A laser Raman spectroscopic study of the interaction of calf-thymus DNA with Cu(II) and Pb(II) ions: metal ion binding and DNA conformational changes

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ABSTRACT

The interaction of calf-thymus DNA with Cu(II) and Pb(II) ions has been investigated in H_2O and D_2O solutions at physiological pH, using laser Raman spectroscopy. The results confirm the destabilizing effect of Cu^{2+} ions, which are shown to bind strongly to the guanine and cytidine bases, perturbing the A-T base pairs and disrupting the double-helical structure of DNA, whose conformation is markedly altered by these interactions. Earlier claims that Pb^{2+} ions destabilize DNA are not supported by the present study. These ions are found to interact only weakly with the nucleic bases, binding to the N7 position of the guanine bases and also interacting with the A-T pairs. Both types of ions are found to interact with the charged phosphate groups of DNA, although these sites are preferred over the nucleic bases by Pb^{2+} ions.

INTRODUCTION

The stabilizing and destabilizing effects of metal ions on the double-helical structure of DNA have been extensively studied (1,2). It has been demonstrated that Cu(II) destabilizes the DNA double helix, whereas alkaline earth metal cations have an opposite effect (3). It is generally believed that metal ions such as Cu^{2+} and Cd^{2+} bind directly to the nucleotide bases, causing a disruption of the hydrogen bond network between the two strands of DNA and lowering its T_m. Metal ions such as Mg^{2+}, on the other hand, bind to the phosphate groups, neutralizing the charges on the DNA backbone and stabilizing the double-stranded structure (3).

Cu(II) binding to native and synthetic DNAs as well as to nucleotides and polynucleotides has been reviewed, and the crystal structures of several copper complexes containing nucleobases have been reported (4). It has been suggested that Cu^{2+} ions bind to the N7 and O6 atoms of guanine and the N3 atom of cytidine in G-C base pairs, with no direct interaction with the A-T pairs of DNA (5, 6). On the other hand, copper coordination to the nitrogen atoms of the purine and pyrimidine bases, as well as to the phosphate oxygen atoms, of DNA components have been indicated from x-ray diffraction and spectroscopic measurements (4). Conflicting results have been obtained on

DNA-Pb interactions in the past. It was originally claimed that Pb^{2+} ions destabilize double-helical DNA (although to a lesser extent than Cu^{2+} ions),suggesting preferential binding to the nucleic bases (7). However, a recent polarographic and CD study (8) has indicated a stabilizing effect of the Pb^{2+} ions at metal:DNA(P) ratios as high as 10, although a lowereing of the T_m was observed at higher concentration ratios (e.g. 50). The preferential binding of Pb^{2+} ions to PO_2^- groups has also been shown to occur with nucleotides, in particular with ATP (9).

Various optical methods, such as NMR, UV, and CD spectroscopy have been widely used to characterize the nature of DNA-Cu interactions (1, 2). However, little use has been made of Raman spectroscopy for this purpose (10, 11). Since this method is a powerful tool in elucidating the nature of biomolecule-metal ion binding and in detecting conformational changes which sometimes result from these interactions, we have used it in the present investigation to better understand the nature of Cu(II) and Pb(II) binding to DNA in aqueous solution.

MATERIALS AND METHODS

The calf-thymus DNA (sodium salt) was purchased from the Sigma Chemical Co. and used as supplied. The D_2O (99.98%) was from Merck and reagent grade $Cu(NO_3)_2 \cdot 3H_2O$ and $Pb(NO_3)_2$ salts were used without further purification.

