

AMINOACYLATION OF tRNA. MAGNESIUM REQUIREMENT AND SPERMIDINE EFFECT

R. THIEBE

*Institut für Physiologische Chemie und Physikalische Biochemie der Universität München,
8000 München 2, Pettenkoferstraße 14a, West Germany*

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1. Introduction

The aminoacylation of tRNA has been formulated as a two step reaction [1]. The first reaction is the activation of the amino acid, and the second is the transfer of the activated amino acid onto the tRNA. Takeda and co-workers have proposed that only the first reaction requires magnesium ions; whereas, the magnesium ions are not necessary for the overall reaction [2,3]. This concept has caused confusion and necessitated a revision of the theory of the two step reaction [5].

We have reinvestigated the effects of spermidine and magnesium ions on the aminoacylation of several tRNAs according to the procedures previously reported [2–4]. The experiments described herein lead to conclusions essentially different from those of Takeda and Igarashi [2]. Magnesium ions are essential in the aminoacylation of most, if not all tRNAs and cannot be substituted by spermidine.

2. Materials and methods

Sodium chloride quality suprapure E. Merck, Darmstadt was used for the dialysis. Phenol had been distilled prior to use. Unfractionated tRNAs from *E. coli* and brewers yeast and ATP disodium salt were purchased from Boehringer Mannheim. Spermidine trihydrochloride was from Fluka, Buchs, and Chelex 100 was from Bio-Rad.

To obtain magnesium-free tRNAs 4 mg of tRNA were dissolved in 2 ml of buffer containing 1 M sodium

chloride, 0.05 M EDTA, saturated with phenol at 4°C, and adjusted to pH 8.0. The tRNA was then dialysed four times against 1 liter of the same buffer at 4°C for 48 hr. This was followed by a three hour dialysis against 1 M sodium chloride and one of five hr against quartz distilled water with several changes of the dialysate. ATP was purified by filtration over a column of Chelex 100 equilibrated with 0.25 M Tris-HCl, pH 7.5. Crude aminoacyl-tRNA synthetases from yeast were prepared as described [6], and synthetases from *E. coli* K 12 were prepared in an analogous procedure. 0.01 M EDTA was added to the buffer for the dialysis of the enzymes. The aminoacylation assay was carried out as described [6]. Unless mentioned otherwise, the incubation mixture contained in 0.1 ml 2.8 A_{260} unit of unfractionated tRNA from *E. coli* or 0.05 A_{260} unit of purified tRNA^{Phe} from yeast, 0.2 A_{260} unit of crude aminoacyl tRNA synthetase, 2.5 μ mol Tris-HCl, pH 7.5, 0.25 μ mol ATP, 50 μ mol of L-[¹⁴C] amino acid, MgCl₂ KCl, and spermidine as indicated in the text. For optimal aminoacylation, 0.1 M KCl and 13 mM MgCl₂ were present. The mixture was incubated for 25 min at 37°C.

3. Results and discussion

The experiments of Takeda et al. [2,3] concerning the substitution of Mg²⁺ by spermidine in the aminoacylation of tRNAs were qualitatively reproducible (table 1) when crude tRNA was employed. However, when the tRNA was exhaustively dialysed (see methods) the results were completely different. The system

Table 1
Effect of cations on the aminoacylation of tRNAs

| Cation added | | pmol of amino acid incorporated per A_{260} unit of tRNA | | |
|------------------------------|------------------------------|--|---------------------------|----------------|
| | | Leu (<i>E. coli</i>) | Phe (<i>E. coli</i>) | Phe (yeast) |
| With un- dialysed tRNA | 13 mM Mg^{++} | 92 | | |
| | 5 mM spermidine | 74 | | |
| With dialysed tRNA | 13 mM Mg^{++} | 0 | | |
| | 5 mM spermidine | 0 | | |
| | 50 mM Tris | 0 | | |
| | 13 mM Mg^{++} , 50 mM Tris | 85 | 44 | 960 |
| | 5 mM spermidine, 50 mM Tris | 1 | 0.5 | 0 |

The experimental conditions were as described in [4]. The incubation mixture contained 1.4 A_{260} unit of unfractionated tRNA from *E. coli* or 0.05 A_{260} unit of purified tRNA^{Phe} from yeast in combination with the homologous enzyme.

