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ON THE BINDING OF Mg^{2+} AND Mn^{2+} TO tRNA

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In this paper previous binding studies of Mg^{2+} and Mn^{2+} ions to tRNA's are reconsidered. Binding data of some representative examples are interpreted including interactions between charges located on the macroion. Both curved and bell-shaped Scatchard plots can be accounted for quantitatively if corrections are made for electrostatic interactions and, if necessary, for the effect of conformational changes on these interactions. It appears that there is no need to invoke more than one class of binding sites on tRNA's, meaning that the experimental binding data can be described using the same intrinsic pK value for all phosphate groups.

1. Introduction

Many biological systems need divalent metal cations, in particular Mg^{2+} ions, for their structural integrity and functioning. Also for tRNA's it is well established that their native structure is promoted in the presence of Mg^{2+} or Mn^{2+} ions in solution [1]. In order to investigate the mode of action of these ions in the stabilization of the native state a number of studies concerning the binding of Mg^{2+} and Mn^{2+} ions have appeared [2–10]. Most authors conclude that there are two classes of binding sites, this being based mainly on the biphasic nature of their Scatchard plots. In some studies "bell-shaped" Scatchard plots were found, indicating that the binding of divalent cations to tRNA is cooperative [5,7,9]. In spite of the fact that most authors find two classes of binding sites there is little agreement about the number of binding sites which are indicated as "strong binding sites". The reported values vary between 1 and 17 [10,7]. Although the high charge density of the ribose-phosphate backbone is well recognised and although it is well understood that this charge density must be partly neutralized to obtain the native structure, it is somewhat surprising, that with one exception [4] the influence of electrostatic interactions has not been taken into account in interpreting Mg^{2+} binding to tRNA's. In this study previous binding studies are reconsidered. It is found that the experimental results can be explained within a simple model, where all phosphate-groups have the same intrinsic binding affinity for magnesium (manganese) ions. Both the curved and the bell-shaped Scatchard plots can be accounted for if corrections are made for electrostatic interactions and for the effect of conformational changes on these interactions.

2. Theoretical aspects

We consider the reversible binding between a macroion and small ions, i.e. tRNA and magnesium ions. In the simplest situation all binding sites are identical and completely independent. In this case the relation between the fraction α of dissociated groups (unoccupied phosphate sites on the macromolecule) and the free Mg^{2+} concentration, $[Mg^{2+}]$, is given by

$$pMg = pK + \log \frac{\alpha}{1-\alpha} \qquad (1)$$

where $pMg = -\log[Mg^{2+}]$ and $pK = -\log K$, K being the dissociation constant. However, in a poly-electrolyte one expects the ionic binding sites to interact with one another in such a way that binding at any site affects the binding affinity at all other sites, since the work required to dissociate a small ion from a macroion is a

function of the charges located on the macroion. Following Linderstrøm–Lang [11] we shall account for this effect by introducing in eq. (1) the term $-0.868\ w'z_iZ$, so that

$$\mathrm{pMg} = \mathrm{pK_{int,Mg}} + \log \frac{\alpha}{1-\alpha} - 0.868\ w'z_iZ \qquad (2)$$

where Z = the total charge of the macroion, i.e. the charge of the tRNA without bound counterions plus twice the number of bound Mg^{2+} ions; z_i = the charge of the small ion; w' = the so called electrostatic interaction factor; $K_{int,Mg}$ = the intrinsic dissociation constant, i.e. the dissociation constant for the situation in which the charge of the macroion is zero. Note that the apparent binding constant is not a real constant. It follows from eq. (2) that

$$\mathrm{pK_{app}} = \mathrm{pK_{int}} - 0.868\ w'z_iZ. \qquad (3)$$

In this treatment counterions like Na^+ ions are supposed not to be involved in site binding [12]. Their screening of the macromolecular charge is taken care of in the electrostatic interaction factor.

The value of the electrostatic interaction factor, w', depends strongly on the model chosen to represent the macromolecular configuration. On the basis of model calculations [11], some general remarks about its behavior can be given. For a chosen model w' is constant as long as the ionic strength, the temperature and the conformation are constant. An increase of ionic strength or temperature will decrease the value of w', the effect of the ionic strength being much more important than that of the temperature. The value of w' will also decrease when the macromolecular conformation is altered from a compact to a more extended form. Such conformational changes will be encountered in the Mg^{2+} binding studies to tRNA.

