used for the reaction. If this were true, presumably the ATP–spermine complex could be a substrate for the reaction.

That the effect of spermine on the exchange reaction is not directly related to tRNA is clear from the data in Table II.

The activation of spermine is more noticeable, at low Mg^{2+} concentration in the formation of threonyl-tRNA. The ratio of rates of PP_i–ATP exchange and aminoacyl-tRNA formation as the Mg^{2+} concentration is varied, in the presence of spermine, has been calculated. Preliminary calculations show that this ratio (PP_i–ATP/aminoacyl-tRNA) varies from 300 to 20 as the Mg^{2+} concentration is diminished. These results suggest that small variations of Mg^{2+} concentrations can have a dramatic effect on the ratios of exchange *versus* transfer rates of aminoacyl-tRNA synthetases, when polyamine effect is studied.

It has already been shown that spermine affects differently several aminoacyl-tRNA synthetases[7]. Threonyl-tRNA synthetase may be just an extreme case in which no activation by spermine alone can be demonstrated. On the other hand, the activating effect of spermine is readily demonstrated when low concentrations of Mg^{2+} are used in the system.

The effects of polyamines in this system may prove useful for further investigations on the mechanism of threonyl-tRNA synthetase.

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