BINDING OF TRANSFER RNA TO POLYAMINES IN PREFERENCE TO Mg^{2+}

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SUMMARY

Binding of spermine and spermidine to tRNA are preferential to that of Mg^{2+}. Polyamines displace Mg^{2+} bound to tRNA while Mg^{2+} does not displace previously bound polyamines. These results suggest that polyamine-tRNA complexes can exist in vivo and can act as substrates in aminoacyl-tRNA formation.

It has been reported that polyamines, such as spermine and spermidine, stimulate that formation of aminoacyl-tRNAs in the absence of Mg^{2+} (refs. 1-5). Previous work in this laboratory showed that the Mg^{2+} required for isoleucyl-tRNA formation in Escherichia coli could be completely replaced by spermine or spermidine[6,7]. It was also demonstrated that the primary function of cations was to bind to tRNA, changing it to an active conformation[8,9].

In this work we studied the bindings of polyamines and Mg^{2+} to tRNA and demonstrated that the former was bound preferentially to the latter.

MATERIALS AND METHODS

Assay for binding of Mg^{2+} to tRNA: Reaction mixtures (0.1 ml)

containing 0.01 M Tris-HCl (pH 7.8), 800 µg of tRNA and cations as indicated were incubated at 37° for the periods specified. Before use, tRNA (E. coli B, Calbiochem, USA) was dialyzed successively for about 16 hours each against 1 mM EDTA in 2.0 M NaCl, 1 mM EDTA and finally distilled water. After incubation the reaction mixture was applied to a Sephadex G-50 column (1 cm x 40 cm) equilibrated with 0.01 M Tris-HCl, pH 7.8. The column was eluted with the same buffer at 4° and 1 ml-fractions were collected. The Mg^{2+} content of each fraction was measured with a Hitachi atomic absorption spectroscopy (model 208) after appropriate dilution.

Assay for binding of ^{14}C-polyamine to tRNA: The reaction mixture was as for binding of Mg^{2+} to tRNA except that ^{14}C-polyamines were used. Fractionation was carried out as described above and then 0.1 ml of each fraction was placed on a paper disc (25 mm diameter) and its radioactivity was counted in a Beckman liquid scintillation spectrometer.

RESULTS AND DISCUSSION

When tRNA was incubated in 15 mM Mg^{2+} at 37° for 30 min and then fractionated on a Sephadex G-50 column, a significant amount of Mg^{2+} was eluted with tRNA (Fig. 1A, line o—o). When tRNA was incubated with 15 mM Mg^{2+} for 15 min and then 3 mM spermine was added and incubation was continued for 15 min, there was a significant decrease in the amount of Mg^{2+} bound to tRNA (Fig. 1B, line •—•). This indicates that Mg^{2+} was released from the tRNA on addition of spermine. When the order of additions of Mg^{2+} and spermine was reversed, adding spermine first, no significant binding of Mg^{2+} was observed (Fig. 1B,

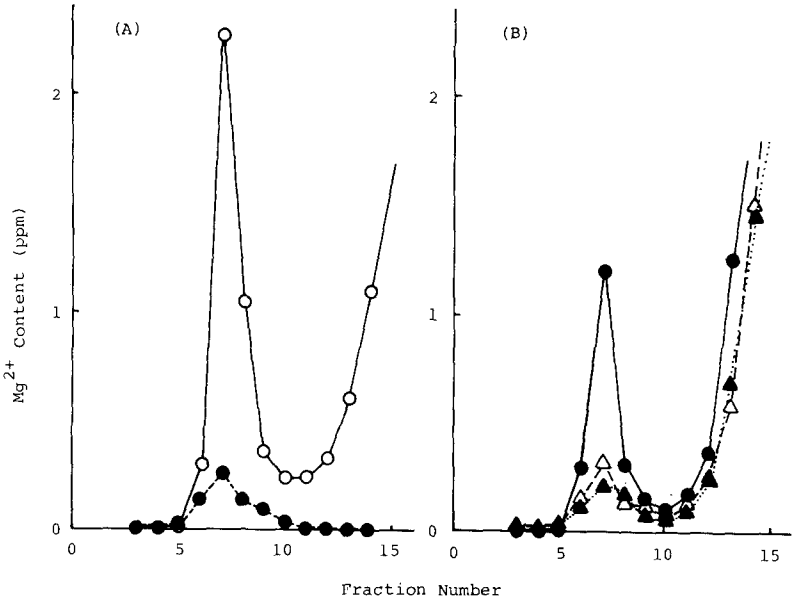

Fig. 1. Binding of Mg^{2+} to tRNA in the presence and absence of spermine. Experimental conditions were as described in the text. The incubation periods were as follows: (A) o—o, 30 min with 15 mM Mg^{2+}; ●--●, 30 min without added cations; (B) ●—●, 15 min with 15 mM Mg^{2+} and then 15 min with 15 mM Mg^{2+} and 3 mM spermine; △--△, 15 min with 3 mM spermine and then 15 min with 3 mM spermine and 15 mM Mg^{2+}; ▲····▲, 30 min with 3 mM spermine and 15 mM Mg^{2+}.

