Vol. 81, No. 3, 1978
April 14, 1978

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 734-742

APPARENT COOPERATIVITY OF CATION BINDING TO PROTONATED TRANSFER RNA MEASURED BY QUANTITATION OF RELEASED PROTONS

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Received December 12,1977

SUMMARY

Magnesium,spermidine and spermine are bound in a cooperative way to protonated tRNA . Experimental evidence suggests that tRNA may be considered as an allosteric molecule.

INTRODUCTION

The effect of cations in folding and stabilising the polynucleotide chains of tRNA molecules has been studied using different experimental approachs/1-8/.The importance of the observed effect depended,however,upon the difference between the initial state of the tRNA and its state after titration with cations/1-5/. The conformation of tRNA at neutral pH in solutions containing Mg^{2+} is usually referred to as the folded,native tRNA structure since it exhibits biological activity.In the case of $tRNA^{Phe}$ from yeast this conformation corresponds very well to its tertiary structure in crystals /9/.

If the initial conformation of the tRNA is different from the native one,the tRNA in its initial state is denatured,and during titration,one observes cooperative binding of cations/1-5/. Binding of cations to the native tRNA is independent/1,3,4/;however,the presence of cations is a prerequisite for the formation of the native structure/1/.At pH values lower than 7 the situation is further complicated since tRNA denatured by extensive dialysis takes up protons/7,8/.As a result the transition from denatured into folded tRNA is accompanied by the release of protons.Starting with

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0006-291X/78/0813-0734$01,00/0

salt-free tRNA,the folded conformation resulting from the binding of cations at pH values close to 7 may be identified with the native one.At about pH 5.5 the folded tRNA undergoes conformational change resulting in the appearance of an acidic folded form of tRNA/10/ i.e. the folded conformation appearing upon cation binding at pH lower than 5.5 cannot be identified with the native structure.Since the acid (protonated) form may have a functional significance/10/,we think it is important that more should be known about its conformation and interactions in solution.

In this paper,we present experimental evidence of cooperative binding of Mg^{2+} and polyamines,such as spermine and spermidine,to protonated tRNA.Cation binding to the salt-free tRNA was observed by measurements of the extent of deprotonation of tRNA throughout titration with cations /7,8/.

MATERIALS AND METHODS

Spermine·4 HCl and spermidine·3 HCl were purchased from Fluka,$MgCl_2$(analytical grade) from Merck,yeast tRNA from Boehringer.Other chemicals were analytical grade from P.O.Ch.Gliwice(PL).

Salt-free tRNA (unfractionated) was prepared as previously described/7/.Concentrations of tRNA in the titrated solutions varied from 10 to 60 A_{260}units per ml(i.e.17.5-105 μM),pH of the salt-free tRNA solution(usually between 4 and 5,depending upon tRNA concentration) was adjusted to the required level by NaOH or HCl.The number of protons released from tRNA during the titration with cations was measured using the pH-stat method as previously described /7/.

RESULTS AND DISCUSSION

Let us take salt-free tRNA at pH 4.5 as the initial solution of tRNA and use the following symbols :

L_t,L_b and L_f to be total,bound and free cation concentration respectively.

H_L^+ ; the number of protons released,at constant pH,by a given cation concentration .

H_{Lmax}^+ ; the maximal number of these protons .

H^+_{OH} ; the number of protons released by NaOH during pH adjustment(at pH 5.0,5.5,6.0 and 6.5) of initial(pH4.5)tRNA solution.

$H_t^+ = H_L^+ + H^+_{OH}$ (therefore at pH 4.5 , $H_t^+ = H_L^+$).

N ; the number of cations bound per tRNA molecule;

$$N = L_b / [tRNA] .$$

n ; the Hill coefficient .

K_L ; the apparent association constant (M^{-1}) .