A solution of DNA (4% w/w, 0.1 M DNA(P)) was prepared by dissolving Na-DNA (40 mg) in a solution of $NaNO_3$ (10^{-2} M) in double distilled water (or D_2O) (1 ml). It was kept in the refrigerator at 5°C for several days, with occasional strirring, to insure the formation of a homogeneous solution. This solution was then mixed with a solution of the metal salt (copper or lead nitrate) 0.04 or 0.08 M (0.5 ml) to give mixtures with the desired metal:DNA ratio (1:2 or 1:1). The pH (pD) of the solutions, measured with a micro-electrode and an Orion Research Model 721 pH meter, was adjusted to 7 ± 0.2 using NaOH (NaOD) (0.1M). After the addition, a greenish blue precipitate was obtained with Cu(II) and a white solid formed with Pb(II). This was transferred with the supernatant solution to a capillary tube for the Raman measurements. The spectra were obtained from the solids immersed in the mother liquor. From their Raman spectra, the supernatant solutions were found to contain no DNA, except in the case of the 1:1 mixture with Cu^{2+}. An atomic absorption determination of the metal content of the supernatant solutions indicated that 55% of Cu^{2+} ions are fixed by the DNA in

a 1:2 (metal:DNA(P)) mixture, as compared with 30% and 25% for Pb^{2+} ions in the 1:2 and 1:1 solutions respectively.

The Raman spectra, recorded on a Spex Model 1400 microcomputer-controlled spectrometer, were excited by the 514.5 nm line from a Spectra Physics Model 2020 argon ion laser. The sealed capillary tubes containing the highly laser sensitive samples were mounted on a motor shaft and rotated at high speed in the laser beam (250 mW at the sample) to prevent decomposition. The 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 here have not been smoothed.

RESULTS AND DISCUSSION
Cu(II)-DNA binding

The Raman spectrum of aqueous calf-thymus DNA in the presence of Cu(II) at a 1:2 metal:DNA(P) ratio is compared with that of the pure nucleic acid in Fig. 1. In this figure we also show difference spectra obtained by subtracting the spectrum of DNA from that of the mixture. Due to the presence of a precipitate as well as of the supernatant solution in the samples studied, it was not possible to use an external intensity standard for the subtraction procedure. Using the phosphate band at 1093 cm^{-1} as an internal intensity standard led to a largely positive difference spectrum (Fig. 1.C). Arbitrarily increasing the weight of the subtracted spectrum by 50% gave a difference spectrum which shows better the differential features associated with band shifts (Fig. 1.D). These same features were observed in the difference spectrum of a 1:1 metal:DNA(P) mixture (not shown). The corresponding spectra in D$_2$O solution were recorded in the 1500 - 1750 cm^{-1} region (Fig. 2) in order to detect the spectral changes which are normally overshadowed by the water band near 1650 cm^{-1}. The weak peaks at 1523 an 1624 cm^{-1} in Fig. 2.B suggest that at least partial deuterium exchange has occurred in the solution of DNA in D$_2$O. However, this should not affect the interpretation of the results, as the main spectral changes observed involve the strong peaks at 1580 and 1672 cm^{-1}, which are not affected appreciably in either frequency or intensity by deuteration (12).

A comparison of the spectra shown in Fig. 1 indicates that Cu^{2+} ions have a pronounced effect on the structure of DNA. The intensity increase of certain bands, namely at ca. 730, 1240, and 1580 cm^{-1} (uncompensated positive