The experimental data on Mg^{2+} binding to tRNA were mostly analysed in terms of Scatchard plots, i.e. a plot of r/c versus r, where r is the (average) number of Mg^{2+} ions bound per tRNA molecule and c is the concentration of free Mg^{2+} ions. In the situation that all binding sites are identical and completely independent the Scatchard formula is given by

$$\frac{r}{c} = \frac{1}{K}(n - r), \qquad (4)$$

where n = the total number of binding sites. Because of the electrostatic interactions this equation has to be modified. Using eq. (3) we get [11]

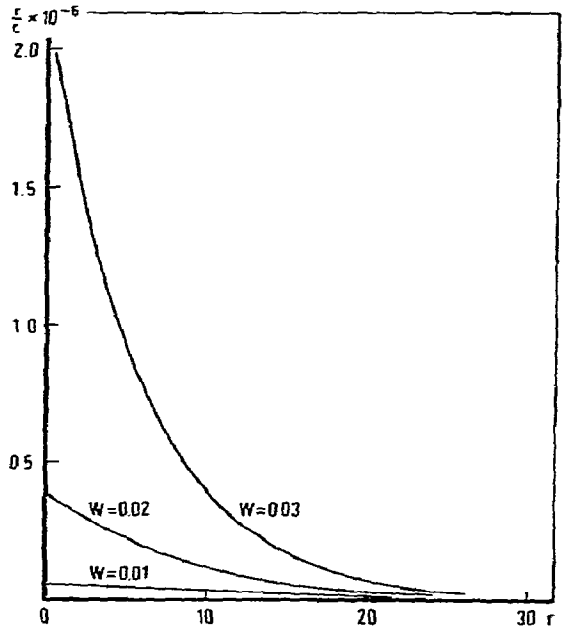

Fig. 1. Scatchard plots demonstrating the influence of electrostatic interactions. The curves were calculated according to eq. (5) with $\mathrm{pK_{int,Mg}} = 2.50$, and assuming that there are 75 phosphate groups in the macromolecule ($Z_{max} = -75$). The values of $w = -0.868\ w'z_i$ are indicated.

$$\frac{r}{c} = \frac{1}{K_{\mathrm{int,Mg}}} e^{-2w'z_iZ}(n - r). \qquad (5)$$

The influence of w' on the form of Scatchard plots is illustrated in fig. 1. In this figure and also in the following sections the term $-0.868\ w'z_iZ$ is replaced by $-wZ$.

3. Calculations

Experimental binding data, influenced by electrostatic interactions, can be linearized, according to eq. (2). A plot of $\mathrm{pMg} - \log \alpha/(1-\alpha)$ versus Z will yield a straight line, provided the experimental conditions are chosen in such a way that the value of w remains constant during titration with Mg^{2+} ions. For the binding of Mg^{2+} to yeast $tRNA^{Phe}$ in a 0.033 M NaCl solution at pH 6 and at 10° and 30°C this condition is satisfied [9]. Using the best fit to the curved Scatchard plot we replotted the data according to eq. (2) (fig. 2). A straight line is obtained which is a

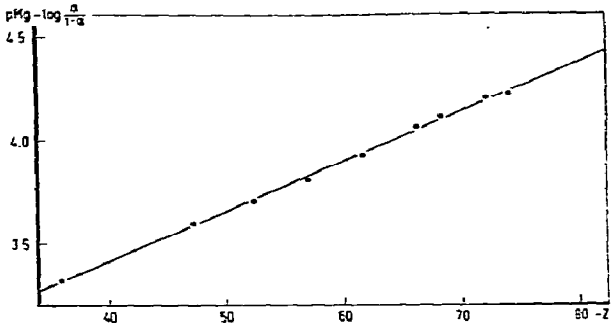

Fig. 2. Plot of pMg − log $\alpha/(1-\alpha)$ versus Z. The closed circles are data from Römer and Hach [9], obtained for Yeast tRNA dissolved in a 0.033 M NaCl solution, pH 6. The experiments were carried out at 10° and 30° C.

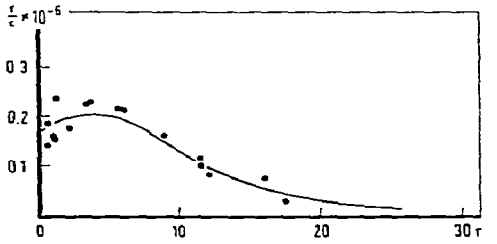

Fig. 4. Scatchard plot calculated according to eq. (5) with $pK_{int,Mg} = 2.45$. In the region where Z varies from −75 to −62, w was linearly varied from 0.016 to 0.022. For values of Z higher than −62 the factor w was 0.022. The dots are experimental data from Römer and Hach [9], obtained for Yeast $tRNA^{Phe}$ dissolved in a 0.033 M NaCl solution at pH 6. The experiments were performed at 45°C.

strong indication that there is only one class of binding sites, i.e. all phosphate groups have the same pK_{int}. The intercept of this line yields $pK_{int,Mg^{2+}} = 2.45$, while from the slope of the line we find $w = 0.024$. Note that these values are not found by iteration, but are uniquely determined by the slope and the intercept of the straight line. From the fact that the experimental curves coincide at 10°C and 30°C it follows also that the pK-value is not (strongly) dependent upon temperature. The condition that w remains constant during titration is also satisfied at elevated temperature (70°C). However, in this situation the tRNA molecule is in a random coil configuration so that the value of w is small. Therefore experimentally a (nearly) straight Scatchard plot was found [9], as predicted by the lower curve with $w = 0.01$ in fig. 1.