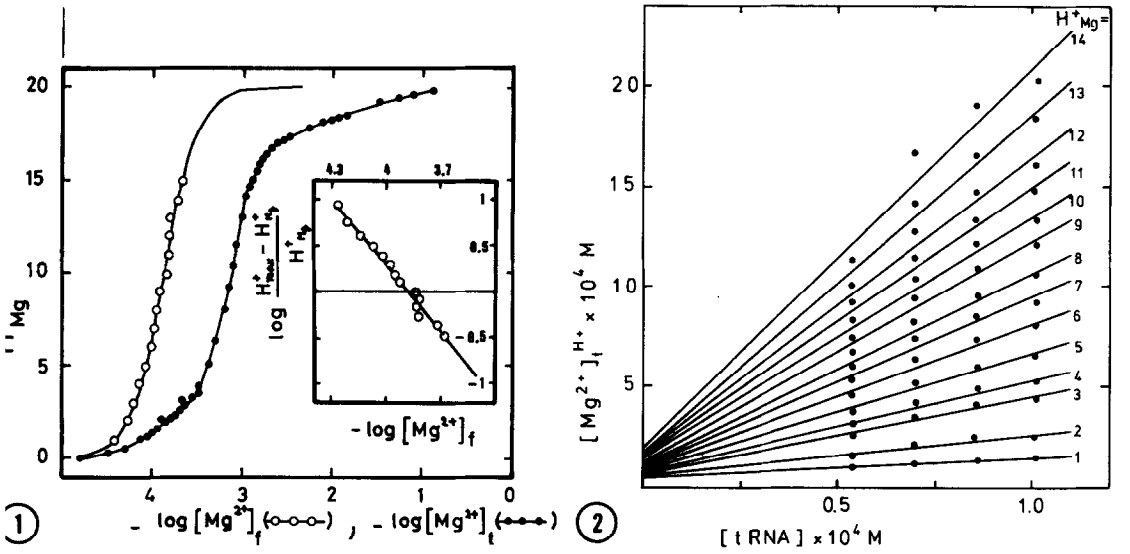

Fig.1 Titration of salt-free tRNA with Mg^{2+} at pH 4.5. •—• experimental curve, o—o theoritical curve calculated according to the parameters given in table 1, open circles denotes the values of $[Mg^{2+}]_f^{H^+}$ obtained as described in the text and shown in fig.2. Inset shows the Hill plot constructed on the basis of these values.

Fig.2 Extrapolation of $[Mg^{2+}]_t^{H^+}$ values obtained at different tRNA concentrations to $[tRNA]=0$.

The index H^+ ($L_f^{H^+}$, $L_b^{H^+}$, $L_t^{H^+}$ or N^{H^+}) denote the value of the described parameter for a given number H_L^+ of the released protons. Since $L_t=L_b+L_f$ then $L_t=N[tRNA]+L_f$ and for a given titration point H_L^+ ;

$$L_t^{H^+} = N^{H^+}[tRNA] + L_f^{H^+} \qquad (1)$$

The apparent titration curve obtained is a plot of H_L^+ versus log L_t (fig.1). In order to construct the plot of H_L^+ versus L_f, it was necessary to obtain L_f values corresponding to a given titration point H_L^+. This was done by performing titrations in solutions in various tRNA concentrations at constant pH (fig.2). Then $L_f^{H^+}$ values corresponding to a given titration point H_L^+ were obtained according to Eq.1, by extrapolation of $L_t^{H^+}$ values obtained for different $[tRNA]$ to $[tRNA]=0$. In this way it was possible to estimate $[Mg^{2+}]_f$ responsible for the release of the first 15 protons ($H_{Mg}^+=1$ to $H_{Mg}^+=15$) out of 20 releasable protons at pH 4.5 and the first 9 protons ($H_{Mg}^+=1$ to $H_{Mg}^+=9$) at pH 5.5 out of 12 releasable protons by Mg^{2+} at this pH.

Table 1. Parameters of cations binding to protonated tRNA.

Cation	pH	$K(M^{-1})$	n
spermine	4.5	60×10^3	2.9
spermidine	4.5	30×10^3	2.8
magnesium	4.5	7.5×10^3	2.3
magnesium	5.5	5.5×10^3	2.2

In the case of spermine and spermidine, titrations at 4.5, the reproductable values of $L_f^{H^+}$ corresponding only to the first 9 (H^+_{Sp}=1 to H^+_{Sp}=9) and 12 (H^+_{Spd}=1 to H^+_{Spd}=12) protons respectively could be obtained, due to tRNA aggregation at high concentrations of polyamines and tRNA itself .