features in the difference spectrum, Fig. 1.D) are similar to those observed upon melting of DNA (13) and point to reduced base stacking and base pairing interactions in the complex. One also notes that the 835 cm^{-1} band characteristic of the B conformation of DNA disappears in the presence of Cu^{2+} ions. This band, which arises from the antisymmetric stretching of the OPO diester group, has been observed to shift to a lower frequency, at ca. 810 cm^{-1}, upon a B to A conformational change (12, 13) and to 800 cm^{-1} in the spectrum of the Z form (14,15). In the present case, a change to the latter is not indicated, as this modification should also cause the guanine band at 680 cm^{-1} to shift to 627 cm^{-1}, which is not observed. Similarly, the A form is excluded as the strong peak characteristic of this conformation at 810 cm^{-1} is not present in the spectrum of the complex. On the other hand, the difference spectrum shows a weak positive feature near 875 cm^{-1}, which has been observed upon metallation of DNA by cis and trans-DDP (dichlorodiammine-
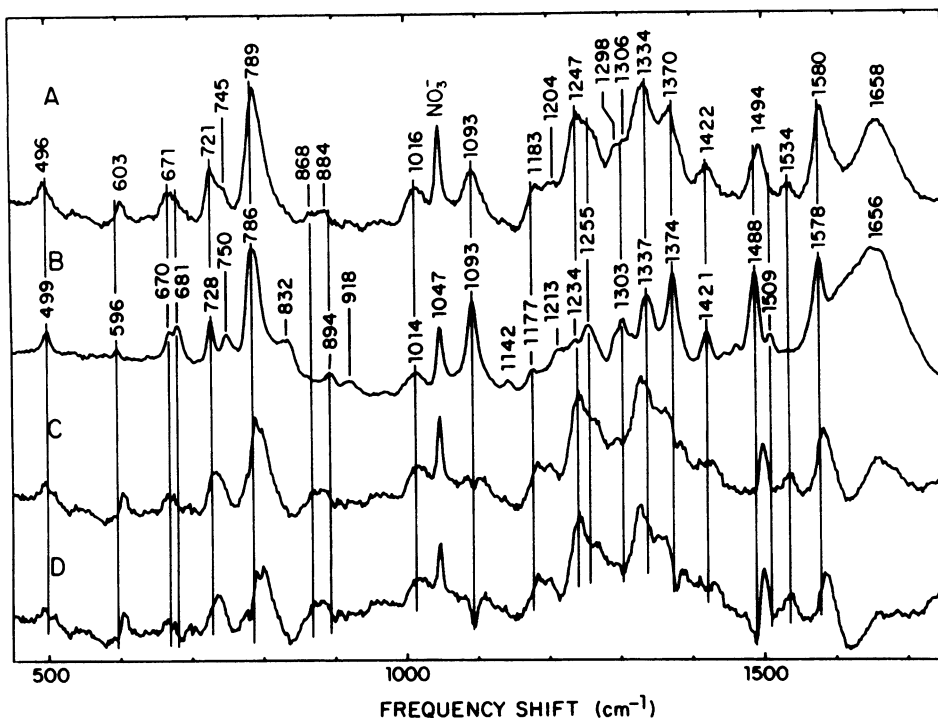

Figure 1. Raman spectra of (A) calf-thymus DNA in the presence of Cu(II) at a 1:2 metal:DNA(P) molar ratio and (B) free aqueous DNA. Difference spectra: (C) A - B and (D) A - 1.5 B.

platinum(II)) (16) and is characteristic of the C form of DNA (17, 18).
However, the symmetric PO_2^- vibration has a rather high frequency (1104 cm^{-1})
in C-DNA (17), whereas no shift of the 1093 cm^{-1} band has been observed here
upon Cu^{2+} interaction. Therefore, we conclude that the altering of the B
conformation of DNA by copper ions is caused by a denaturation process rather
than a change to another specific ordered structure of the macromolecule.

The difference spectra in Figs. 1 and 2 contain several derivative
features due to band shifts which occur upon metallation of DNA by Cu(II).
In particular, the two bands at 1486 and 1578 cm^{-1}, which are mostly due to
guanine vibrations (12), shift to higher frequencies upon complex formation,
an effect which is opposite to that observed upon DNA melting (13). Previous
studies on 5'-GMP have shown that the effects on the Raman spectrum of the
binding of different electrophiles at the N1 and N7 positions are quite
similar (16, 19). The major difference between these two types of
interactions is the reduced intensity of the 1578 cm^{-1} band associated with
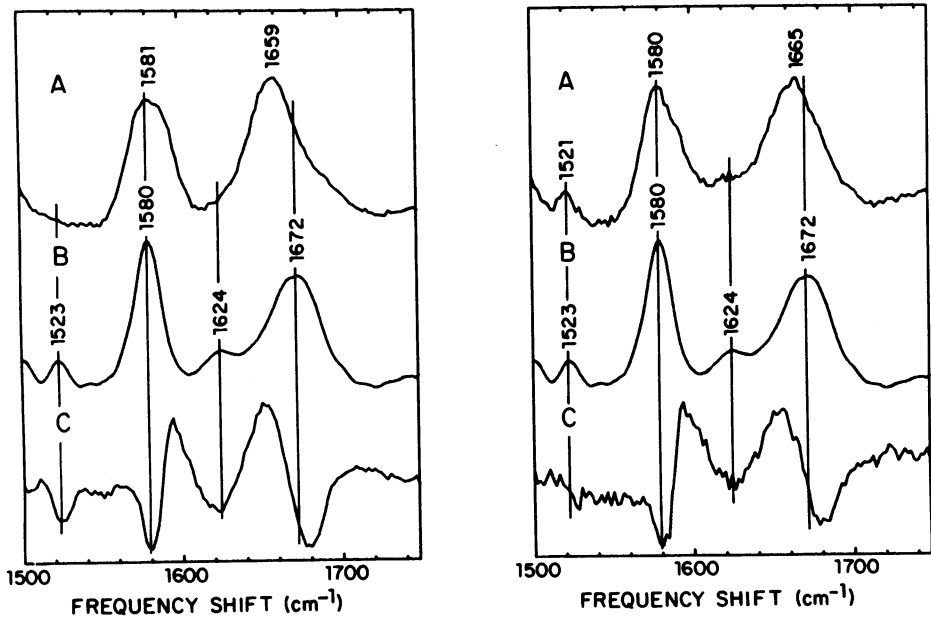N1 metallation. In the present case, the difference spectra (Fig. 1)

