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ARTICLE *in* BIOPOLYMERS · JANUARY 1990

Impact Factor: 2.39 · DOI: 10.1002/bip.360300709 · Source: PubMed

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Raman Spectroscopic Study of the Effects of Ca^{2+}, Mg^{2+}, Zn^{2+}, and Cd^{2+} Ions on Calf Thymus DNA: Binding Sites and Conformational Changes

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SYNOPSIS

The interaction of Mg^{2+}, Ca^{2+}, Zn^{2+}, and Cd^{2+} with calf thymus DNA has been investigated by Raman spectroscopy. These spectra reveal that all of these ions, and particularly Zn^{2+}, bind to phosphate groups of DNA, causing a slight structural change in the polynucleotide at very small metal : DNA(P) concentration ratio (*ca.* 1 : 30). This results in increased base-stacking interactions, with negligible change of the B conformation of DNA. Contrary to Zn^{2+} and Cd^{2+}, which interact extensively with the nucleic bases (particularly at the N7 position of guanine), the alkaline-earth metal ions are bound almost exclusively to the phosphate groups. The affinity of both the Zn^{2+} and Cd^{2+} ions for G·C base pairs is comparable, but the Cd^{2+} ions interact more extensively with A·T pairs than Zn^{2+} ions. Interstrand cross-linking through the N3 atom of cytosine is suggested in the presence of Zn^{2+}, but not Cd^{2+}.

INTRODUCTION

The stabilizing and destabilizing effects of various metal ions on duplex DNA were quickly recognized in earlier studies, as the melting temperature of DNA, usually determined from the change of optical density at 260 nm in the uv spectrum, was found to depend very strongly on the nature of the metal ion.[1,2] Divalent metal ions were classified in the decreasing order Mg^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, and Cu^{2+} for their relative ability to bind to the phosphate groups rather than to the bases, and consequently, to stabilize the double-helical structure of DNA by neutralizing the negative charges on the polynucleotide backbone.

The above results reflect a very complex situation, as metal ions can interact at many different sites on the nucleic bases, with varying effects on the stability and on the conformation of the double-helical biopolymer. Furthermore, as was recently pointed out, the melting temperature of DNA is not only function of the binding of metal ions at specific sites, but it also involves a temperature-labilizing effect as, with increasing temperature, metal ions can have access to binding sites that are normally protected in the duplex, causing an additional weakening of the nucleic acid structure.[3]

The effects of metal ions on the structure of DNA have been studied by a number of particularly sensitive methods. For example, CD, uv, and sedimentation studies have provided very useful information on the overall structure of the nucleic acid (conformation, stacking interactions, flexibility, etc.). However, the determination of specific sites of interaction through such methods is usually very indirect. Vibrational (particularly Raman) spectroscopy, on the other hand, although less sensitive, is often better adapted for this type of studies since it can simultaneously provide information on binding sites, stacking interactions, and conformation. We have recently used Raman spectroscopy to clarify the modes of interaction of Pb^{2+} and Cu^{2+} ions with DNA, the latter having a very high affinity for the bases.[4] In the present paper we use the same method to examine the influence of two types of metal ions, Mg^{2+} and Ca^{2+}, which are generally believed to in-

CCC 0006-3525/90/7-80743-10 $04.00
Biopolymers, Vol. 30, 743–752 (1990)
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teract exclusively with the phosphate groups of nucleic acids. We have also investigated the effect of Zn^{2+} and Cd^{2+} ions, which have a higher affinity for the bases and are particularly interesting due to the fact that they can bring about the renaturation of thermally denaturated DNA.[2,5]

EXPERIMENTAL

The calf thymus DNA (sodium content ranging from 7.2 to 9%) was purchased from the Sigma Chemical Co. and used as supplied. The D_2O (99.98%) was from Merck Frosst Canada, Inc., and the metal salts incorporated in the mixtures were reagent grade, used without further purification.

The DNA/metal–salt mixtures were prepared as follows: A solution of DNA [8% w/w, 0.2 *M* DNA(P)] was first prepared by dissolving Na-DNA in water (or D_2O). It was kept in the refrigerator at 5°C for *ca.* 24 h, with occasional cautious stirring, to ensure the formation of a homogeneous solution. Solutions of the metal salts ($MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, $ZnCl_2$, or $CdCl_2$), 0.005–1.0 *M*, were also prepared in distilled water and their pH, measured with a microelectrode and an Orion Research Model 721 pH meter, was adjusted to 7 ± 0.2 using aqueous NaOH (NaOD). In the final step, the appropriate salt solution was added dropwise to the DNA solution, with constant stirring, to give a mixture with the desired metal : DNA(P) ratio (1 : 40 to 5 : 1), at a final DNA concentration of 4% w/w [0.1 *M* DNA(P)]. It was found extremely important to add the salt solutions to the DNA in a very progressive manner, to ensure the formation of mixtures giving reproducible spectral results.

The Raman spectra of the samples contained in sealed capillary tubes were excited by the 514.5-nm line of a Spectra Physics Model 2020 argon ion laser (at *ca.* 400 mW of laser power at the sample) and recorded on a Spex Model 1400 microcomputer-controlled spectrometer. These spectra were typically recorded at 5-cm^{-1} slit width, with a 2-s integration time at each 2-cm^{-1} frequency increment. They were routinely background corrected by subtracting an appropriate third-degree polynomial function from the original curve. The spectra reproduced in this paper have not been smoothed.