did not contain sufficient Tris to support aminoacylation even in the presence of Mg^{++} . Aminoacylation could be restored by the addition of Tris. Mg^{++} was necessary for aminoacylation and could not be entirely substituted by spermidine (table 1). The effects of spermidine and Mg^{++} on the aminoacylation of tRNAs from different sources are represented in fig.1

and 2. Especially in the *E. coli* system (fig.1), spermidine strongly stimulated the aminoacylation of the tRNA. Potassium chloride has qualitatively a very similar effect. The slight residual activity in the presence of spermidine at zero Mg^{++} is probably due to a contamination of the incubation mixture with about 10^{-9} mol Mg^{++} . This value was calculated from the extrapolation

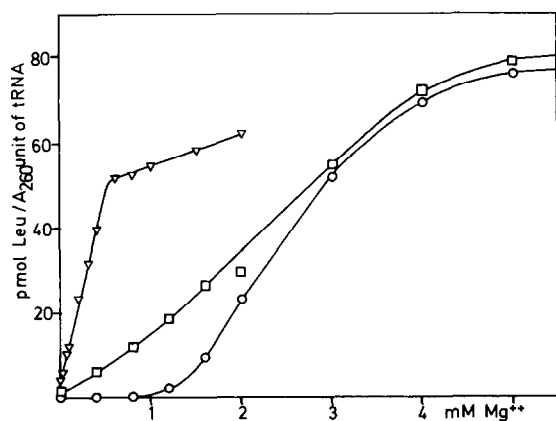


Fig.1. Magnesium dependence of the formation of Leu-tRNA^{Leu} from *E. coli*. Each sample contained 2.8 A_{260} unit of unfractionated *E. coli* tRNA. (○ — ○) without any addition, (□ — □) with 0.1 M KCl, (▽ — ▽) with 5 mM spermidine.

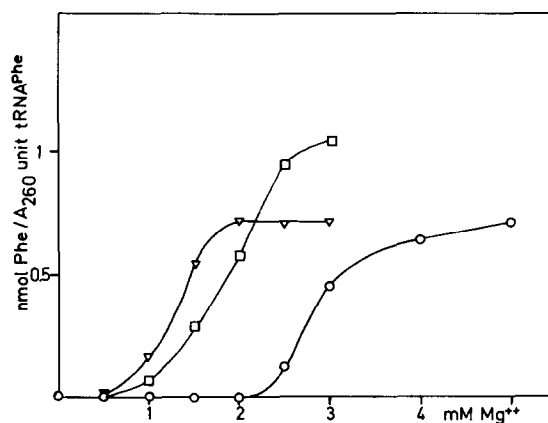


Fig.2. Magnesium dependence of the formation of Phe-tRNA^{Phe} from yeast. Each sample contained 0.05 A_{260} unit of purified yeast tRNA^{Phe}. (○ — ○) without any addition, (□ — □) with 0.1 M KCl, (▽ — ▽) with 5 mM spermidine.

of the curve in fig.1. When spermidine is present, the addition of EDTA to the aminoacylation assay does not eliminate the trace Mg^{++} to stop the reaction.

Earlier conclusions that Mg^{++} is not necessary when spermidine is present [2,3], are based upon experiments in which the tRNA was not free from Mg^{++} . As shown in fig.1, in the presence of spermidine and 1 mM Mg^{++} , one observes a good aminoacylation of dialysed tRNA; whereas, in the absence of spermidine, no reaction can be measured at this Mg^{++} concentration.

Without magnesium ion there is practically no amino acid activation reaction [7]. However there are indications that the sensitivity to Mg^{++} varies with the different aminoacyl-tRNA synthetases [8,9]. It has been shown by several authors that the transfer reaction (step two) does not necessarily require Mg^{++} [10–12]. Furthermore, spermidine is bound only to the tRNA and not to the aminoacyl-tRNA synthetase [13]. Spermidine has a structural effect on the tRNA [14] and can substitute for Mg^{++} in the transfer reaction, but Mg^{++} is essential in the ATP-consuming activation of the amino acid, probably with most of the aminoacyl tRNA synthetases.

There is no more disagreement as to the Mg^{++} requirement between the formation of the aminoacyl adenylate, the transfer of the amino acid from the adenylate onto the tRNA and the over-all reaction. This disagreement had been one serious argument against the conception of the aminoacyl adenylate synthetase complex as an obligate intermediate in the aminoacylation of tRNA. We will see if other arguments against this theory hold further.

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