Figure 2. Raman spectra in D_2O solution of (A) calf-thymus DNA in the
presence of Cu(II) at a metal:DNA(P) molar ratio of 1:1 (left) and 1:2
(right) and (B) free DNA. (C) Difference spectra (= A - B)

indicate similar intensity changes for the 1486 and 1578 cm^{-1} bands, which suggests that Cu^{2+} ions bind to the N7 position of the guanine bases, in the major groove of DNA. This type of complexation appears to be similar to that taking place with cis-DDP (16), which is known from crystallographic studies to bind at the N7 site of guanine (20). However, binding of Cu(II) to the N1 position of guanine bases cannot be discarded, and the present results are also be compatible with interstrand bridging through simultaneous binding to the N3 position of cytidine. Interstrand crosslinking of DNA by Cu(II) has been suggested previously (6) and is consistent with the observed renaturation of DNA upon removal of the metal ions (3).

The cytidine bases are also purturbed by copper ion interactions, as evidenced by the shift to higher frequency of the strong band at 786 cm^{-1}. This band, which is displaced to lower frequency upon DNA melting (13), has been observed to shift in the opposite direction upon N3 coordination of CH$_3$Hg$^+$ (16). The change in the intensity of the band at 1522 cm^{-1} (D$_2$O), assigned to the cytidine bases (12), also indicates the involvement of these bases in the Cu(II) coordination process. Note that N3 of cytidine is normally hydrogen-bonded to guanine in the DNA double helix, but coordination of Cu(II) at this site has been shown by x-ray diffraction to occur in a 2:1 cytosine-copper chloride complex (21).

Two main models have beeen proposed for the Cu(II) - DNA interaction. In one of these models, a copper ion is bound to N7 of guanine and to N3 of cytidine in the same base pair, the former being flipped over from its normal position to make such coordination possible (5, 6). In the second model, a Cu^{2+} ion interacts with two adjacent guanines in the same strand (likely at the N7 site), with a charge transfer to the N3 atom of the complementary cytosine bases (22). Both of these models involve interactions of copper at the guanine N7 and cytidine N3 sites. It is not possible to favor any one of these two models from the present observations which are in general agreement with both possibilities.

As seen in Fig. 2, a very large shift of the thymine band of DNA at 1672 cm^{-1} (in D$_2$O) occurs in the presence of Cu^{2+}, to 1665 and 1659 cm^{-1} respectively at 1:2 and 1:1 Cu^{2+}:DNA(P) ratios. This behavior parallels that observed in dTMP (1663 to 1648 cm^{-1}) (19) and in 1-methylthymine (1664 to 1654 cm^{-1}) (23) upon N3 coordination of CH$_3$Hg$^+$. The shift of the 1374 cm^{-1} band to 1370 cm^{-1} is also similar to that of 1-methylthymine (1374 to 1368 cm^{-1}) upon mercuration (23). However, the melting of DNA gives the same spectral changes (1673 to 1658 cm^{-1} in D$_2$O and 1374 to 1371 cm^{-1} in H$_2$O) (13)

and it is difficult to conclude from the observed frequency shifts in the presence of Cu(II) that these changes are due to metal coordination rather than to a denaturation of the double helix. Note that the N3 position of thymine is recessed in the minor groove of the double helix of DNA and that the substitution of the proton on this atom necessitates the breaking of A-T base pairs.