As the various salts studied in this work did not modify appreciably the Raman spectrum of DNA, we have concentrated our efforts on the difference spectra, obtained by subtracting the spectrum of free DNA from that of the mixtures, in order to better show the spectral changes caused by metal–DNA interactions. The subtraction procedure normally requires the presence in the spectra of a reference peak, due to an internal standard (*e.g.* NO_3^-) that is present at the same concentration in the two samples. This peak is annulled in the difference spectrum by an appropriate choice of the multiplication constant in the computer-assisted operation ([DNA + salt] − constant × [DNA]). The use of this technique gave unreliable results with the solutions containing the Zn^{2+} and Cd^{2+} salts. This was due to the tendency of DNA to form heterogeneous mixtures upon addition of the salt solutions. Careful measurements using the 1047-cm^{-1} marker band of NO_3^- ions (10^{-2} *M*) in a 1 : 1 Ca^{2+}–DNA mixture, which forms an homogeneous solution, showed that within experimental error the 786-cm^{-1} peak of DNA was canceled in the conditions in which the marker band was annulled. The latter was therefore chosen in this work as the marker band for the subtraction procedures, with good internal consistency in the results obtained with the various solutions studied. The difference spectra usually showed negative features whose amplitude was less than 25% of the original peaks, with an estimated error of ± 5% (abs.). In the case of Zn^{2+} and Cd^{2+} solutions, where the results were less reliable because of sample heterogeneity, this degree of accuracy was achieved by averaging the results obtained in several independent experiments.

RESULTS AND DISCUSSION

Characteristic difference spectra, obtained by subtracting the spectrum of free DNA from those of the polynucleotide in the presence of Mg^{2+}, Ca^{2+}, Zn^{2+}, and Cd^{2+} ions at various metal : DNA(P) molar ratios, are reproduced in Figures 1–3. The main features in these difference spectra consist of negative peaks, at 1093, 1337, 1374, 1488, and 1578 cm^{-1}. The 1093-cm^{-1} band is related to the phosphate groups, whereas the others arise from the nucleic bases. The intensity changes of the various peaks in the spectra can be measured directly from the figures, taking into account the factor (× 3 or × 6) by which the difference spectra have been amplified in order to best show the changes occurring in the presence of the metal ions.

Binding to Phosphate Groups

Metal binding to the phosphate groups in DNA are best reflected in the Raman spectra by the decrease in intensity of the 1093-cm^{-1} band, which arises

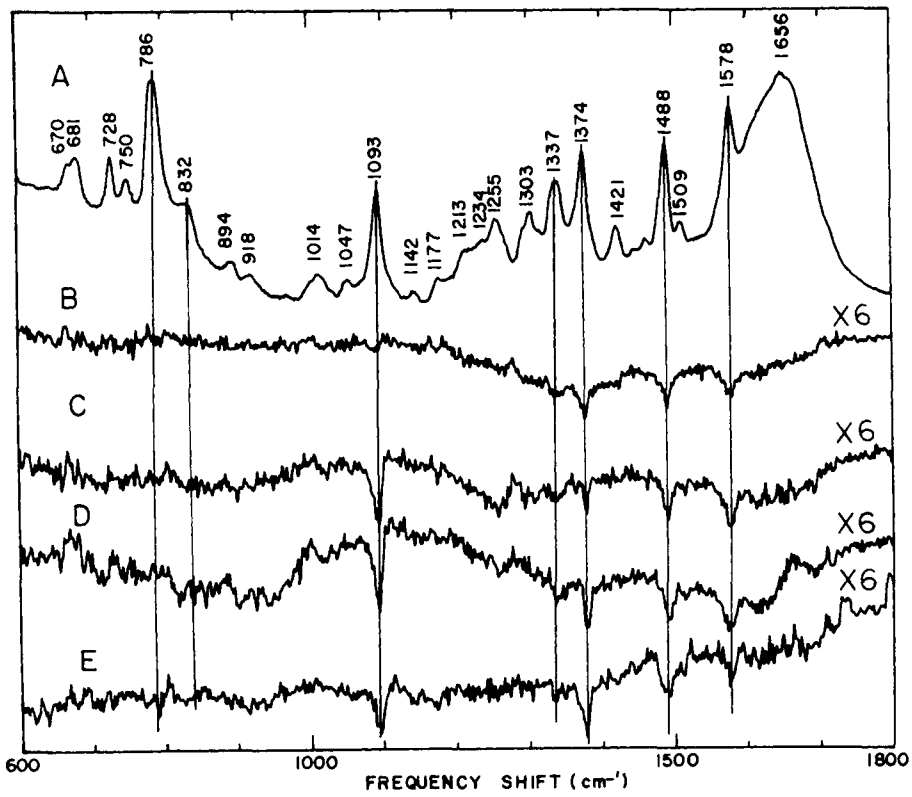

Figure 1. (A) Raman spectrum of aqueous (4% w/w) calf thymus DNA. Difference spectra obtained by subtracting the spectrum of an aqueous solution of DNA from the corresponding spectrum in the presence of Ca^{2+} ions at a (B) 1 : 5, (C), 1 : 1, and (D) 5 : 1 metal : DNA(P) ratio. (E) Difference spectrum for Mg^{2+}-DNA, at 1 : 1 metal : DNA(P) ratio.

from the symmetric PO_2^- stretch. This effect is observed to various extent in all spectra shown in Figures 1–3, depending on the type of metal ion and on its relative concentration. A similar behavior was observed in our previous Raman study with Pb^{2+} and Cu^{2+} ions,[4] and a decrease in intensity of the 1088-cm^{-1} ir band, relative to the antisymmetric mode at 1225 cm^{-1}, was also detected upon binding of Cd^{2+} ions by calf thymus DNA.[6]

There are a number of ways in which a metal ion can interact with the negatively charged PO_2^- groups of DNA, depending on the nature and on the exact site of this interaction. Purely electrostatic interactions of the phosphate groups with the hydrated metal ions are not believed to modify appreciably, in either intensity or frequency, the 1093-cm^{-1} band of DNA. More likely, the observed intensity decrease in the Raman spectra is caused by a much more direct type of interaction (covalent binding) with one particular oxygen atom of the phosphate group, giving a $-P(=O)-O-metal^+$ type of complex. The formation of a $-PO_2^- \cdots metal^{2+} \cdots N7(purine)$ chelate, previously suggested,[7] would also be consistent with the experimental results.