Having obtained $L_f^{H^+}$ values, we are allowed to use empirical Hill equation or Scatchard representation/1,11/for further analysis of the titration data. The linear plot obtained according to the Hill equation (inset to fig.1),

$$\log \frac{H^+_{Lmax} - H^+_L}{H^+_L} = n \log L_f + \log K \quad (2)$$

reveal different information depending upon its slope. In our case, $n > 1$ obtained for Mg^{2+} (fig.1 and table 1), spermine and spermidine (table 1) indicates the cooperativity of these cation bindings to protonated tRNA. This is further evidenced by the Scatchard plots which were obtained. Fig.3 shows the plots of H^+_L versus H^+_L/L_f for Mg^{2+} (at pH 4.5 and 5.5) as well as for spermine and spermidine (at pH 4.5). Despite high distribution of the experimental points their pattern clearly shows a positive curvature demonstrating cooperative character of the cation binding to protonated tRNA. Using the simplest model (Eq.2) for quantitative description of the titration data, Scatchard parameters are given by :

$$H^+_L = \frac{H^+_{Lmax} K^n L_f^n}{1 + K^n L_f^n} \quad \text{and} \quad H^+_L/L_f = \frac{H^+_{Lmax} K^n L_f^{n-1}}{1 + K^n L_f^n} \quad (3\ a,b)$$

In fig.3, the experimental values are indicated by points, solid lines are theoretical curves calculated according to Eqns 3a,b, using the parameters summarised in table 1 .

In our case binding of cations was observed by indirect method of

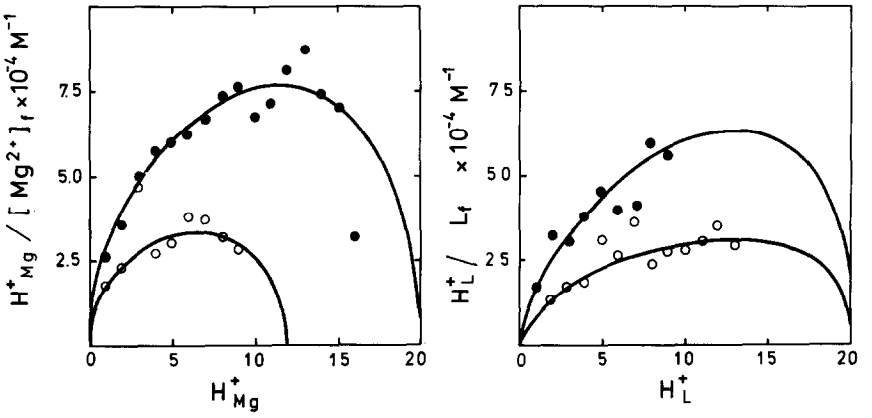

Fig.3 Scatchard representation of Mg^{2+} (●—●, pH 4.5 and o—o pH 5.5). spermine ●—● and spermidine o—o binding data .

cation binding measurement/1/i.e.by measurement of changes in the extent of tRNA deprotonation upon binding of cations/7,8/.The release of protons from tRNA is connected with the decrease in apparent pKs of base ionisations following the increase in ionic strength of the solvent.The"abnormal"base ionisation,with pK_{H^+} around 6,has been demonstrated in $tRNA^{Ile}$/5/.A similar effect observed in low salt solutions of polyI.poly C was found to be a consequence of the existence of local pH prevailing in the vicinity of the polymer,due to its negatively charged phosphate groups/12/.On the other hand,it is known that the release of protons from tRNA is accompanied by substantial structural rearrangements of the tRNA molecule/7,10,13/.

It should be therefore concluded that the initial,protonated form of tRNA shows a higher affinity for protons than the final state,RL,appearing upon cation binding i.e.the equilibrium $RL \rightleftharpoons RH^+L$ is shifted to the left when compared with $R \rightleftharpoons RH^+$.On the other hand, cooperativity of cation binding to the protonated form of tRNA demonstrates that the initial state of RH^+ shows a lower affinity for cations than the final form RL i.e. that the equilibrium $RL \rightleftharpoons R$ is shifted to the left when compared with $RH^+L \rightleftharpoons RH^+$.
This concurs with the results obtained for linear polynucleotides and condensation screening theory of Manning /14,15/.