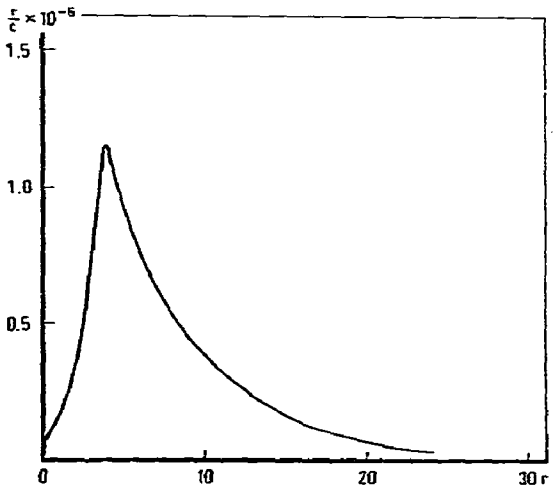

Fig. 3. Scatchard plot demonstrating the influence of a conformational change of the macromolecule during binding. The curve was calculated according to eq. (5) with $pK_{int,Mg} = 2.5$. The factor w was linearly varied from 0.01 to 0.03 in the region where Z varies from −75 to −68. For higher values of Z, w was kept constant at 0.03.

When magnesium (or manganese) binding studies are performed at elevated temperature and at relatively low ionic strengths or with large monovalent cations like tri-ethanolamine, which have a relatively small screening effect, the configuration of the tRNA molecule may change during titration. This situation has been encountered in several instances by different investigators [5,7,9,13]. As a result of the conformational change the electrostatic interaction factor w' will change during the titration.

Fig. 3 shows the type of Scatchard plot one predicts for such a situation. The pK_{int} value was held constant during the calculation, while the value of w was linearly changed from 0.01 to 0.03 in the region where Z varies from $Z = -75$ to $Z = -68$. Thus in this region it was assumed that w is the weighed average of the electrostatic interaction factors representative of the different conformations present in solution. With a value of Z higher than −68 the molecule was assumed to have reached its native structure and consequently the value of w was kept constant. This

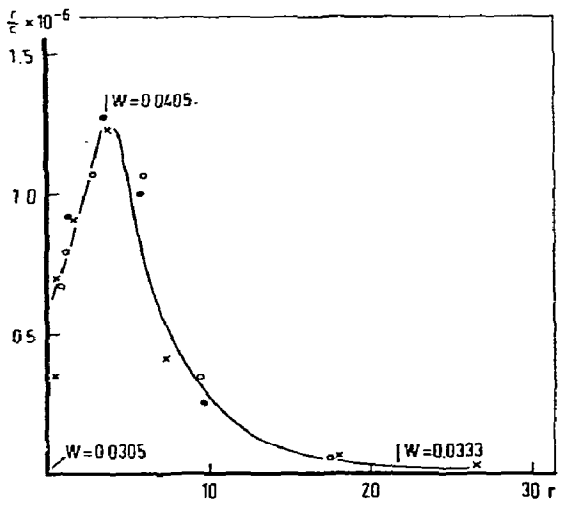

Fig. 5. Scatchard plot calculated according to eq. (5) with $pK_{int,Mn^{2+}} = 1.85$. The factor w varies linearly from 0.0305 to 0.0505 when Z varies from −76 to −68. In the region, where Z varies from −68 to −32, w linearly varies from 0.0405 to 0.0333 (see text). The experimental data were taken from Danchin [5]. × Yeast $tRNA^{Phe}$. ○ E. Coli $tRNA^{fMet}$. ● E coli $tRNA^{Val}$.

analysis was applied to the Mg^{2+} binding to Yeast $tRNA^{Phe}$ obtained at 45°C under the solution conditions indicated in fig. 2 (data from Römer and Hach [9]). It is known that under these conditions the TψC and the DHU stems are intact, while the tertiary structure, the acceptor stem and the anticodon stem are melted out [14]. Addition of Mg^{2+} ions results in a Scatchard plot exhibiting a maximum. Optically monitoring of the structure as a function of temperature shows that during the Mg^{2+} titration the native structure is restored. Our best fit to the Mg^{2+} binding data at 45°C is shown in fig. 4. The curve was obtained with $pK_{int} = 2.45$, while w was varied linearly between 0.016 to 0.022 in the region where Z varies from −75 to −62. In this region the change in conformation takes place. Note that the pK value is the same as that obtained in fig. 2, as was to be expected since the pK value was found to be rather insensitive to changes in temperature. The value of $w = 0.022$ valid for the right hand side of the Scatchard plot (fig. 4) is only slightly lower than the value used in fig. 2.

