Interaction of Magnesium Ions with Poly A and Poly U

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Synopsis

The binding of Mg^{++} to poly A and poly U has been measured quantitatively by using the metallochromic indicator calmagite. The method is described in detail. It is shown that there is electrostatic interaction between the binding sites, viz., the phosphate groups, and the intrinsic association constant for the specific binding can be determined. After extrapolation to zero ionic strength we find that, for the binding of Mg^{++} to poly A, $k_{int} = 4 \times 10^4$ and for that, to poly U, $k_{int} = 3 \times 10^4$. The intrinsic enthalpy of association is negative. The effect of Mg^{++} on the secondary structure of poly A and poly U has been studied by measuring the ultraviolet absorbance, optical rotatory dispersion and viscosity as a function of the amount of added Mg^{++} ions. It was found that Mg^{++} promotes the formation of a more ordered secondary structure by neutralizing or screening the negative charges. It is concluded from the absorbance measurements that for poly A at $pH \geq 7$ and for poly U at $pH > 9$ this ordering involves stacking of the bases. Likewise, in solutions of UDP with a pH around 10, base stacking occurs on addition of Mg^{++} .

Introduction

Metal ions, especially Mg^{++} ions, play an important role in the functioning of nucleic acids. Although much work has been done in recent years on the interaction between Mg^{++} and nucleic acids¹⁻³ the phenomena are so complex that our understanding of this subject is far from complete. Much of this complexity is caused by the interplay between the binding of Mg^{++} to the phosphate moieties of the ribose phosphate backbone and the alterations in secondary structure that occur by the screening of the charges. Therefore, besides our quantitative binding studies we have examined the effect of Mg^{++} ions on the secondary structure of poly A and poly U.

Originally there was no agreement about the identity of the binding site of Mg^{++} ions but it is now generally accepted that Mg^{++} does not bind to the bases⁴ but to the negatively charged phosphate groups.⁵ However, the nature of this binding is still not fully elucidated. Some authors⁶ conclude that the binding is specific, others⁷ that diffuse binding predominates. In this paper it is concluded that the binding is specific. This conclusion is based directly on experimental results and not on any complicated polyelectrolyte theory with numerous assumptions.

There is much confusion about the definitions of specific and diffuse binding. In conformity with Lyons and Kotin⁷ we mean by specific or site binding an association of counterions with specific groups on the polymer, which can be described by the law of mass action. On the other hand, we mean by diffuse binding all nonspecific electrostatic interactions between counterions and polyion due to the electric potential of the latter. This interaction is often called ionic atmosphere binding. In most binding experiments the sum of specific and diffuse binding is measured.

Materials and Methods

Poly A and poly U were synthesized by polymerization of the corresponding nucleoside diphosphates.⁸ Special attention was paid to the purification of the polynucleotides. After repeated phenol extraction the aqueous solutions were dialyzed against EDTA and many charges of de-ionized water.

The concentration of the polynucleotides was determined spectrophotometrically after diluting with 0.025*M* Tris, pH 8.5. The molar absorptivity in this medium at the wavelength of the maximum was measured by phosphate analyses.⁹ We found for poly A, $\epsilon_{257} = 10,240 \pm 120$ l./mole-cm and for poly U, $\epsilon_{260.5} = 9250 \pm 40$ l./mole-cm.

Calmagite was a product from Noury-Baker, Deventer, The Netherlands.

The concentration of the titrant solutions was determined by titration with EDTA.

Absorbances were measured with a Zeiss PMQ II spectrophotometer, equipped with accessories so that the solutions in the cuvetts could be magnetically stirred and thermostatted with a temperature constancy better than 0.5°C. Optical rotatory dispersion (ORD) curves were recorded on a Jasco automatic spectropolarimeter model ORD/UV-5. Viscosities were measured with an Ubbelohde capillary viscometer. pH was measured directly in the cuvetts with a Pusi pH meter type 11 Z using a combined calomel-glass electrode (Radiometer, type GK 2024 C). The titrant was added with an Agla micrometer syringe or with a Manostat Digi-pet.