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line △--△) compared to that in the control (Fig. 1A, line ●--●). Moreover, when Mg^{2+} and spermine were added at the same time, no significant Mg^{2+}-binding was observed after incubation for 30 min (Fig. 1B, line ▲····▲).

Similar results were obtained using 3 mM spermidine in place of 3 mM spermine (Fig. 2).

Figure 3 shows that spermine can bind to tRNA both in the presence and absence of Mg^{2+}. When tRNA was incubated with 15 mM Mg^{2+} for 15 min and then 3 mM ^{14}C-spermine was added and incubation was continued for 15 min, all the ^{14}C-spermine added were eluted with tRNA (Fig. 3, line o—o). The ^{14}C-spermine was

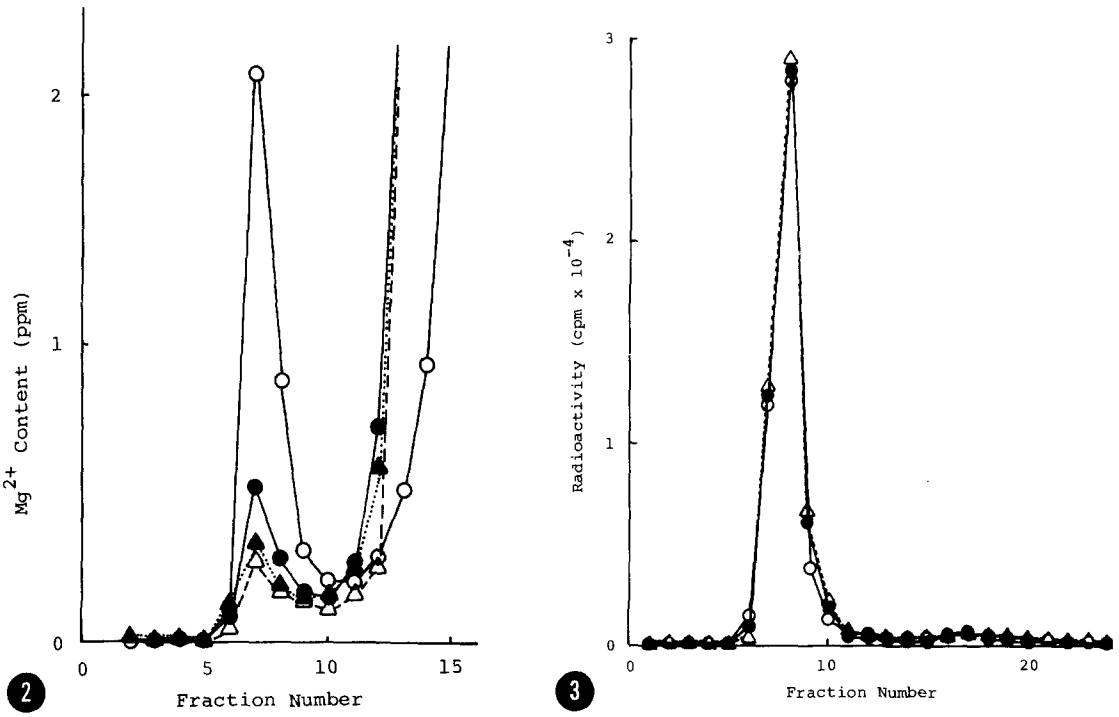

Fig. 2. Binding of Mg^{2+} to tRNA in the presence and absence of spermidine. Experimental conditions were as described in the text. The incubation periods: o—o, 30 min with 15 mM Mg^{2+}; ●—●, 15 min with 15 mM Mg^{2+} and then 15 min with 15 mM Mg^{2+} and 3 mM spermidine; △--△, 15 min with 3 mM spermidine and then 15 min with 3 mM spermidine and 15 mM Mg^{2+}; ▲····▲, 30 min with 3 mM spermidine and 15 mM Mg^{2+}.

Fig. 3. Binding of ^{14}C-spermine to tRNA in the presence of Mg^{2+}. Experimental conditions were as described in the text. Total counts of ^{14}C-spermine added were 5.6×10^5 counts/min. Incubation periods: o—o, 15 min with 15 mM Mg^{2+} and then 15 min with 15 mM Mg^{2+} and 3 mM ^{14}C-spermine; ●—●, 15 min with 3 mM ^{14}C-spermine and then 15 min with 3 mM ^{14}C-spermine and 15 mM Mg^{2+}; △-△, 30 min with 3 mM ^{14}C-spermine and 15 mM Mg^{2+}.

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also all bound to tRNA when the order of additions of cations was reversed (Fig. 3, line△--△), or when Mg^{2+} and ^{14}C-spermine were added at the same time (Fig. 3, line ●—●).