Assuming that the decrease in intensity of the 1093-cm^{-1} band is proportional to the amount of metal directly bound to the phosphate groups, it is possible to compare the relative affinity of the different metal ions for these groups. For both Mg^{2+} and Ca^{2+}, a value of a few percent is found at a relative metal : DNA(P) ratio of 1 : 5, reaching near 10% at 1 : 1 ratio and leveling off at a slightly higher value at a 5 : 1 concentration ratio (Figure 1). In the same conditions, the proportion of phosphate-bound Ca^{2+} is comparable to that of Mg^{2+}. The amount of bound metal as a function of its relative concentration is apparently proportional to the observed increase of the melting temperature of DNA, at least in the case of Mg^{2+} and Ca^{2+}, for which the effect is more important in the lower metal concentration range.[1,2]

Earlier thermal denaturation studies of DNA in the presence of metal ions have indicated that the ratios of base/phosphate binding affinities of the

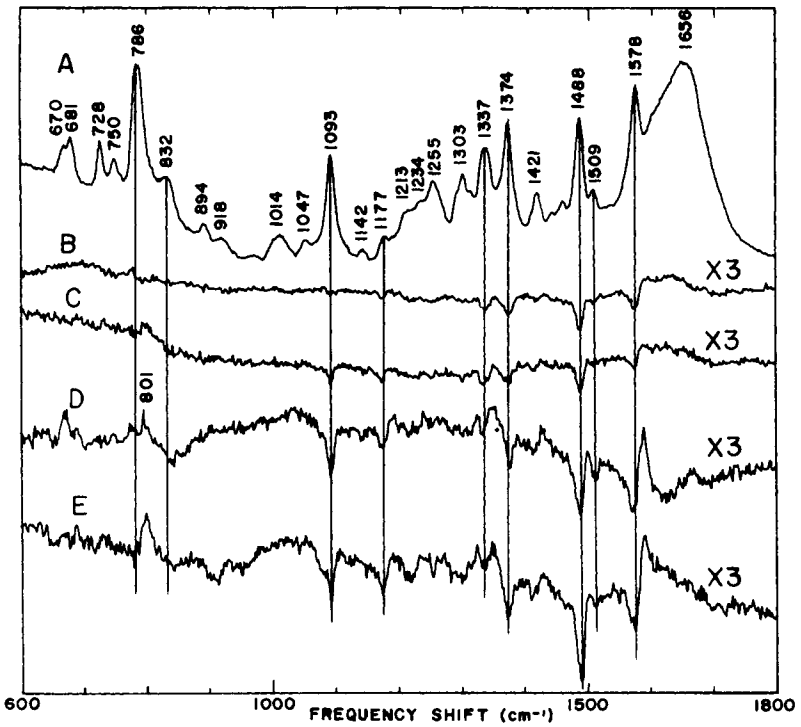

Figure 2. (A) Raman spectrum of aqueous (4% w/w) calf thymus DNA. Difference spectra obtained by subtracting the spectrum of an aqueous solution of DNA from the corresponding spectrum in the presence of Zn^{2+} ions at a (B) 1 : 5, (C) 1 : 1, (D) 4 : 1, and (E) 5 : 1 metal : DNA(P) ratio.

metal ions investigated here increased in the order $Mg^{2+} < Zn^{2+} < Cd^{2+}$.[2] The present results (Figure 2) further indicate that the affinity of Zn^{2+} ions for the phosphate groups is higher than that of the alkaline–earth ions: the proportion of phosphate-bound Zn^{2+} ions at a 5 : 1 metal : DNA(P) ratio (20%) is practically twice that of Mg^{2+} and Ca^{2+}. This is in agreement with the conclusion from a recent differential pulse-polarographic study of the effect that phosphate groups are the preferential binding sites for Zn^{2+} ions at low metal : DNA(P) ratio.[3] That the relatively hard Zn^{2+} ions interact more strongly with the phosphate groups than the softer Cd^{2+} ions is expected from the theory of hard, intermediate, and soft acids and bases. However, the preferential binding of Zn^{2+} ions, compared to the harder Mg^{2+} and Ca^{2+} species, is unexpected. This could mean that the mode of interaction of the latter is partly of the electrostatic type, which is not expected to have an appreciable effect on the intensity of the 1093-cm^{-1} band.

It was concluded from a recent ir study[6] that Cd^{2+} ions do not bind, except in a loose electrostatic manner, to phosphate groups at metal : DNA(P) ratios < 3. Our findings do not support this conclusion. We find that the ability of Cd^{2+} ions to bind directly to phosphate groups is comparable to that of Mg^{2+} and Ca^{2+} ions, at least for metal : DNA(P) ratios ≤ 4 (Figure 3). At a ratio of 5 : 1, the Raman spectrum becomes very perturbed and the amount of bound Cd^{2+} drops to a very low value (*ca.* 5%). As will be discussed later, these changes result from a denaturation of DNA and the migration of Cd^{2+} ions from phosphate groups to new binding sites that become available after the opening of the DNA double helix.

Interaction of Mg^{2+} and Ca^{2+} Ions with the Bases

The stabilizing effect of Mg^{2+} (and, presumably, Ca^{2+}) ions on the structure of DNA, shown by thermal denaturation experiments,[1,2] has been interpreted by its ability to bind to the phosphate groups rather than to the nucleic bases. This conclusion was confirmed from CD[7] and uv[7,8] measurements, and from a study of the stability constants of Mg^{2+}–nucleoside phosphate systems.[9] Although alkaline–earth ions have very little affinity for the nucleic bases, metal complexes involving these ions have