Determination of Mg^{++} Binding Using Calmagite

The metallochromic indicator calmagite¹⁰ was used to determine the concentration of free Mg^{++} in solutions containing a polynucleotide and Mg^{++} . Our method is a refinement of the procedure of Shack and Bynum¹¹ and of Lansink,¹² who used the less stable Erio T. If the undissociated form of calmagite is represented by H_3D , the equilibrium (1) between calmagite and Mg^{++} exists around pH 10:

with the association constant

$$k = \frac{\gamma_{\text{MgD}^-} \gamma_{\text{H}^+}}{\gamma_{\text{HD}^-} \gamma_{\text{Mg}^{++}}} \cdot \frac{[\text{MgD}^-] [\text{H}^+]}{[\text{HD}^-] [\text{Mg}^{++}]} \quad (2)$$

in which γ is the activity coefficient and the square brackets denote molarity of the ion. At constant pH and ionic strength, eq. (2) can be written:

$$k' = [\text{MgD}^-]/([\text{HD}^-] [\text{Mg}^{++}]) \quad (3)$$

When the fraction of calmagite that is converted into MgD^- is α , we get

$$k' = \frac{\alpha}{(1 - \alpha)} \frac{1}{[\text{Mg}^{++}]} \quad (4)$$

If k' is known, the free Mg^{++} concentration can be determined by measuring α . This is done by absorbance measurements. Representing the absorbance of pure HD^- , pure MgD^- and a mixture of these two (all solutions with the same total calmagite concentration) at a certain wavelength by A_{HD^-} , A_{MgD^-} , and A , respectively, we have

$$\alpha = (A - A_{\text{HD}^-})/(A_{\text{MgD}^-} - A_{\text{HD}^-}) \quad (5)$$

A problem arises because k' is not known in the medium of our measurements. Also the total calmagite concentration ($[\text{D}]$) is unknown because the commercial calmagite used was rather impure and efforts to obtain a chromatographically pure product were without success. However it was possible to calculate k' and $[\text{D}]$ from the blank titrations, which consisted essentially of adding Mg^{++} to calmagite in such a way that $[\text{D}]$ remained constant (see below) and measuring the absorbance after each addition. Representing the total Mg^{++} concentration by $[\text{Mg}^{++}]_{\text{tot}}$ we have

$$[\text{Mg}^{++}] = [\text{Mg}^{++}]_{\text{tot}} - \alpha[\text{D}] \quad (6)$$

Substitution of eq. (6) in eq. (4) and some rearrangement gives

$$[\text{Mg}^{++}]_{\text{tot}} [(1 - \alpha)/\alpha] = [\text{D}] (1 - \alpha) + (1/k') \quad (7)$$

or

$$[\text{Mg}^{++}]_{\text{tot}}/\alpha = (1/k') [1/(1 - \alpha)] + [\text{D}] \quad (8)$$

When the left side of eq. (7) is plotted versus $1 - \alpha$ or the left side of eq. (8) versus $1/(1 - \alpha)$, straight lines are obtained from which k' and $[\text{D}]$ can be derived as illustrated in Figure 1. When this paper was in preparation, a paper appeared by Momoki et al.¹³ who investigated this method for determining association constants more systematically.

It is assumed in the foregoing that calmagite is only present as HD^- or MgD^- . Considering the pK values of calmagite, viz., $\text{pK} = 8.14$ for $\text{H}_2\text{D}^- \rightleftharpoons \text{HD}^- + \text{H}^+$ and $\text{pK} = 12.35$ for $\text{HD}^- \rightarrow \text{D}^{3-} + \text{H}^+$,¹⁰ this condition is satisfied for more than 99% only between pH 10.14 and pH 10.35. So it is a drawback of this method that our measurements had to

Fig. 1. Determination of the total calmagite concentration and the association constant of the MgD^- complex at $5^\circ C$ in $0.01M$ trimethylamine HCl, pH 10.2: (a) according to eq. (7), $k' = 4.17 \times 10^5$, $[D] = 5.55 \times 10^{-5}$; (b) according to eq. (8), $k' = 4.10 \times 10^5$, $[D] = 5.56 \times 10^{-5}$.

be confined to this narrow pH range. Equation (5) is based on Beers' law. It was verified that this law is operative for HD^- and MgD^- at the wavelength of the measurements. It has been found by Lindstrom and Diehl¹⁰ that Mg^{++} and calmagite form a 1:1 complex. This is confirmed by the fact that straight lines are obtained in Figure 1. This fact is also a justification for using unpurified calmagite. It means that the impurities do not react with Mg^{++} and have either no absorbance at all at the wavelength of the measurements or an absorbance that cancels by the method of measurement in which all absorbances are differences between solutions containing exactly the same amount of calmagite.