Interaction of Cu^{2+} with adenine is indicated by the shift of the 1338 cm^{-1} band to 1334 cm^{-1} and the very strong intensity increase of this band in the spectrum of the complex. This intensity change, together with the decrease in intensity of the peak at 1374 cm^{-1}, due to thymine, is similar to that observed upon platination of DNA (24). It is very difficult to determine from the Raman spectra which binding sites of adenine are involved (16, 25, 26). The N7 position is readily accessible in the major groove of the duplex and the N1 site would be available after denaturation. Interaction of Cu(II) with the A-T base pairs of DNA has been suggested in previous studies (22, 27).

Other binding schemes for the interaction of Cu(II) with DNA have been proposed, such as a chelation between N7 of guanine and the closest phosphate group in the same strand (22) or a binding to phosphate groups only (28). The only indication of Cu^{2+} interaction with phosphate groups comes from the difference spectrum shown in Fig. 1.C, where a large positive value is obtained in all regions when the 1093 cm^{-1} phosphate band is taken as the reference. We believe that there is in fact a decrease in the intensity of this characteristic band following the binding of some of the copper ions to phosphate groups.

Pb(II)-DNA binding

The Raman spectrum of a mixture of 0.1 M DNA(P) with Pb(II) at a 1:1 molar ratio is shown in Fig. 3. As was the case with Cu^{2+} (Fig. 1), the subtraction spectrum obtained using the 1093 cm^{-1} phosphate band as an internal intensity standard (Fig. 3.C) gives positive features in the regions where vibrational bands occur. The presence of these features at ca. 790 and 1580 cm^{-1} cannot be ascribed to denaturation in this case, as this type of transformation would also be characterised by a very strong band near 1240 cm^{-1} in the difference spectrum. The subtraction spectrum shown in Fig. 3.D, which seems more appropriate, was obtained so as to best cancel out the DNA bands.

The Raman spectra indicate that the mode of binding of Pb^{2+} by DNA is quite different from that of Cu^{2+} and that its effects on the conformation of

the macromolecule is far less important. In fact, as revealed by the 835 cm^{-1} region of the spectrum, the B conformation of DNA remains essentially unchanged in the adduct. Furthermore, as was explained above, the results indicate that the coordination process does not affect the double-helical structure of the biopolymer, the base pairs remaining stacked in the complex precipitate.

The difference spectrum in Fig. 3.D indicates a decrease in the intensity of the 1093 cm^{-1} peak, which is believed to reflect the binding of Pb^{2+} ions by phosphate groups of DNA, as a similar effect has been observed in the Raman spectrum of ATP following the interaction of divalent metal ions with its PO$_2^-$ groups (29, 30). There are several indications that the Pb^{2+} ions interact almost exclusively with the phosphate groups of DNA at the relative concentrations used in the present study. The most convincing proof comes from the difference spectra in Figs. 1 and 3, which show that the
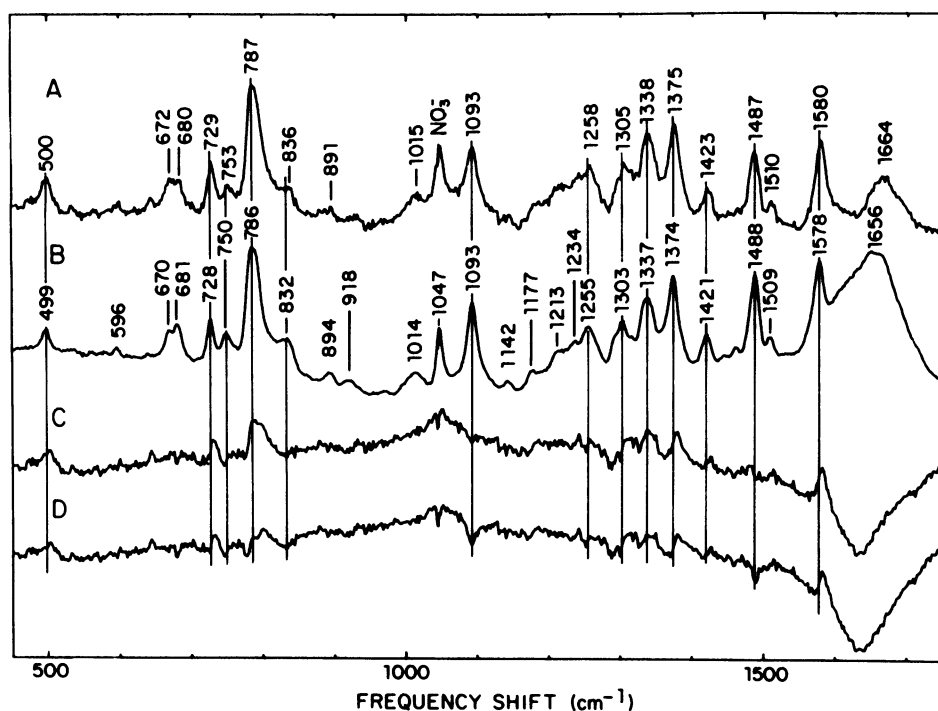