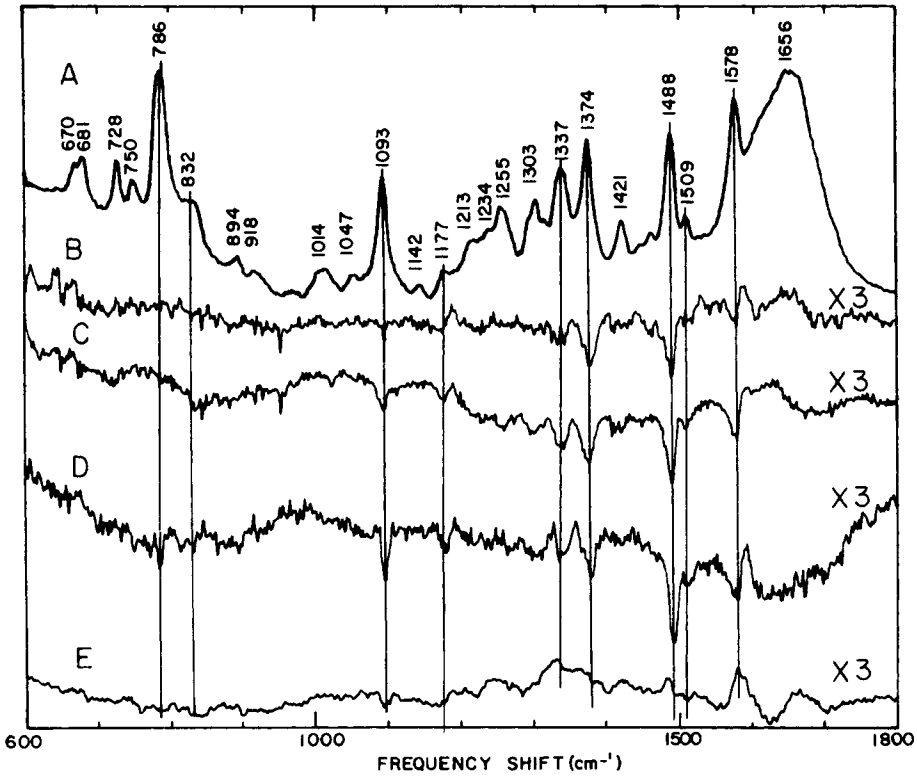

Figure 3. (A) Raman spectrum of aqueous (4% w/w) calf thymus DNA. Difference spectra obtained by subtracting the spectrum of an aqueous solution of DNA from the corresponding spectrum in the presence of Cd^{2+} ions at a (B) 1 : 5, (C) 1 : 1, (D) 4 : 1, and (E) 5 : 1 metal : DNA(P) ratio.

been isolated and characterized. In Ca–TMP · $6H_2O$, the metal ions interact only with the phosphate group,[10] but in Ca–IMP · $6.5H_2O$, Ca^{2+} ions are bound to the N7 atom of inosine, as well as the PO_3^{2-} group and O2 and O3 sugar hydroxyl groups.[11] Magnesium ions bind directly to the N7 sites of guanine in poly(dG · dC) · poly(dG · dC), with an indirect metal–phosphate interaction, stabilizing the left-handed Z-DNA.[12] They are also bound indirectly to the N7 and O6 position of guanine (*via* water molecules) in the crystalline d(CpGpCpGpCpG) polymer, resulting in Z-DNA formation.[13] The possibility of a similar type of interaction in DNA has been suggested from ir studies on thin films prepared by evaporation of a 2.5 : 1 Mg-DNA solution.[8]

Besides the negative peak at 1093 cm^{-1}, which characterizes the binding of Ca^{2+} and Mg^{2+} ions to the phosphate groups of DNA, the difference Raman spectra obtained with Mg^{2+}-DNA and Ca^{2+}-DNA mixtures at relative concentrations ranging from 1 : 40 to 5 : 1 [metal : DNA(P)] show negative features in some of the regions where strong DNA bands occur, namely at 1337, 1374, 1488, and 1578 cm^{-1} (Figure 1), indicating a decrease in the intensity of these peaks of the nucleic bases in the presence of the metal ions. That these changes could be due to binding by the nucleic bases, as in the examples cited above, is partly substantiated from recent quantum-mechanical calculations,[14,15] which have indicated that several sites on the DNA bases are favorable for Mg^{2+} and Ca^{2+} binding, namely the N7-O6 position of guanine and the N3 atom of adenine. However, the intensity modifications detected here are not very large (5% on average) and they occur even at very low metal : DNA(P) ratios (*e.g.*, 1 : 10), with little change upon increasing the relative metal concentration. This behavior is not that expected for normal binding of the metal ions by the phosphate groups of DNA, which is shown from the behavior of the 1093-cm^{-1} peak to increase progressively as the metal concentration is raised. Since the above bands are known to decrease in intensity with increasing base-stacking interactions (Raman hypochromism),[16] we believe the observed spectral modifications are for the most part caused by a structural change in DNA, which occurs at low metal concentration, as will be discussed further below.

Binding of Zn^{2+} Ions by the Bases

Zinc ions have a dual action on DNA; up to a metal : DNA(P) ratio of 1 : 1 they stabilize the double helix, after which a destabilizing effect takes place.[2] These ions are also quite special in that they bring about the total reversibility of the denaturation of DNA, without the need of increasing the ionic strength of the solution after recooling.[5] This means that Zn^{2+} ions interact with the DNA bases in such a way as to keep the complementary bases in close proximity in the unwound stage.

The Raman difference spectra of Zn^{2+}-DNA solutions (Figure 2) are very similar to those obtained with Mg^{2+} and Ca^{2+}; they show a negative peak at 1093 cm^{-1}, characteristic of phosphate bonding, and several other features at frequencies corresponding to bands of the nucleic bases. The latter become visible at very low metal : DNA(P) ratios (*e.g.*, 1 : 20) and they can be explained in part by a hypochromic effect associated with increased base-pair stacking interactions, as in the spectra with the alkaline-earth ions. However, the intensity changes with Zn^{2+} are more pronounced than with Mg^{2+} and Ca^{2+}. For example, the effects on the 1488- and 1578-cm^{-1} bands are on the order of 15% at low Zn^{2+} : DNA ratio, increasing to *ca.* 30% at high metal concentrations, whereas these changes are *ca.* 5% with Mg^{2+} and Ca^{2+} over the whole concentration range.