For the binding experiment we made a solution containing polynucleotide, calmagite, and buffer and salt to adjust the ionic strength. In most cases we used trimethylamine HCl as the buffer and tetraethylammonium chloride as the salt, for it is to be expected that the large monovalent cations are less tightly bound electrostatically than, for example, K^+ . To one part of this solution a Mg^{++} solution was titrated containing exactly the same concentration of calmagite, buffer, and salt. After each addition the absorbance was measured, the other part being used as reference. Thus we were able to measure directly the differences in eq. (5). For the determination of A_{MgD^-} we added enough Mg^{++} so that the absorbance remained constant on further addition. With eq. (5) α was calculated and plotted versus $[Mg^{++}]_{tot}$ (see Fig. 2). A blank titration was per-

Fig. 2. Spectrophotometric titration of $1.01 \times 10^{-3}M$ poly A in the presence of $4.5 \times 10^{-5}M$ calmagite with $9.85 \times 10^{-3}M$ $MgCl_2$ at $25^\circ C$ in $0.01M$ trimethylamine HCl, pH 10.1, wavelength $530 m\mu$: (—) free Mg^{++} concentration as function of α as calculated by eq. (4); (a) concentration of free Mg^{++} ; (b) concentration of Mg^{++} bound to calmagite; (c) concentration of Mg^{++} bound to poly A.

formed correspondingly. It was checked that during titration the decrease in pH was no more than 0.1.

It appears from Figure 2 that the curve for the titration of poly A is shifted to the right as compared with the blank. Since a part of the Mg^{++} is bound to poly A this is quite understandable. According to eq. (4), α is a measure of the free Mg^{++} concentration and in the presence of poly A more Mg^{++} has to be added to reach the same α as in the absence of poly A. From the horizontal difference between the two curves and the polynucleotide concentration the number ν of Mg^{++} ions bound per monomeric unit can be calculated. This is correct only if there is no interaction between calmagite and the polynucleotide. The existence of such an interaction could not be detected by absorbance, ORD, or conductivity measurements. In Figure 3 ν is plotted versus $\log [Mg^{++}]$ for the binding of Mg^{++} to poly A and poly U. For comparison the results of experiments with ADP and AMP are also given. It appears that Mg^{++} is scarcely bound to AMP in the region of free Mg^{++} examined as a result of the low association constant of the $MgAMP$ complex.¹⁴ However, this region can not be extended because it is fixed by the properties of calmagite. If it is assumed that the binding sites on ADP and AMP are occupied at $\nu = 1.0$ and on the polynucleotides at $\nu = 0.5$, it follows from Figure 3 that the order of decreasing affinity for Mg^{++} is: poly A > poly U > ADP > AMP. This agrees with results from con-

Fig. 3. Number of Mg^{++} ions bound per monomeric unit (per molecule for ADP and AMP) as a function of the free Mg^{++} concentration. Experimental conditions as in Figure 2.

Fig. 4. Result for the binding of Mg^{++} to ADP from Figure 3 and of a binding experiment with citrate in 0.002*M* trimethylamine HCl, pH 10.0, plotted according to eq. (9).

ductometric titrations.¹⁵ Similar results were obtained for the binding of Ca^{++} to the nucleotides. Because the association constant of CaD^- is about 100 times smaller than that of MgD^- ,¹⁰ these measurements were not very accurate and will not be discussed further.

TABLE I
Association Constant and Number of Binding Sites for the
 Mg^{++} Complexes of Citrate and ADP

	$k \times 10^{-3}$	n	pH	Medium	Ref.
Citrate	5.2	1.22	10.2	0.1M $\text{NH}_4\text{OH-HCl}$	This work
	18	1.12	9.8	0.002M trimethylamine HCl	This work
	16	1.16	10.0	0.002M trimethylamine HCl,	Fig. 4,
	3.6		7.6	$I = 0.16$	17
ADP	13	1.17	10.1	0.01M trimethylamine HCl,	Fig. 4,
	9 ± 2		8.7	$I = 0.004$	18

Discussion

If the binding sites for Mg^{++} on a polynucleotide chain are identical and completely independent, the relation (9) holds:

$$\nu/[\text{Mg}^{++}] = kn - k\nu \quad (9)$$

where k is the association constant and n the number of binding sites per monomeric unit. Plotting $\nu/[\text{Mg}^{++}]$ versus ν gives a straight line from which k and n can be calculated. (Scatchard plot¹⁶).