Fig.4 a,b shows the comparison of the deprotonating effect of Mg^{2+} at different pH values.Mg^{2+} concentrations required for the release of protons denoted by the same number H^+_L but at different pH values are compared on fig.4 a,and Mg^{2+} concentrations required

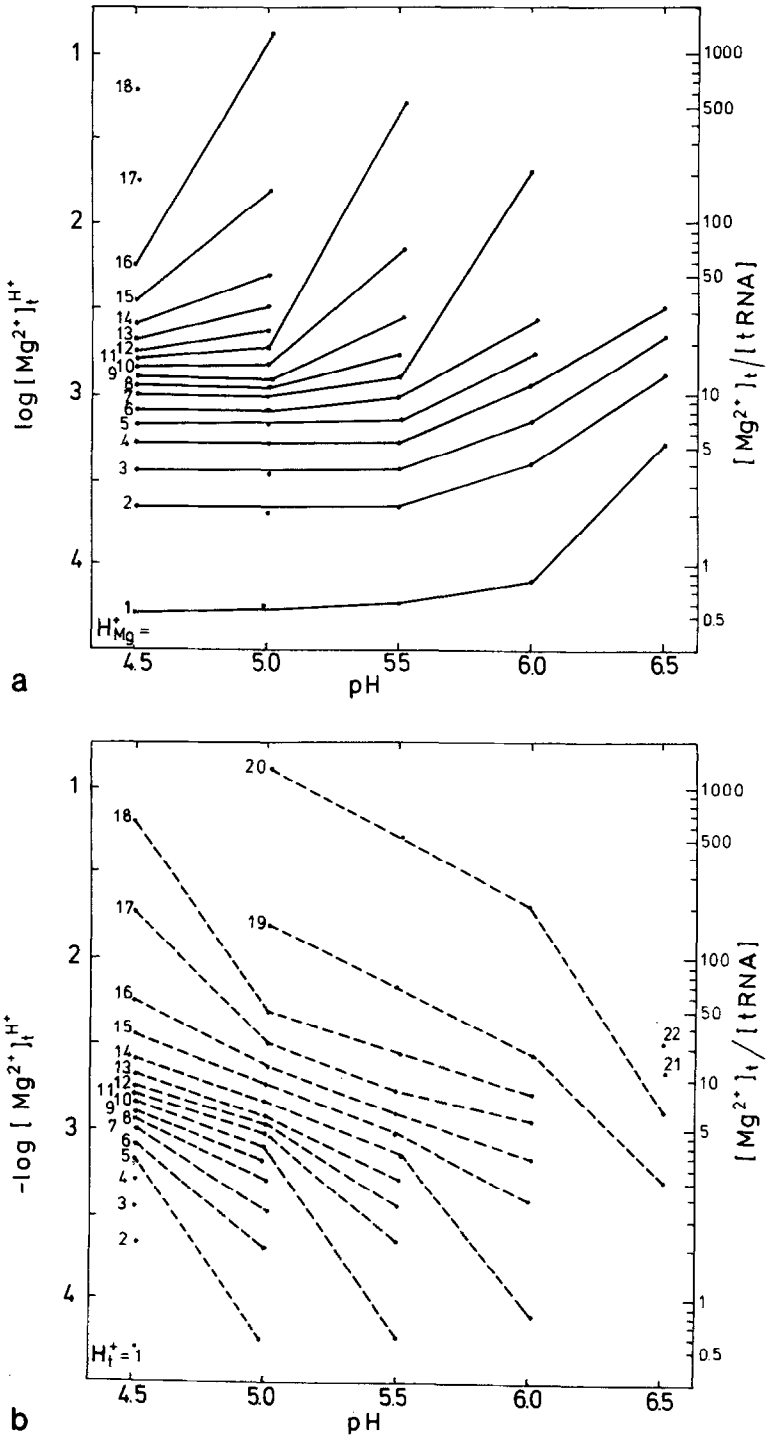

Fig.4 Comparaison of Mg^{2+} concentrations and $[Mg^{2+}]_t / [tRNA]$ required for release of a given number of protons H^+_{Mg} (fig. 4a) and H^+_t (fig.4b) at different pHs. [tRNA] = 1.035 x 10^{-4} M.

