MAGNESIUM IONS STILL NECESSARY IN ISOLEUCYL–tRNA FORMATION

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1. Introduction

The concept that the aminoacylation of tRNA proceeds in two steps, activation of the amino acid and transfer of the activated amino acid onto the tRNA, has been open to question since Takeda and Igarashi [1] reported in 1969 that only the first reaction requires magnesium ions, whereas, the magnesium ions are not necessary for the overall reaction.

This problem has been further investigated [2–14] with contradictory conclusions. Takeda and Ogiso [15] reconfirmed their previous reports on isoleucyl–tRNA formation in *Escherichia coli,* reactivating the discussion about a one step or a two step mechanism.

The results of the present paper are in favour of a two step mechanism. Mg^{2+} has at least two functions in the aminoacylation of tRNA and spermine cannot substitute for Mg^{2+} in the activation of the amino acid as well as in the overall aminoacylation reaction. The results of Takeda and Ogiso [15], correctly interpreted, support this concept.

2. Materials and methods

The preparations of magnesium free *E. coli* tRNA and of the crude synthetase from *E. coli* MRE 600 were as previously described [12]. The dialysis of the enzyme against EDTA containing buffer was followed by a dialysis against EDTA free buffer. ATP disodium salt from Boehringer Mannheim was used without further purification. Spermine had been purchased from Fluka, Buchs.

The aminoacylation assay was carried out as described [16]. The incubation mixture contained in 0.1 ml: 0.5 A_{260} unit unfractionated tRNA from *E. coli,* 0.3 A_{260} unit crude aminoacyl tRNA synthetase, 2.5 μmol Tris–HCl, pH 7.5, 0.25 μmol ATP, 5 μmol L-[^{14}C]isoleucine, $MgSO_4$ and spermine as indicated in the legend of the figure. The mixture was incubated for 20 min at 30°C. The pyrophosphate exchange reaction was measured according to the method described in [17]. The incubation mixture contained in 0.1 ml: 2.5 μmol Tris–HCl, pH 7.5, 1 μmol ATP, 5 μmol isoleucine, 10 μmol [^{32}P]pyrophosphate, 0.3 A_{260} unit crude aminoacyl tRNA synthetase, $MgSO_4$ and spermine as indicated in the legend of the figure. The mixture was incubated for 5 min at 30°C.

3. Results and discussion

The effect of spermine and Mg^{2+} on the aminoacylation of $tRNA^{Ile}$ from *E. coli* and on the Ile-dependent pyrophosphate exchange is represented in fig.1. In the presence of spermine the stimulation of the pyrophosphate exchange by Mg^{2+} coincides with the increase in aminoacylation, whereas in the absence of spermine both reactions need very different Mg^{2+} concentrations for an optimal process. These results indicate that Mg^{2+} has at least two different functions in the aminoacylation of the tRNA. In the activation of the amino acid, as measured by the pyrophosphate exchange, about 0.2 mM Mg^{2+} is required which cannot be replaced by spermine; in the transfer of the activated amino acid onto the tRNA, approximately 1 mM is additionally required. Only this additional Mg^{2+} can be replaced by spermine and some other cations

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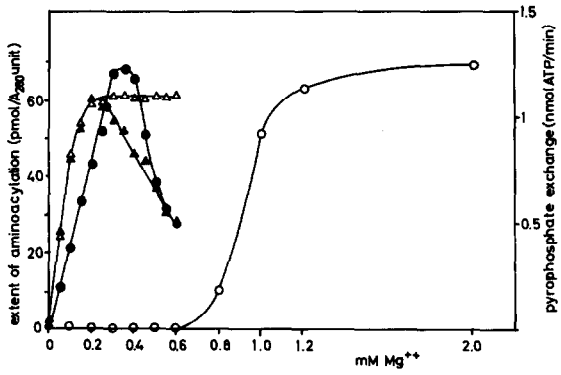

Fig.1. Influence of Mg^{2+} on the aminoacylation of $tRNA^{Ile}$ and on the Ile-dependent ATP pyrophosphate exchange. (●–●) pyrophosphate exchange in the absence of spermine, (▲–▲) pyrophosphate exchange in the presence of 3 mM spermine, (○–○) aminoacylation in the absence of spermine, (△–△) aminoacylation in the presence of 3 mM spermine.

[18–20], its function probably being the stabilisation of the tertiary structure of the tRNA.

In contrast to the aminoacylation reaction the pyrophosphate exchange reaction shows a rather sharp optimal Mg^{2+} concentration (fig.1). The decrease observed at higher Mg^{2+} concentration is evidently due to the decrease of the concentration of free pyrophosphate, according to a dissociation constant of 10^{-5} M of the Mg^{2+} pyrophosphate complex at 30°C and pH 7.5. (This constant was measured by conductometric titration of sodium pyrophosphate with magnesium sulphate.)

Takeda and Ogiso claimed that the discrepancies between their results and those of other authors [12,14] might be due to an inhibition by EDTA. However the experimental data coincide very well, only the interpretations differ.

Takeda and Ogiso carefully measured the Mg^{2+} concentration in their aminoacylation mixture and found it to be 18 μM. At this Mg^{2+} concentration one would expect from the results depicted here in fig.1, no aminoacylation in the absence of spermine and in its presence an aminoacylation of about 5 pmol Ile/A_{260} unit tRNA. Compared with an optimal aminoacylation of about 70 pmol Ile/A_{260} unit, this represents only a residual activity. Takeda and Ogiso found under similar conditions in the presence of 3 mM spermine an incorporation of approximately 3200 cpm Ile in 400 μg tRNA, but they did not compare it with the aminoacylation at optimal Mg^{2+} concentration. Assuming a counting efficiency of 80% in the liquid scintillation spectrometer, the 3200 cpm would be about 4–5 pmol Ile/A_{260} unit tRNA. This residual activity is in very good agreement with the one expected from the contamination with Mg^{2+}.

The paper of Takeda and Ogiso does not confirm the replacement of Mg^{2+} requirement by polyamines in isoleucyl–tRNA formation. It rather supports that Mg^{2+} is essential in the aminoacylation of $tRNA^{Ile}$ and cannot be substituted by spermine.

Mn^{2+} can substitute for Mg^{2+} as well in the Ile-dependent pyrophosphate exchange as in the aminoacylation of $tRNA^{Ile}$ (experiments not shown). However no conditions have been found that permit aminoacylation of tRNA without pyrophosphate exchange. The concept of the aminoacyl adenylate synthetase complex as an obligatory intermediate in the aminoacylation of most, if not all, tRNAs is still valid.

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