Fig. 5. Results for the binding of Mg^{++} to poly A and poly U from Figure 3 and to poly U in 0.1M KCl plotted according to eq. (9); I = ionic strength.

This is illustrated in Figure 4 for the binding of Mg^{++} to ADP and citrate. The calculated values of k and n given in Table I together with some other results are in good agreement with results from the literature.

In Figure 5 the Scatchard plots for the binding of Mg^{++} to poly A and poly U are given. The lines are curved so that it must be concluded that the condition of identical and independent sites is not fulfilled. This is not surprising, because the negative phosphate groups are the binding sites, so there must be electrostatic interaction between the identical sites. The affinity for Mg^{++} decreases as more binding sites are occupied. The apparent association constant for the specific binding is a function of the electrostatic free energy of the association. This can be expressed by the following relation.^{19,20}

$$k = k_{\text{int}} \exp \left\{ -ze\psi/k_B T \right\} \quad (10)$$

where T is the absolute temperature, k_B the Boltzmann constant, e the proton charge, and z the valence of the Mg^{++} ions. ψ is the electrostatic potential at a binding site caused by the charges of the other binding sites; thus ψ is dependent on the number of bound Mg^{++} ions, and $\psi = 0$ when $\nu = n$. The intrinsic association constant (k_{int}) can thus be interpreted as the association constant for occupation of the last site on the polymer, i.e., when $\psi = 0$. In this way we do not take into account the charges of the bases in the definition of ψ . It is therefore to be expected that k_{int} will be dependent on pH. Unfortunately, our method did not allow variations in pH so that we could not verify this statement. It is furthermore clear that the association constant can also be dependent on the secondary structure. According to our definition k_{int} is determined by the structure of the polynucleotide at $\nu = n$.

Combination of eqs. (9) and (10) gives, after some conversion,

$$\log \{ \nu / [\text{Mg}^{++}] (n - \nu) \} = \log k_{\text{int}} - 0.868 (e\psi/k_B T) \quad (11)$$

On substituting in (11) the value $n = 0.5$, since one Mg^{++} ion is bound by two phosphate groups, the left side of this equation is solved and can be plotted against ν (see Figs. 6 and 7). These figures can also be regarded as plots of ψ versus ν . By extrapolation to $\nu = 0.5$ we find the value of $\log k_{\text{int}}$.

It appears from Figures 6 and 7 that there is much resemblance between the results for poly A and poly U. An abrupt fall in the logarithmic term at low ν is followed by a gradual decrease. The value of ν at which the abrupt fall occurs is different for poly A and poly U. However we do not attribute this to a specific distinction between the two polynucleotides, for the same difference was found for different preparations of the same polymer. Because of the decreasing accuracy of the measurements with increasing values of ν no reliable results could be obtained for $\nu > 0.3$. So the extrapolation to $\nu = 0.5$ to obtain k_{int} is not entirely without objections.

It can be seen from the figures that the Mg^{++} binding is strongly dependent on ionic strength. This is not surprising, because electrostatic

Fig. 6. The binding of Mg^{++} to poly A at different ionic strengths plotted according to eq. (11). The ionic strengths of 0.055 and 0.275 were obtained by addition of tetraethylammonium chloride; the poly A concentration was $2.53 \times 10^{-3}M$. The other experimental conditions were as in Figure 2. See text for the meaning of the vertical lines.

forces play an important role in this binding. The extrapolated values of k_{int} are not true thermodynamic constants, in agreement with the fact that in eq. (11) concentrations, not activities are written. It is possible to calculate k_{int} at zero ionic strength if we assume that the ionic strength dependence of the binding of Mg^{++} to poly A and poly U is the same as that of the binding of Mg^{++} to ADP, which has been determined by Phillips et al.¹⁸ Inasmuch as our measurements were performed at constant ionic strength we may suppose that as a result of such a calculation the curves are only shifted vertically and do not change their shape. The magnitude of this shift is given in Figures 6 and 7 by vertical lines. Considering the assumptions and the accuracy of the measurements, we find at zero ionic strength for poly A that $\log k_{int}$ is approximately 4.6 and for poly U $\log k_{int}$ is about 4.5. For poly A this association constant refers to specific binding only, because at $v = 0.5$ there cannot be diffuse binding to the phosphate groups, and adenine is uncharged at the pH of our

measurements. (It has been shown by Skerjanc and Strauss⁶ that in the case of DNA diffuse binding can be neglected over the whole titration range.) The pK of the base in poly U is about 10,²¹ so about half of the bases are negatively charged at the pH of our measurements. Therefore there will be diffuse binding of Mg^{++} even when the phosphate groups are completely occupied. However it will be shown in the next section