The loss in intensity of the band at 1488 cm^{-1}, which is predominantly due to a guanine vibrational mode with a strong N7-C8 stretching component,[17] is of particular interest as a similar intensity change was observed in DNA alkylated in the N7 position of guanine.[18] The change in Zn^{2+}-DNA solutions is therefore a clear indication of bonding of Zn^{2+} ions at the N7 position of the guanine bases. The intensity change in the 1578-cm^{-1} region, accompanied by a slight shift to higher frequency, is also consistent with binding at either the N7 or N1 position,[19] although the latter is probably not involved, being engaged in interstrand hydrogen bonding and therefore protected against metal attack in duplex DNA.

Binding of Zn^{2+} ions to different types of DNA has been shown by uv difference spectroscopy to be proportional to their G·C content, and CD measurements have indicated the suppression of protonation of G·C base-pair region at acidic pH on the addition of transition metal ions.[7] These observations were interpreted in terms of preferential binding of these ions by chelation of the N7 atom of guanine and a neighboring phosphate group, as in crystalline $[Zn(IMP)]_n$.[20] Quantum-mechanical calculations have also indicated that simultaneous coordination to N7 and C6=O of guanine is energetically favored among the various possibilities for the binding of Zn^{2+} ions by A·T and G·C base pairs.[21] Although the present results clearly indicate coordination at the N7 position of guanine, it is not possible to tell if this is accompanied by simultaneous binding to the C6=O group or to a neighboring phosphate group. Similarly, the results are consistent with, but no proof of, preferential binding along specific sequences of bases (GpG and GpC) in the same DNA strand, as proposed on various occasions.[22-24]

The Raman difference spectra give clear indications that A·T base pairs are also involved in the metalation process. The intensity changes are not very important for adenine bands: the weak peak at 1509 cm^{-1} clearly loses some intensity with increased ion concentration, and that at 1338 cm^{-1} shows a constant loss over the whole concentration range. This latter effect is but slightly larger than with Ca^{2+} ions and it probably also arises from a hypochromic effect associated with increased stacking interactions in the presence of the metal ions. On the other hand, the 1374-cm^{-1} peak (mostly due to thymine, but with a small contribution from adenine) decreases in intensity with increased metal concentration, to more than 15% at a 5 : 1 metal : DNA(P) ratio (compared to 5% or less with Mg^{2+} and Ca^{2+}). An important loss of intensity is also seen in the small thymine band at 1177 cm^{-1}. Finally, the intensity ratio of the 1580-1672-cm^{-1} bands (*ca.* 1.2 in D_2O solution) is much lower than with the alkaline-earth ions (*ca.* 1.4) as a result of the decreased intensity of the 1580-cm^{-1} guanine band and the 1672-cm^{-1} peak is shifted to lower frequency by 3 cm^{-1} (compared to 2 cm^{-1} with Ca^{2+} ions). Quantum-mechanical calculations have indicated that metal binding at either N7 or N3 of A in A·T pairs induces a positive charge at N6 and H6, thus strengthening the hydrogen bond with O4 of the T complementary base.[21] This, in turn, decreases the frequency of the 1672-cm^{-1} band, which is due to a coupled stretching motion of the C4=O and C5=C6 bonds.[25] Such an effect clearly occurs in 1-methylthymine, where the band at 1664 cm^{-1} in aqueous solution shifts to 1647 cm^{-1} in the solid, in which the molecules are tightly bound in pairs through N-H···O4 hydrogen bonds.[26]

Thermal denaturation studies have shown that Zn^{2+} ions progressively destabilize the DNA double helix at metal : DNA(P) ratios > 1,[2] but the exact cause of this effect is still unknown. Quantum-me-

chanical calculations[21] have indicated that binding of Zn^{2+} ions at either the N7 or N3 positions of G, which are the most likely targets for metal attack in G·C base pairs, should stabilize these units. Binding of Zn^{2+} ions to N7 of A, and O2 of T, in A·T pairs is not expected to occur to a large extent, and it should not influence appreciably the stability of these pairs. On the other hand, the calculations have indicated that binding at N3 of A is favored and that it leads to a weakening of the hydrogen bond between N1 of A and H3–N3 of T, resulting in a slight overall destabilization of A·T base pairs.[21] However, this conclusion appears to be inconsistent with the observation that Zn^{2+} ions continuously increase the T_m of the DNA-like polymer poly[d(A·T)] with increasing metal concentration (from *ca.* 46 to 55°C for a 3 : 1 metal : polymer ratio).[5] It is therefore likely that the overall destabilization of DNA at high metal concentrations is due to many different factors, including metalation at sites normally protected by interstrand hydrogen bonding in the double helix.

Several models have been proposed to explain the metal-facilitated rewinding of DNA by ions such as Cu^{2+}, Cd^{2+}, and Zn^{2+}. In some of these models, interstrand cross-linking has been proposed, such as with the N3 atom of C and N7 of G.[22,27,28] A charge transfer to the N3 atom of C has also been suggested.[23] These models have been questioned, as the binding specificity of the labile metal species originally linking complementary base pairs in denatured DNA is not very high, so that slippage of the nucleic acid strands along these binding points would be expected to occur.[24] However, they find support in the present findings, since a positive peak near 800 cm^{-1} in the difference spectra of Zn^{2+}-DNA solutions at metal : DNA(P) ratios $\geq$ 1 (Figure 3) is consistent with binding at N3 of C.[19] Note that this effect is not observed in Cd^{2+}-DNA solutions.

