THE INTERACTION OF NUCLEIC ACID WITH Mg-IONS

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SUMMARY

It is a commonly accepted notion that nucleic acid invariably binds divalent cations on the negatively charged sites of their phosphate groups. Evidence is presented here to support the contention that although the negatively charged sites contribute to the energy of binding they are not always the actual sites of binding. Experiments on the relative affinities of native and denatured DNA for divalent cations suggest that the divalent cations are bound much more strongly by the nitrogenous base groups. The most likely groups involved are adenine and guanine because they contain sites favourable for the formation of five-membered chelates. Added support for the involvement of adenine is given by the loss of a substantial portion of the binding in the presence of formaldehyde which is believed to combine with the adenine amino group. These findings have important implications on the interactions of divalent cations with synthetic polynucleotides and naturally occurring nucleic acids which will be dealt with elsewhere.

INTRODUCTION

In a recent paper ZUBAY AND DOTY[1] have demonstrated that native deoxyribonucleic acid (DNA) binds Mg^{++} weakly, whereas thermally denatured DNA binds it strongly to the extent of about 0.71 charge equivalents of Mg^{++} per DNA phosphate. Since thermal denaturation is believed to result in the breakage of hydrogen bonds between the purine and pyrimidine groups, it was suggested that the Mg^{++} was being bound by these basic groups, in particular, the purines adenine and guanine. These groups seemed particularly relevant to the binding problem because they supply molecular configurations suitable for chelation. This paper contains more discussion and evidence in favour of such an interpretation.

EXPERIMENTAL DETAILS

Conductometric titrations were done using an RC-type conductivity bridge (Cambridge Instrument Co. Ltd., England) at 25°.

The DNA used in these experiments was prepared from calf thymus gland according to the method of SIMMONS[1] and subsequently denatured by boiling a salt-free 0.1% solution for 15 min. The formaldehyde-treated DNA was prepared by

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exposure to 2.5 % formaldehyde, pH 6.8, and 25° for 48 h which is the procedure for complete reaction recommended by FRAENKEL-CONRAT[2].

RESULTS AND DISCUSSION

At first glance it might seem strange that Mg^{++} does not bind to native DNA, if the chosen sites are correct, for it appears that the normal DNA hydrogen bond pairing would not interfere with Mg^{++} chelation to the N_7 and N_{10} in adenine or the N_7 and O_{10} in guanine as indicated in Fig. 1. Closer examination, however, reveals good reasons why this is not so. In the first instance Mg^{++} cannot chelate with adenine in native DNA because the H not hydrogen-bonded on the N_{10} is in the plane of

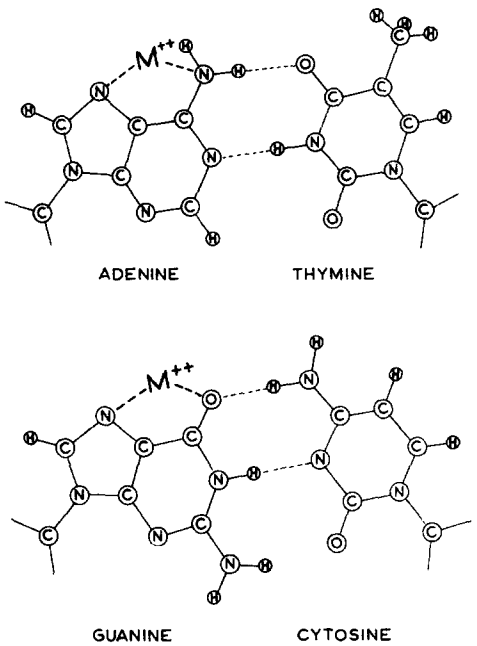

Fig. 1. Illustrating the difficulty of chelating divalent cation (M^{++}) to the purines when they are hydrogen bonded as in native DNA.

chelation preventing the approach of Mg^{++} to form a five-membred ring. Consequently the formation of a five-membered chelate with Mg^{++} requires the rotation of the amine hydrogens by about 90°*, which in turn necessitates the breakage of the hydrogen bond between the adenine N_{10} and the thymine O_{10}. In the second instance, involving the chelation of Mg^{++} to guanine a survey of known Mg^{++}

* It is possible that the amino hydrogens prefer to remain in the plane of the adenine because of the partial double-bond character of the C_6–N_{10} bond. In adenine HCl the C_6–N_{10} bond has a length of 1.27 Å (W. COCHRAN, *Acta Crystallographica*, 4 (1951) 81) corresponding to more than 50 % double-bond character. Unfortunately adenine is not directly comparable with adenine HCl since the formation of a resonant hybrid with a C_6–N_{10} double bond involves a separation of charge in the former. Thus one would expect the C_6–N_{10} to have more double-bond character in the hydrochloride. It seems clear that there will be some shortening of the C_6–N_{10} in adenine over the single-bond length but in view of our ignorance as to its exact amount it is most difficult to say how much stabilization energy will result from this partial double-bond character.

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chelates[3] suggests that Mg^{++} should have a much higher affinity for guanine in the enol form than in the keto form present in native DNA. In order to get Mg^{++} binding therefore, it would first be necessary to convert guanine to the enol form* which would entail breaking the hydrogen bonds between guanine and cytosine. Thus it is not surprising to find a qualitative difference in the affinity of native and denatured DNA for Mg^{++} since in both the cases of adenine and guanine destruction of the hydrogen bonding found in native DNA is a prerequisite to binding.