Similar results were obtained when tRNA was incubated with

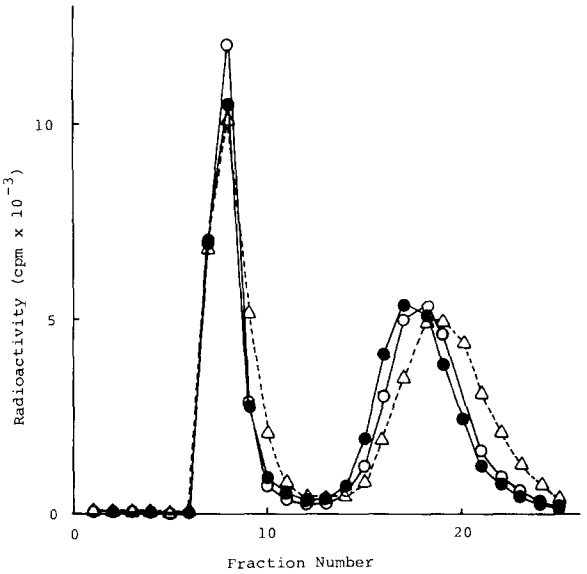

Fig. 4. Binding of ^{14}C-spermidine to tRNA in the presence of Mg^{2+}. Experimental conditions were as described in the text. Total counts of ^{14}C-spermidine added were 4.6 x 10^5 counts/min. Incubation periods: o—o, 15 min with 15 mM Mg^{2+} and then 15 min with 15 mM Mg^{2+} and 3 mM ^{14}C-spermidine; ●—●, 15 min with 3 mM ^{14}C-spermidine and then 15 min with 3 mM ^{14}C-spermidine and 15 mM Mg^{2+}; △--△, 30 min with 3 mM ^{14}C-spermidine and 15 mM Mg^{2+}.

15 mM Mg^{2+} and 3 mM ^{14}C-spermidine except that only about two thirds of the ^{14}C-spermidine added was eluted with tRNA (Fig. 4). The order of addition of cations did not affect the amount of binding of ^{14}C-spermidine to tRNA.

These results indicate that the bindings of spermine and spermidine to tRNA are preferential to that of Mg^{2+}. Polyamines displace Mg^{2+} bound to tRNA while Mg^{2+} does not displace previously bound polyamines. Thus, when polyamines and Mg^{2+} were added together to tRNA, only the polyamines were bound to tRNA.

When 800 µg of tRNA were incubated with various amounts of ^{14}C-spermine, it was found that the binding of ^{14}C-spermine to tRNA increased with the amount of ^{14}C-spermine added to a maximum with 3 mM spermine (Fig. 5). Assuming that the molecular

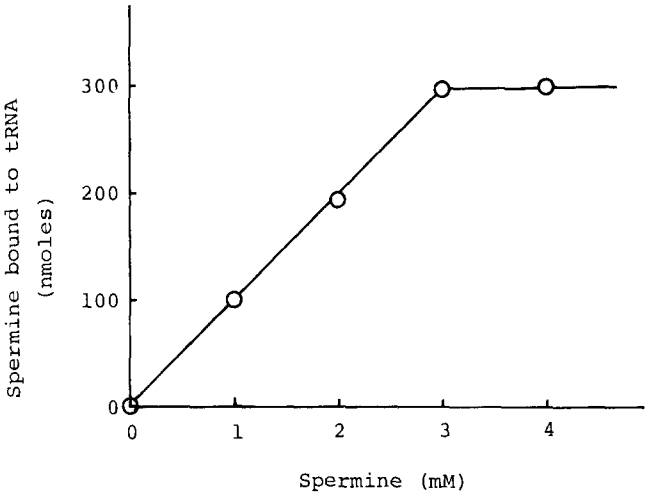

Fig. 5. Binding of spermine to tRNA. Experimental conditions were as described in the text. Various amounts of ^{14}C-spermine as indicated were added. The total amount of spermine bound to tRNA was calculated as the sum of the counts/min in each fraction.

weight of tRNA is 2.5 x 10^4, it was calculated from this that about 6 molecules of spermine bind to one molecule of tRNA under these experimental conditions.

Maximal binding of ^{14}C-spermidine was obtained with about 2 mM spermidine (Fig. 4). Based on this figure, about 4 molecules of spermidine bind to one molecule of tRNA.

Cohen[10] demonstrated that one molecule of tRNA has 10 binding sites for spermidine, and that 2 or 3 molecules of spermidine are tightly bound to tRNA. Under our experimental conditions, the binding capacities of tRNA for spermidine and spermine were about 4 and 6 molecules per molecule, respectively. On the other hand, it has been suggested that with optimal concentrations of spermidine and spermine for isoleucyl-tRNA formation in the presence of 800 μg of tRNA, about 10-12 molecules of spermidine or spermine interact with one molecule of tRNA[11]. These results suggest that some molecules of polyamines

are not tightly bound to tRNA so that they dissociate from the latter during gel filtration.

It has been reported that both Mg^{2+} and polyamines interact with tRNA, converting it from an inactive to an active conformation[9,10,12,13]. Evidence for the *in vivo* binding of some polyamines to tRNA has also been reported [14]. However, little is known of the relative affinities of polyamines and Mg^{2+} for tRNA. The present work shows that the bindings of spermine and spermidine to tRNA are preferential to that of Mg^{2+}, suggesting that polyamine-tRNA complexes can exist *in vivo* and can act as substrates in aminoacyl-tRNA formation.

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