Binding of Cd^{2+} Ions by the Bases

The effect of Cd^{2+} ions on the melting temperature of DNA is somewhat similar to that of Zn^{2+} ions, except that the destabilization effect starts at a lower metal : DNA(P) ratio (0.5 as compared to 1 in the case of Zn^{2+}) and it is more severe, lowering the T_m by 10°C at a 3 : 1 metal : DNA(P) ratio.[2] This has been interpreted by a higher affinity of Cd^{2+} ions for the bases. Cd(II) has a strong affinity for N7 of G (and I), as in the complexes $Cd_2(IMP)_3 \cdot 12\ H_2O$[29] and $Cd(GMP) \cdot 5\ H_2O$,[30] but it can also react with A, as in a binuclear complex of adenine, in which Cd^{2+} is coordinated to the N3 atom of A.[31]

Up to a Cd^{2+}-DNA concentration ratio of 4 : 1, the Raman spectra (Figure 3) indicate that the mixtures have the same general behavior as the Zn^{2+}-DNA solutions, although the magnitude of the intensity changes are not exactly the same. As mentioned above, the intensity decrease of the 1093-cm^{-1} band indicates that the binding of Cd^{2+} ions by phosphate groups is somewhat less than of Zn^{2+} ions. The loss in intensity of the 1337- and 1374-cm^{-1} bands, on the other hand, is larger (by 25–50%) with Cd^{2+} than with Zn^{2+}, and the shift of the thymine band at 1672 cm^{-1} in D_2O is also larger, at high metal concentration, with Cd^{2+} solutions (4 cm^{-1} instead of 3 cm^{-1} for Zn^{2+}), suggesting that Cd^{2+} ions have a higher affinity for A·T pairs. Binding of Cd^{2+} ions to G is also shown from the intensity changes of the 1488 and 1578 bands, which are, on the average, comparable to those with Zn^{2+}. However, these intensity changes do not increase linearly with metal ion concentration, and they seem to level off for metal : DNA(P) ratios > 2 : 1. This is probably due to a slight but progressive denaturation of DNA, which is known to cause an intensity increase of the 1488- and 1578-cm^{-1} bands,[16] offsetting the decrease caused by binding to G.

From the above observations, the lesser stability of Cd^{2+}-DNA adducts with respect to the equivalent complexes with Zn^{2+} can be explained by a lesser affinity of Cd^{2+} ions for the phosphate groups and, possibly, by a higher affinity for A·T pairs. With increasing metal concentration, denaturation occurs at a metal : DNA(P) ratio $\geq$ 5 : 1, as indicated by major changes in the Raman spectrum. Contrary to Zn^{2+}-DNA solutions, Cd^{2+}-DNA mixtures [at metal : DNA(P) ratios $\leq$ 4] do not show in their difference spectra any positive feature near 800 cm^{-1}, indicative of the involvement of N3 of C in an interstrand metal bridge or in the formation of a charge transfer complex capable of holding the two DNA strands together during thermal denaturation.

If Cd^{2+} ions can allow complete renaturation of DNA, even in the absence of interstrand metal bridging, then the same effect has to be achieved from metal interactions within individual strands. Several models have been proposed in the past, in which the metal ions are assumed to be bound preferentially to specific sequences of bases in DNA, such as GpC[22] and GpG.[23,24] This type of binding has been observed in $Zn(MeGMP)_2 \cdot 4H_2O$ and $Zn(MeIMP)_2 \cdot 4H_2O$, where two adjacent molecules of GMP and IMP are linked by a Zn^{2+} ion through coordination at the N7 position.[24] Quantum-mechanical calculations have shown that metal-ion

(Zn^{2+}) binding to guanine by coordination at the N7 and C6=O positions reinforces all hydrogen bonds between the G · C base pairs.[21] It seems therefore possible that the preferential binding of metal ions to G and the resulting increase in hydrogen-bond energy in G · C pairs transform G · C-rich segments of DNA into anchor points that would not be broken in the normal thermal denaturation process. This would provide an alternate explanation beside metal bridging for the complete renaturation of DNA after cooling.

The difference spectrum obtained with the 5 : 1 Cd^{2+}-DNA mixture (Figure 3E) is much more complex that for the 4 : 1 metal : DNA(P) ratio and it points to partial denaturation of DNA. In particular, the increased intensity of the peaks at 1488, 1578, and *ca.* 1240 cm^{-1} (positive features in the difference spectrum) and the decrease of the 832-cm^{-1} band, characteristic of the B-form conformation, indicate appreciable unstacking of the bases[16] and some loss of ordered structure at this high metal concentration. Spectral changes in the 1300–1400-cm^{-1} region, rich in A and T vibrations, and in the 1600–1700-cm^{-1} region (in D_2O solution the 1672-cm^{-1} band shows a red shift of 8 cm^{-1}), where several vibrations of T are active, suggest that base pairs in A · T-rich regions of DNA become separated. Normally protected groups, such as N1 of A, and N3-H and C4=O of T, are now available for metal binding, and some ions migrate from the phosphate groups to fill these new sites.

The situation here is somewhat similar to that with Cu^{2+} at a 1 : 2 metal : DNA(P) ratio,[4] the latter type of ion having a much more pronounced destabilizing effect on double-helical DNA.[2] One major difference, however, is found in the region of the guanine bands at 1488 and 1578 cm^{-1}, which are shifted to higher frequencies (1494 and 1580 cm^{-1}) with Cu^{2+}, whereas only a slight red shift of the 1488-cm^{-1} band, probably due to partial denaturation,[16] is observed with Cd^{2+}. Another characteristic difference is found with Cu^{2+} that, unlike Cd^{2+}, gives a large positive peak near 800 cm^{-1} in its difference spectrum with DNA; this is similar to the Zn^{2+} case, although the effect is more pronounced. This suggests that Cu^{2+} ions, contrary to Cd^{2+}, causes G · C base pairs to pull apart, this being followed by metalation at the N1 position of G and N3 of C.

The present results on Cd^{2+}-DNA mixtures are at variance with some of the conclusions reached in a recent Raman and ir study of this system.[6] In particular, it was concluded in this latter study that metal direct binding on PO_2^- groups does not occur until a metal : DNA(P) ratio of 1 is reached and that no significant complexation on the bases occurs at low metal concentrations. These restrictions are not confirmed by our own observations. Note, however, that the earlier study was done with 0.15 *M* NaCl solutions and that it was restricted to metal : DNA(P) ratios ≤ 3, so that the large spectral changes associated with denaturation at ratios ≥ 5 were not observed.