Fig. 7. The same results as in Figure 5 for the binding of Mg^{++} to poly U plotted (●) according to eq. (11); (○) reduced viscosity of solutions with exactly the same composition as in the binding experiments (η and η_0 are the viscosity of the solution and the solvent, respectively). See text for the meaning of the vertical lines.

that this diffuse binding is very weak, so that the value of k_{int} refers mainly to specific binding. This is in accordance with the great similarity between the binding results for poly A and poly U, for the binding sites on both polymers are identical. In the foregoing also the specific binding of the monovalent cations to polynucleotides is neglected. In the literature there is still much disagreement whether or not such a binding exists, but even the authors that presume this binding find very low association constants.

Fig. 8. Binding of Mg^{++} to poly A at 10 and 25°C in 0.01M trimethylamine HCl, pH 10.1, plotted in such a way that the intrinsic enthalpy of the association can be calculated by eq. (12).

The change in reduced viscosity of poly U by addition of Mg^{++} ions as a measure of the change in secondary structure is also given in Figure 7. At low ionic strength Mg^{++} promotes the formation of a more compact structure; at high ionic strength this is already effectuated by the monovalent cations. The same results were obtained with poly A.

From the temperature dependence of the Mg^{++} binding the intrinsic enthalpy of the association (ΔH_{int}) can be calculated by a formula derived by Tanford:²²

$$\Delta H_{\text{int}} \approx R \left[\frac{\partial(\ln[\text{Mg}^{++}])}{\partial(1/T)} \right]_{\nu} \quad (12)$$

in which R is the gas constant.

This is illustrated in Figure 8, in which $\log[\text{Mg}^{++}]$ is plotted versus ν at different temperatures. At each value of ν the quotient $\Delta(\log[\text{Mg}^{++}])/\Delta(1/T)$ can be taken, after which ΔH_{int} can be calculated with eq. (12). We find for poly A, $\Delta H_{\text{int}} = -2$ to -3 kcal/mole and for poly U $\Delta H_{\text{int}} =$

-4 to -5 kcal/mole. The accuracy of the measurements is too small to attach much value to this difference. However it is certain that ΔH_{int} is negative, in contrast to the positive value found for the enthalpy of the association of Mg^{++} with ADP and ATP.¹⁸

Effect of Mg^{++} Ions on the Secondary Structure of Poly A and Poly U

It is well known that Mg^{++} ions affect the ultraviolet absorbance of nucleic acids. Since Mg^{++} does not bind to the bases this alteration is

Fig. 9. Spectrophotometric titration of $10^{-4}M$ poly U in $0.025M$ trimethylamine HCl, pH 10.9: (●) with $MgCl_2$; (+) with $MgCl_2$ in the presence of $3.5 \times 10^{-4}M$ NaCl; (O) with NaCl. Wavelength $270 m\mu$. On the vertical axis the percentage hypochromic effect is plotted: A = absorbance after addition of metal ions, A° = absorbance before addition of metal ions.

Fig. 10. Spectrophotometric titration of $3 \times 10^{-5}M$ UDP in $0.025M$ trimethylamine HCl, pH 10.7, with $MgCl_2$ and with NaCl. Wavelength $270 m\mu$. See also Figure 9.

Fig. 11. ORD of poly A in 0.01*M* acetate buffer, pH 4.7, in absence ($r = 0$) and presence ($r = 1$) of Mg^{++} . The poly A concentration was $10^{-4}M$. On the vertical axis the molar rotation $[M] = 10 \alpha / lc$ is plotted, where α is the measured rotation in degrees, l the optical path length in decimeters, and c the molarity of the solution.

caused by stacking of the bases that becomes possible after the negative charges of the phosphate groups have been screened.