In the same fashion other "bell-shaped" Scatchard plots can be interpreted. The analysis was for instance applied to the manganese binding data of Danchin [5]. The interpretation of his results is somewhat more involved, however, since the binding experiments were performed at such a low ionic strength (0.005 triethanolamine) that during the addition of Mn^{2+} ions not only the tRNA conformation but also the ionic strength of the solution changes. The best fit to Danchin's data is given in fig. 5. The following parameters were obtained: $pK_{int,Mn^{2+}} = 1.85$, w varies linearly from 0.0305 to 0.0405, when Z varies from −76 to −68. This change of w is due mainly to the conformational change of the tRNA's. In the region where Z varies from −68 to −32, w varies from 0.0405 to 0.0333. This change in w comes from the changes in ionic strength of the solution during titration.

4. Discussion

The present treatment shows that the biphasic Scatchard plots which have been found for the Mg^{2+} and Mn^{2+} binding to native tRNA can be explained by taking into account electrostatic interactions. In other words there is no need to invoke strong and weak Mg^{2+} (or Mn^{2+}) binding sites with different pK_{int} values on tRNA's. Upon binding of Mg^{2+} the apparent binding constant gradually diminishes because the electrostatic contribution to the binding becomes less [see eq. (3)].

"Bell-shaped" Scatchard plots have been shown to occur when during the binding experiments the tRNA structure changes [9]. This consequently produces a change in the electrostatic interaction factor w'. It is interesting to note that after the molecule returns to its native configuration a value of w' is obtained which is the same as found at lower temperature for this structure. Moreover, since such different forms of Yeast $tRNA^{Phe}$ as the intact molecule and the molecule with only the TψC and the DHU stem intact have the same intrinsic binding constant (see preceding section), it seems that Mg^{2+} and Mn^{2+} mainly act by neutralizing negative charge of the tRNA. In this respect it is interesting to note that Mg^{2+} or Mn^{2+} ions are not an absolute requirement to obtain the native structure, at least in solution. It has been demonstrated by Cole et al. [15], Römer and Hach [9] and by Reid and

Robillard [16] that monovalent cations e.g. Na^+ ions at high enough concentration can serve the same purpose.

Electrostatic interactions have also been discussed by Sander and Ts'o [4]. They determined the intrinsic pK value from the "straight part" of the Scatchard plot at relatively low values of r. The curved part of the Scatchard plot at higher values of r was explained by the introduction of an exponential correction term accounting for electrostatic interactions, aggregation effects and changes in ionic strength in the Scatchard equation. However, correction for electrostatic interactions are expected to be most important at lower values of r, where the influence of the charge of the macromolecule is most effective. Consequently the intrinsic pK values presented by these authors represent the pK_{int} plus the complete correction factor for electrostatic interaction [see eq. (3)].

We are well aware of the fact that a good fit to the experimental data can also be achieved with different approaches as for instance given by Schreier and Schimmel [7] and McGhee and von Hippel [17]. In our opinion however the effect of electrostatic interactions will play a dominant role for Mg^{2+} and Mn^{2+} binding to tRNA's. To illustrate this point we compare binding data of Rialdi et al. [6] with results of Römer and Hach [9]. Under the condition that Mg^{2+} binding to native Yeast $tRNA^{Phe}$ was studied, curved Scatchard plots were obtained by both groups. However, at lower ionic strength ($[Na^+] \approx 0.01$) Rialdi et al. found a much higher intercept of the Scatchard plot than Römer and Hach at $[Na^+] = 0.033$. Such a difference in binding behavior to native tRNA can be easily explained on the basis of electrostatic interactions. As was pointed out in relation to fig. 1 a decrease in ionic strength will result in an increase of w and consequently leads to a higher value of the intercept in the Scatchard plots. McGhee and von Hippel show that the binding of ligands covering more than one binding site (as do Mg^{2+} ions) results in non-linear Scatchard plots. Their model cannot however explain the large differences in binding behavior upon a change of ionic strength as described above. Moreover, since straight Scatchard plots are obtained for unfolded tRNA [6,9], effects like those described by McGhee and von Hippel are negligible.

Note: The importance of electrostatic interactions in the binding of Mg^{2+} and Mn^{2+} to tRNA has been independently recognized by J.L. Leroy and M. Guéron (private communication).

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