for the release of protons denoted by the same total number H_t^+, at different pH values, on fig.4b. From this figure, it is seen that Mg^{2+} concentrations necessary to release a given proton, H_t^+, increase with decreasing pH. This is important because the same H_t^+ can be presumed to have the same pK_{H^+} and consequently to be the same protonation sites. Fig.4a shows that Mg^{2+} at pH 5.0, 5.5 and partly at pH 6.0 is as effective in deprotonation of tRNA as in pH 4.5. The deprotonating effect of Mg^{2+} depends upon Mg^{2+} affinity of tRNA, which decreases with decreasing pH, and upon the population of protonated sites, which increases with decreasing pH. This effect is similar at pH 4.5, 5.0 and 5.5, particularly if we compare the first 75 % of protons released by Mg^{2+} at these pH values. At pH values higher than 5.5, Mg^{2+} becomes less effective in tRNA deprotonation, due to the decrease in population of protonated sites in tRNA at these pHs.

It is interesting that the number of bound cations N^{H^+} (Eq.1) per number of the released protons H_L^+ is almost constant for a given cation over the first 75% of the titration curve/13/(fig.4a, pH4.5-5.5), and is a function of the valence of cations. The N^{H^+} values of about 12, were obtained for magnesium, and 9 to 6 for spermidine and spermine, respectively corresponding to $H_L^+ = 10$ at pH 4.5. This gives an additional evidence of the role of phosphates (binding sites for cations) in the protonation of the salt-free tRNA.

The ability of tRNA to form local high negative charge density has already been demonstrated in $tRNA^{Phe}$ crystals and explain the existence of strong cation binding sites/16/. In the absence of cations in the apparently salt-free solution of tRNA, the same may be responsible for "abnormal" high proton affinity of tRNA. The formation of negative charge clusters in tRNA, connected with the formation of tertiary structure in native tRNA, can only be achieved in the presence of a positive charge which neutralizes the repulsion between negatively charged phosphates. The positive charge may be that of Mg^{2+} or other cation which fits to tRNA structure. A similar effect may, however, be achieved inside the tRNA molecule due to protonation of bases in the absence of a suitable external positive charge down in salt-free tRNA. The effect of "abnormal" base ionisations occurs to a greater extent in salt-free tRNA and relatively low pH values (pH 4.5). However, this also occurs at more physiological pH values (pH 6, 6.5) and in the presence of Mg^{2+} (fig.4).

Binding of cations depress the tRNA affinity for protons and vice versa. In addition, the binding of cations to the protonated tRNA is cooperative. tRNA should be therefore considered as an allosteric molecule/17/. Although the functional significance of this allosteric effect is not yet known, it may be of importance during interactions of tRNA with other macromolecules, namely on ribosomes where local variations in charge density can be responsible for protonation and, consequently, conformational transitions of tRNA during the process of translations/18/.

The cooperative character of cation binding to tRNA was explained by an allosteric (concerted) model/4/. Mg^{2+} binding is preceded by the structural transition of the denatured form of tRNA, showing a low affinity for Mg^{2+}, into native conformation, which in turn binds cations in two classes of independent binding sites, strong and weak. The alternative sequential model/2/, also shown by experiment, assumes the sequential binding of cations to the denatured tRNA which result in native structure formation. In our case the situation is further complicated due to tRNA protonation, and therefore both of these models could be inadequate. The most similar to our case is, however, that described for $tRNA^{Ile}$ at pH values below 7/5/ where dissotiation of the protonated form RH^+ precedes sequential binding of Mg^{2+} followed by structural rearrangements of the R form. If this is so, the "concerted" transition $RH^+ \rightleftharpoons R$ (when deprotonation takes place at more than one site) should be more pronounced to allow for tetra- or trivalent cation bindings to R form than for divalent cation binding, explaining higher apparent cooperativity (table 1) in the former binding than in the latter binding. This also explains the apparent discrepancy between our results and Lynch & Schimmel results/4/, who observed the decrease in cooperativity of Mg^{2+} binding with pH. The effect they measured was mainly due to sequential binding of Mg^{2+} observed by means of fluorescence, whereas that observed by us was principally due to deprotonation step .

Acknowledgements : This work was supported by a grant from the Polish Academy of Sciences within the project 09.7.1.

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