On addition of Mg^{++} to poly A and poly U a hypochromic effect was observed at the wavelength of the maximum near 260 $m\mu$ without a shift of this wavelength. For poly A in alkaline medium the hypochromic effect reached its maximal value at $r \approx 1$ (r is the number of Mg^{++} ions added per monomeric unit). As expected, a hypochromic effect was not

Fig. 12. ORD of poly A in 0.02M 2-amino-2-methylpropane-1,3-diol buffer, pH 9.0, in absence ($r = 0$) and presence ($r = 1$) of Mg^{++} . The poly A concentration was $10^{-4}M$. See also Figure 11. For comparison the ORD of AMP in the same medium is given.

observed in acid medium since poly A has a double helical conformation in this medium so that base stacking is already maximal. Poly U shows a hypochromicity only at pH > 9. As can be seen in Figure 9 it reaches a maximum value at $r \approx 10$. Hypochromicity can also be obtained with Na^+ ions, but the monovalent ions are much less effective than the divalent Mg^{++} ions. We believe that the negative charge of uracil ($pK \approx 10$)²¹ plays a dominant role in causing this phenomenon. As a result of the repulsion between these charges, stacking is impossible. Screening the charges promotes base stacking, and a hypochromic effect results. Since a large excess of Mg^{++} is necessary for maximal base stacking, it is concluded that the diffuse binding of Mg^{++} to the negative uracil is very weak. It is clear that the same effect can be brought about by other cations. It is rather remarkable that no hypochromic effect is observed at low pH, though poly U has a random coil conformation in that medium and though the affinity for Mg^{++} of poly U is about the same as that of poly A as shown in the preceding section. It seems quite possible that this difference between poly A and poly U is connected with the greater association tendency of purines in comparison with pyrimidines.²³ As already

mentioned, the hypochromicity of poly A becomes constant at $r \approx 1$, in agreement with the high value of the association constant.

To a less extent, the same base stacking as in poly U is also possible in UDP, as shown in Figure 10.

In contrast to the findings of Cheng²⁴ for DNA, the change in the ORD of the polynucleotides by Mg^{++} ions is less than the change in the ultra-violet absorbance. In acid medium the effect of Mg^{++} on the ORD of poly A falls within the limits of experimental accuracy (Fig. 11). In alkaline medium the molar rotation changes about 20% (Fig. 12). These findings agree with the results of the absorbance measurements. Comparison of Figures 11 and 12 shows that in alkaline medium in the presence of Mg^{++} the rotation is much less than in acid medium. However one cannot conclude that little stacking is introduced by the Mg^{++} ions in alkaline medium, since the shape of the ORD curve in acid medium is also determined by the protonation of adenine. For poly U we could not detect an effect of Mg^{++} on the ORD, either at pH 4.8 or at pH 10.0.

Conclusion

There is no difference in the binding of Mg^{++} to poly A or to poly U. There is electrostatic interaction between the binding sites. By extrapolation to complete occupation of the binding sites the intrinsic association constant for the specific binding is found. This constant is of the same magnitude as that of the complex between Mg^{++} and ADP.

It appears from the figures that the electrostatic potential ψ is not a linear function of the charge of the polynucleotides. This is caused, among other things,²⁵ by changes in secondary structure as a result of the addition of Mg^{++} ions. It may be imagined that at low ν the polynucleotides have an extended conformation owing to the mutual repulsion of the negative phosphate groups; at higher values of ν the structure is more compact with stacking of the bases. This stacking is limited to rather short fragments of the polynucleotide chain so that the molecule as a whole has a flexible structure.

It has been suggested by some authors^{6,7} that in native DNA one Mg^{++} ion binds to only one phosphate group owing to the large distance between the phosphate groups in helical DNA. Molecular model building with Courtauld models showed that in our polynucleotides one Mg^{++} ion can bind to two phosphate groups at the same time.

Though Skerjanc and Strauss⁶ also found that the binding of Mg^{++} to DNA is specific, their value of k_{int} is much lower than ours. Besides the large distance between the phosphate groups in DNA this can be caused by the fact that they calculated k_{int} with a formula like eq. (10) by using a theoretical value of ψ .²⁶

Since it is known that Mg^{++} and Mn^{++} bind approximately equally to nucleic acids there is good agreement concerning the value of k_{int} between our results and that of Eisinger et al.²⁷ and of Cohn et al.,²⁸ who determined the specific binding of Mn^{++} to poly A, poly U and some

other polynucleotides by proton relaxation studies. An important difference is however that they found straight lines in the Scatchard plots.

For the binding of Mg^{++} to ribosomal RNA, Goldberg²⁹ found Scatchard plots that bear much resemblance to ours, both in the shape of the curves and the effect of the ionic strength. The binding he measured was about 100 times as weak as in our case.

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