Figure 3. Raman spectra of (A) calf-thymus DNA in the presence of an equimolar amount of Pb(II) and (B) free aqueous DNA. Difference spectra: (C) A - B and (D) A - 1.3 B.

perturbations to the nucleic bases are much more important in'the case of the mixture with copper than that with lead. This occurs in spite of the fact that the proportion of bound metal, as determined by the atomic absorption measurements on the supernatant solutions (see the experimental section) is higher in the 1:1 Pb-DNA mixture (1 Pb/3 DNA bases) than in the 1:2 Cu-DNA mixture (1 Cu/4 DNA bases). The present findings are at variance with the earlier claim that Pb^{2+} ions destabilize double-helical DNA at nearly equal metal and DNA(P) concentration (7). They are, however, in agreement with the recent polarographic and CD measurements which indicated that at metal:DNA(P) ratios lower than approximately 15 the interactions take place mostly with the phosphate groups (8). This type of preferential binding also occurs with nucleotides, such as with ATP (9, 30).

The weak features occuring in the region of the vibrational bands of DNA in the difference spectra (Figs. 1.D and 3.D) show that Pb^{2+} ions interact much more weakly than Cu^{2+} ions with the DNA bases. Furthermore, the spectral changes observed suggest that the sites of interaction may be different for the two types of ions. For example, the spectrum of the Pb(II)
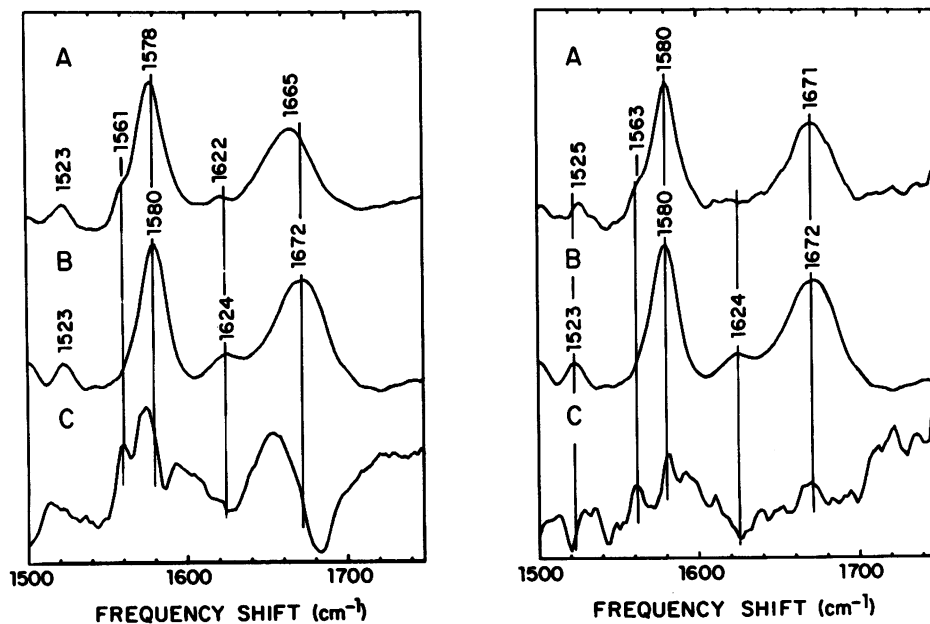