Effect of Divalent Metal Ions on the Conformation of DNA

Many studies, mostly by uv and CD spectroscopy, have indicated that aqueous DNA undergoes a conformational change from the B form to a "C-like" structure in the presence of divalent metal ions.[7,22,32,33] Our results do not support such a drastic change in structure, although it is not certain that the comparison is meaningful, as the measurements were made in quite different conditions: the Raman spectra were recorded at DNA concentrations that are several orders of magnitude higher than those for the absorption and CD studies, and at metal : DNA(P) ratios that are generally much lower.

The Raman spectrum of C-DNA differs from that of the B form mainly in that the 832-cm^{-1} band is not present in the former and the phosphate band at 1094 cm^{-1} in B-DNA is shifted to 1104 cm^{-1} in the spectrum of the C form.[34] No such shift of the 1093-cm^{-1} band was detected in any of the spectra presented here, although a decrease in intensity of the 832-cm^{-1} band was observed with the Cd^{2+} solutions at metal : DNA(P) ratios ≥ 1 and, to a smaller extent, with the Zn^{2+} solutions. This loss of B-type double-helical structure is interpreted by a partial denaturation of DNA in the present case. A change to the A form can also be considered, especially since the differential curves obtained from the solutions with Zn^{2+} show a positive peak near 800 cm^{-1} that corresponds approximately to the characteristic peak of A-DNA at 806 cm^{-1}.[35] However, the phosphate band occurs at 1099 cm^{-1} with this type of structure, giving negative and positive peaks at 1092 and 1102 cm^{-1}, respectively, in the difference spectrum derived from the B and A forms. This is not observed in our spectra. Finally, a change to the Z form can also be excluded, as this should be indicated by a clear negative peak at 615 cm^{-1} and positive feature at 682 cm^{-1} in the differential spectrum.[36] Note, however, that in view of the experimental error associated with our data and the intensity of the bands characteristic of DNA structure, any departure from B conformation would

probably have to affect at least 10% of the nucleic acid to be detected.

Independently of the changes that could occur in DNA at relatively high metal concentration, the spectra suggest that some modifications take place at very low metal : DNA(P) ratios. These are probably not related to a denaturation process, as they also occur with the alkaline-earth metal ions. One of the most striking effect in this regard is the decrease in intensity of several Raman bands of the nucleic bases, which occurs at a metal : DNA(P) ratio as low as 0.1. Another parameter, which can be monitored very accurately by the technique of "true" Raman difference spectroscopy,[37] is the frequency shift of the 1672-cm^{-1} thymine band, measured in D_2O solutions. As shown in Figure 4 (in which the log scale is used for convenience), this indicates that a well-defined modification occurs in DNA solutions at a metal : DNA(P) ratio of *ca.* 1 : 30.

The above phenomenon could be explained by preferential interactions of the metal ions with certain sequences of DNA, such as GpG or CpG, as suggested in certain models.[22-24] Since these sites are not numerous (*e.g.*, 4% GpG sequences in a single strand of calf thymus DNA, on a statistical basis), they would quickly become saturated and the effect would be restricted to low metal : DNA(P) ratios. The spectral changes observed could then be explained by a slight distortion of the DNA double-helical structure as a result of this type of binding. The weak point in this argument is the fact that the observed spectral changes also occur with alkaline-earth ions, which are not believed to interact very strongly with the nucleic bases.

A more likely explanation of these results is a cooperative structural change in the DNA structure resulting from a partial neutralization of charges on the phosphate backbone. The effect would then be nearly independent of the nature of the divalent metal ions, inasmuch as their ability to bind to PO_2^- groups is not too different. Whatever the nature of this structural change, no major departure of the B type of conformation of DNA is indicated by the spectra. An increase in base-stacking interactions is suggested, however, possibly resulting from a small change in the winding angle of the biopolymer. But it is not clear if the structural changes suggested from the Raman spectra are related to the transition to a C-like structure suggested from the uv absorption and CD spectra.

Summary

The following conclusions are derived from our study of the Raman spectra of solutions of calf thymus DNA in the presence of Mg^{2+}, Ca^{2+}, Zn^{2+}, and Cd^{2+} ions at various metal : DNA(P) ratios:

—All of these metal ions can bind in a covalent manner to the phosphate groups, the affinity being roughly the same for Mg^{2+}, Ca^{2+}, and Cd^{2+}, and somewhat higher for Zn^{2+}. This stabilizes the double-helical structure of DNA and,

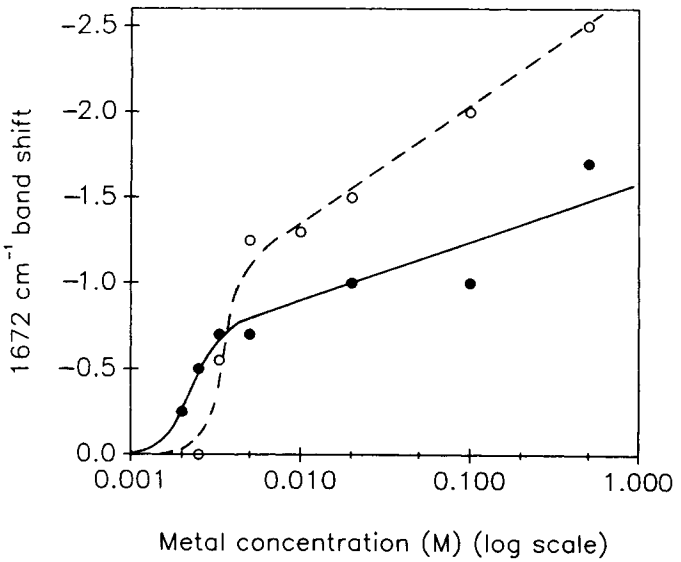

Figure 4. Shift of the 1672-cm^{-1} band of DNA (in D_2O) in the presence of Zn^{2+} (O---O) and (● —— ●) Ca^{2+} ions, as a function of the metal : DNA(P) ratio.

at a very small concentration of the metals [metal : DNA(P) ratio *ca.* 1 : 30], causes a slight structural change in the polynucleotide, resulting in increased base-stacking interactions.