In most chelates Mg^{++} depends on excess negative charge in the ligand for binding[3]. Here also, this seems to be the case for, although it is now reasonable to believe the Mg^{++} is associated with the purines, there can be no doubt that the phosphate groups contribute to the binding energy by virtue of their negative charge. Indeed the fact that denatured DNA binds less than 1 charge equivalent of Mg^{++} may be explained by the fall off of this electrostatic contribution to the binding energy as the charge becomes neutralised. This lack of saturation of the available purine binding sites is further suggested by the fact that copper, which is known to form stronger chelates than Mg^{++}, binds to a greater extent (0.83 equivalents) under the same conditions[1].

Despite the foregoing evidence as to the site of Mg^{++} binding, there remains some doubt about the affinity of adenine for Mg^{++} because Mg^{++} prefers to chelate with oxygen-containing compounds especially in bidentates. As further evidence that adenine is in part responsible for the Mg^{++} binding, denatured DNA was treated with 2.5% formaldehyde prior to titration. FRAENKEL-CONRAT[2] has shown that formaldehyde reacts selectively with the amine groups of nucleic acid and consequently formaldehyde should reduce the chelating power of the adenine. The results of titrating denatured DNA with $MgCl_2$ before and after formaldehyde addition are

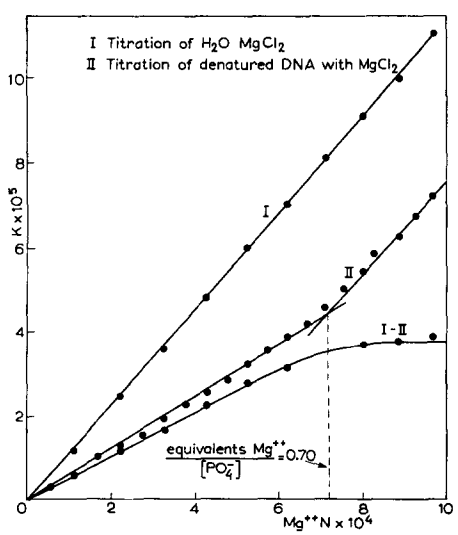

Fig. 2. Titration of denatured DNA with $MgCl_2$.

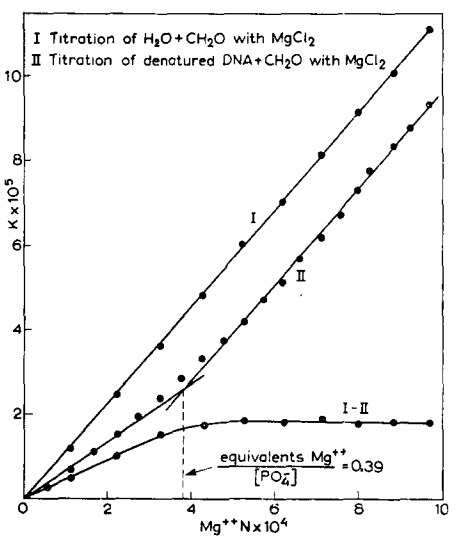

Fig. 3. Titration of denatured DNA + CH_2O with $MgCl_2$.

* Although guanine may be mostly in the keto form when free in solution, the energy necessary to go from one form to the other is probably less than 2.5 kcal or considerably less than the binding energy which may be estimated for the Mg^{++} complex.

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shown in Figs. 2 and 3 where the conductance increment curves for the addition of $MgCl_2$ to both solvent and DNA solution are given. In these graphs the ordinate K represents the specific conductance in units of ohm^{-1} cm^{-1}. The relative decrease in conductance and the position of the break in the conductance increment curves demonstrates that about half the sites for binding Mg^{++} have disappeared in the presence of formaldehyde, and we attribute this to the reaction of formaldehyde with the adenine amino group. In thymus DNA guanine comprises 21 mole % of the basic groups and if all these groups were available for titration in the presence of formaldehyde we should expect to find 0.41 equivalents of Mg^{++} binding per DNA phosphate. In agreement with this expectation the experimental break in the conductance curve occurs at about 0.39.

With the advent of pure synthetic polynucleotides it is now possible to test the proposal made here more directly. This has not been done fully but a step in this direction has been made by STEINER AND BEERS[4] who have observed significant binding of Ca^{++} to polyadenylic acid *via* its effect on the acid titration curve of the polymer.

The suggestion that the adenine could chelate metals in certain appropriately modified chemical forms has appeared elsewhere[5-7]. SZENT-GYÖRGYI[5] was the first to suggest that adenine in conjunction with the triphosphate grouping in ATP was capable of forming a quadridentate with Mg^{++}. This suggestion has received support from the work of EPP, RAMASARMA AND WETTER[6] who have found modification in the infra-red spectra of ATP and ADP (but not AMP) in the purine- and phosphate-absorbing regions in solutions containing Mg^{++}.

The discovery of the point of attachment of Mg^{++} in nucleic acid suggests the manner in which divalent cations might be operative in various stable and dynamic nucleic acid structures. The divalent cation has a profound stabilizing influence on the three-stranded polynucleotide complex discovered by FELSENFELD AND RICH[8] and the site of Mg^{++} binding limits the possible structures the three chains may assume. This point will be elaborated elsewhere[9]. The divalent cation also has a unique stabilizing influence on the ubiquitous microsomal particles[10] which contain about 50 % RNA and it is not unlikely that the metal is bound according to the rules we have suggested. Proceeding to the more speculative dynamic potentialities of Mg^{++} it may be not unreasonable to visualize metal acting as an intermediate complexing agent in the unwinding of the two DNA chains prior to doubling of DNA content and cell duplication or to envisage the metal as playing a role in stabilizing three-stranded polynucleotide structures akin to poly AUU which might be involved in the formation of RNA on a double-stranded DNA template[11].

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