Figure 4. Raman spectra in D_2O solution of (A) calf-thymus DNA in the presence of Pb(II) at a metal:DNA(P) molar ratio of 1:1 (left) and 1:2 (right) and (B) free DNA. (C) Difference spectra (= A - B)

complex in D_2O solution (Fig. 4) shows a new band occuring as a shoulder at
ca. 1560 cm^{-1} on the low frequency side of the 1580 peak, whereas the
corresponding spectrum with Cu(II) (Fig. 2) shows instead a high-frequency
shoulder near 1595 cm^{-1}. This possibly indicates that Pb^{2+} ions interact
preferably with A-T base pairs, as the 1550 - 1580 cm^{-1} region to the
spectrum of adenine is known to be very sensitive to metal coordination (25,
26). Interactions with A-T pairs is also suggested from the shift of the
thymine band from 1672 to 1665 cm^{-1} in the 1:1 metal:DNA mixture and from the
small shifts of most bands, which are attributable to thymine and guanine
bases, in the 1200 to 1400 cm^{-1} region.

Some degree of interaction of Pb^{2+} ions with the guanine bases is also
indicated from the spectra. In particular, the difference spectrum (Fig.
3.D) shows that the guanine band at 1488 cm^{-1} loses some of its intensity
upon interaction of Pb(II) with DNA. This intensity change is similar to
that observed in DNA alkylated in the N7 position of guanine (31) and
probably reflects Pb^{2+} binding at this same position. In the case of Cu^{2+}
binding, a similar mode of interaction was suggested above, but the decrease
in intensity to the 1488 cm^{-1} band could not be observed as it was masked by
an opposite change caused by the denaturation of the double helix. An
important spectral difference in the interaction of Cu(II) and Pb(II) with
DNA is the appreciable shift of the 1488 cm^{-1} band (to 1495 cm^{-1}) in the Cu
complex, an effect which does not occur in the presence of Pb(II). This
could be due to the binding of Cu(II), but not of Pb(II), at the N1 position
of guanine.

CONCLUSION

Although the Cu^{2+} and Pb^{2+} ions are both classified as Lewis acids of
intermediate strength, the behavior of Pb(II) in the presence of DNA shows
that it is harder than Cu(II). Consequently, it binds preferentially to the
charged phosphate groups of DNA, stabilizing its double-helical structure
without affecting its conformation. It interacts also weakly with the
nucleic bases, most likely at the N7 positions of guanine and adenine in the
major groove and to the O2 atom of thymine in the small groove of the
macromolecular duplex. We could not, however, confirm its binding to the O2
atom of cytidine, which has been suggested as a major site of interaction of
the nucleotide in Me$_2$SO (32). Cu^{2+} ions behave somewhat differently: they
also bind to the phosphate groups of DNA, but, contrary to the Pb^{2+} ions,
their interactions with the nucleic bases is extremely important,

particularly with the G-C base pairs. They can bind at sites which are normally engaged in interstrand hydrogen bonding, disrupting the double-helical structure of the biopolymer and affecting its conformation. Our results are also consistent with the possibility of interstrand interaction at the G-C base pair level, which allows for the renaturation of DNA upon removal of the metal ions.