—The alkaline-earth metal ions interact very little, if at all, with the nucleic bases.

—The preferred site of interaction for Zn^{2+} and Cd^{2+} ions is the N7 position of the guanine bases, but these two types of metal ions also interact with the exposed sites on the A · T base pairs, the amount of bound ions becoming progressively more important as the sites on G · C pairs become saturated at the higher metal concentrations.

—Zn^{2+} and Cd^{2+} ions have different relative affinities for the G · C and A · T base pairs, and this could have an effect on their relative destabilizing effect on DNA at high metal concentration.

—Cross-linking interactions, involving the N3 position of cytidine bases, is also suggested in the presence of Zn^{2+}, but not Cd^{2+}

We are indebted to the Natural Sciences and Engineering Research Council of Canada and to the Ministère de l'Education du Québec for financial support of this work.

REFERENCES

1. Eichhorn, G. L. (1962) *Nature* **194,** 474–475.
2. Eichhorn, G. L. & Shin, Y. A. (1968) *J. Am. Chem. Soc.* **90,** 7323–7328.
3. Swiatek, J., Pawlowski, T., Gasiorowski, K. & Kozlowski, H. (1987) *Inorg. Chim. Acta* **138,** 79–84.
4. Tajmir-Riahi, H. A., Langlais, M. & Savoie, R. (1988) *Nucleic Acids Res.* **16,** 751–762.
5. Shin, Y. A. & Eichhorn, G. L. (1968) *Biochemistry* **7,** 1026–1032.
6. Alex, S. & Dupuis, P. (1989) *Inorg. Chim. Acta* **157,** 271–281.
7. Zimmer, Ch., Luck, G. & Triebel, H. (1974) *Biopolymers* **13,** 425–453.
8. Bhattacharyya, R. G., Nayak, K. K. & Chakrabarty, A. N. (1988) *Inorg. Chim. Acta* **153,** 79–86.
9. Frey, C. M. & Stuehr, J. E. (1972) *J. Am. Chem. Soc.* **94,** 8898–8904.
10. Trueblood, K. N., Horn, P. & Luzzati, V. (1961) *Acta Crystallogr.* **14,** 965–982.
11. Brown, E. A. & Bugg, C. E. (1980) *Acta Crystallogr.* **B36,** 2597–2604.
12. Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., Van Boom, J. H., Van der Marel, G. & Rich, A. (1979) *Nature (London)* **282,** 680–686.
13. Gessner, R. V., Quigley, G. J., Wang, A. H.-J., Van der Marel, G. A., Van Boom, J. H. & Rich, A. (1985) *Biochemistry* **24,** 237–240.
14. Sagarik, K. P. & Rode, B. M. (1983) *Inorg. Chim. Acta* **76,** L209–L212.
15. Sagarik, K. P. & Rode, B. M. (1983) *Inorg. Chim. Acta* **78,** 177–180.
16. Erfurth, S. C. & Peticolas, W. L. (1975) *Biopolymers* **14,** 247–264.
17. Majoube, M. (1984) *J. Chim. Phys.* **81,** 303–315.
18. Mansy, S. & Peticolas, W. L. (1976) *Biochemistry* **15,** 2650–2655.
19. O'Connor, T., Bina, M., McMillin, D. R., Moller Haley, M. R. & Tobias, R. (1982) *Biophys. Chem.* **15,** 223–234.
20. DeMeester, P., Goodgame, D. M. L., Jones, T. & Skapski, A. C. (1974) *Biochim. Biophys. Acta* **353,** 392–394.
21. Anwander, E. H. S., Probst, M. M. & Rode, B. M. (1987) *Inorg. Chim. Acta* **137,** 203–208.
22. Förster, W., Bauer, E., Schütz, H., Berg, H., Akimenko, M., Minchenkova, L. E., Evdokimov, Yu. M. & Varshavsky, Ya. M. (1979) *Biopolymers* **18,** 625–661.
23. Richard, H., Schreiber, J. P. & Daune, M. (1973) *Biopolymers* **12,** 1–10.
24. Miller, S., VanDerveer, D. G. & Marzilli, L. G. (1985) *J. Am. Chem. Soc.* **107,** 1048–1055.
25. Susi, H. & Ard, J. S. (1974) *Spectrochim. Acta Part A* **30,** 1843–1853.
26. Guay, F., Beauchamp, A. L., Gilbert, C. & Savoie, R. (1983) *Can. J. Spectrosc.* **28,** 13–20.
27. Eichhorn, G. L. (1981) *Adv. Inorg. Biochem.* **3,** 1–46.
28. Zimmer, Ch., Luck, G., Fritzsche, H. & Triebel, H. (1971) *Biopolymers* **10,** 441–463.
29. Goodgame, D. M. L., Jeeves, I., Reynolds, C. D. & Skapski, A. C. (1975) *Nucleic Acids Res.* **2,** 1375–1379.
30. Aoki, K. (1976) *Acta Crystallogr. B* **32,** 1454–1459.
31. Wei, C. H. & Jacobson, K. B. (1981) *Inorg. Chem.* **20,** 356–363.
32. Hanlon, S., Brudno, S., Wu, T. T. & Wolf, B. (1975) *Biochemistry* **14,** 1648–1662.
33. Hanlon, S., Chan, A. & Berman, S. (1978) *Biochim. Biophys. Acta* **519,** 526–536.
34. Erfurth, S. C., Kiser, E. J. & Peticolas, W. L. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 938–941.
35. Prescott, B., Steinmetz, W. & Thomas, G. J., Jr. (1984) *Biopolymers* **23,** 235–256.
36. Benevides, J. M. & Thomas, G. J., Jr. (1983) *Nucleic Acids Res.* **11,** 5747–5761.
37. Savoie, R., Langlais, M. & Beauchesne, P. (1987) *Appl. Spectrosc.* **41,** 671–674.

Received August 21, 1989
Accepted May 7, 1990