REFERENCES
1. Barton,J.K. and Lippard,S.J. (1980) In Spiro,T.G. (ed.) Nucleic Acid-Metal Ion Interactions, Wiley, New York, pp. 31-113.
2. Marzilli,L.G., Kistenmacher,T.J., and Eichhorn,G.L. (1980) In Spiro,T.G. (ed.) Nucleic Acid-Metal Ion Interactions, Wiley, New York, pp. 179-250.
3. Eichhorn,G.L. and Shin,Y.A. (1968) J. Am. Chem. Soc. **90**, 7323-7328.
4. Aoki,K. (1982) J. Cryst. Soc. Japan **23**, 370-395, and references cited therein.
5. Eichhorn,G.L. and Clark,P. (1965) Proc. Natl. Acad. Sci. U.S.A. **53**, 586-593.
6. Zimmer,Ch., Luck,G., Fritzsche,H., and Triebel,H. (1971) Biopolymers **10**, 441-463.
7. Eichhorn,G.L. (1962) Nature **194**, 474-475.
8. Gasiorowski,K., Swiatek,J., and Kozlowski,H. (1985) Inorg. Chim. Acta **106**, L1-L5.
9. Sigel,H., Fischer,B.E., and Farkas,E. (1983) Inorg. Chem. **22**, 925-934.
10. Huong,P.V., Carmona,P., and Lorosch,J. (1986) In Peticolas,W. and Hudson,B. (eds) Proceedings of the Tenth International Conference on Raman Spectroscopy, University of Oregon, pp. 4.3-4.4.
11. Maskos K. (1978) Acta Biochim. Polon. **25**, 101-111 and 113-127.
12. Prescott,B., Steinmetz,W., and Thomas,Jr.,G.J. (1984) Biopolymers **23**, 235-256.
13. Erfurth,S.C. and Peticolas,W.L. (1975) Biopolymers **14**, 247-264.
14. Benevides,J.M., Wang,A.H.-G., van der Marel,G.A., van Boom,J.H., Rich,A., and Thomas,Jr.,G.J. (1984) Nucleic Acids Res. **12**, 5913-5925.
15. Benevides,J.M., and Thomas,Jr.,G.J. (1983) Nucleic Acids Res. **11**, 5747-5761.
16. O'Connor,T., Bina,M., McMillin,D.R., Moller Haley,M.R., and Tobias, R.S. (1982) Biophys. Chem. **15**, 223-234.
17. Erfurth,S.C., Kiser,E.J., and Peticolas,W.L. (1972) Proc. Natl. Acad. Sci. USA **69**, 938-941.
18. Goodwin,D.C. and Brahms,J. (1978) Nucleic Acids Res. **5**, 835-850.
19. Mansy,S. and Tobias,R.S. (1974) J. Am. Chem. Soc. **96**, 6874-6885.
20. Cramer,R.E., Dahlstrom,P.L., Seu,M.J.T., Norton,T., and Kashigawi,M. (1980) Inorg. Chem. **19**, 148-154.
21. Carrabine,J.A. and Sundaralingam,M., (1968) Chem. Comm. 746-746.
22. Richard,H., Schreiber,J.P., and Daune,M. (1973) Biopolymers **12**, 1-10.
23. Guay,F., Beauchamp,A.L., Gilbert,C., and Savoie,R. (1983) Can. J. Spectrosc. **28**, 13-20.

24. Mansy,S., Chu,G.Y.H., Duncan,R.E., and Tobias,R.S. (1978) J. Am. Chem. Soc., **100,** 607-616.
25. Savoie,R., Jutier,J.-J., Prizant,L., and Beauchamp,A.L. (1982) Spectrochim. Acta, Part A **38,** 561-568.
26. Savoie,R., Poirier,D., Prizant,L., and Beauchamp,A.L. (1981) J. Raman Spectrosc. **11,** 481-486.
27. Bemski,G., Rieber,M., and Wust,M. (1971) FEBS Lett. **14,** 117-120.
28. Ropars,C. and Viovy,R. (1965) J. Chim. Phys. **62,** 408-417.
29. Lanir,A. and Yu,N.-T. (1979) J. Biol. Chem. **254,** 5882-5887.
30. Harrison,P.G. and Healy,M.A. (1983) Inorg. Chim. Acta **80,** 279-285.
31. Mansy,S. and Peticolas,W.J. (1976) Biochem. **15,** 2650-2655.
32. Marzilli,L.G., de Castro,B., Caradonna,J.P., Stewart,R.C., and Van Vuuren,C.P. (1980) J. Am. Chem. Soc. **102,** 916-924.