The Binding of Manganese(II) and Zinc(II) to the Synthetic Oligonucleotide d(C–G–C–G–A–A–T–T–C–G–C–G)$_2$. A ^{1}H NMR Study

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The interaction of the synthetic oligonucleotide d(C–G–C–G–A–A–T–T–C–G–C–G)$_2$ with two different transition-metal ions has been investigated in aqueous solution by means of ^{1}H NMR spectroscopy. The effects on the DNA due to the presence of manganese(II) or zinc(II) have been monitored by observing the paramagnetic broadening and diamagnetic shifts of the non-exchangeable proton resonance lines, respectively.

The ^{1}H NMR spectra acquired during the course of the manganese(II) titration show very distinct broadening effects on certain DNA resonance lines. Primarily, the H8 resonance of G4 is affected, but also the H5 and H6 resonances of C3 are clearly affected by the metal. The results imply that the binding of manganese(II) to DNA is sequence specific.

The ^{1}H spectra obtained during the zinc(II) titration reveal diamagnetic shift effects which largely conform with the paramagnetic broadening effects due to the presence of manganese(II), although this picture is somewhat more complex. The H8 resonance of G4 displays a clearly visible high-field shift, while for the other guanosine H8 protons this effect is absent. The H1′ and H2′ protons of C3 show an effect of similar strength, although in the opposite direction, while H5 and H6 of C3 are only slightly affected.

Local differences in the structure of the DNA and the basicities of potential binding sites on different base steps in the sequence might account for the observed sequence selectivity.

During the last few decades, the biological significance of the interaction between metal ions and nucleic acids has become a rather well established fact. We may mention the observed necessity for the presence of metals in many natural processes where nucleic acids play the dominant role,[1] the discovery of platinum-based chemotherapeutic drugs whose useful effect probably originates from their attack on DNA,[1–3] and finally the toxicity of certain metals, which may be explained by their interaction with DNA, since they are often shown to induce mutations.[1] These intriguing phenomena have inspired many researchers in their attempts to understand the nature of the interaction between various metal ions and nucleic acids and their constituents. Both X-ray crystallography[4–6] and NMR methods[4,7] have been used extensively to reveal the binding patterns in monomer complexes in the solid state and solution, respectively. These studies provide us with an important result. Different metals show pronounced binding selectivity towards the various parts of a nucleotide. In qualitative terms the hard-/softness of the metal and ligand, together with the required coordination geometries for the metal, could indicate the preferred binding site.

Having obtained a fairly clear picture of the binding properties at the monomer level, it is natural to address the even more important question of how these metal-dependent binding patterns influence the structure and function of the nucleic acid polymers. Some early UV spectroscopic studies on the melting and renaturing behaviour of native DNA in the presence of rather high concentrations of various divalent metals ions showed an interesting variation through the series of metals.[8] These observations could be explained in terms of a varying degree of affinity towards the phosphate backbone and the base-pairs, and in the difference in the total affinity towards the nucleotide. Likewise, some divalent metal ions are able to induce B-Z helix coil transitions in synthetic DNA polymers, while others do not, as monitored by CD spectroscopy.[9] However, these methods only indirectly indicate possible binding behaviour. With the advent of automatic procedures for synthesizing DNA in relatively large quantities, model compounds in the shape of DNA oligomers of sizes suitable for NMR studies are now readily available. At the same time, the structure determination of DNA in solution, based on

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two-dimensional NMR methods,[10-13] is developing quickly towards acceptance as a method of equal power and importance as X-ray crystallography in the solid state.

Several NMR studies have already been undertaken in order to investigate the nature of the interaction of certain DNA oligomers with different platinum complexes[14-16] or other metal-containing drugs.[17,18] Since these studies are concerned with metal-containing drugs only, a closer look at the interaction between 3d transition metals and DNA at this level of complexity is in order.

In this study we have investigated the binding behaviour of Mn(II) and Zn(II) in their interaction with the palindrome oligonucleotide d(C–G–C–G–A–A–T–T–C–G–C–G)$_2$ used as a DNA model. The effects from titrating the DNA with Mn(II) have been monitored by observing paramagnetic line-broadening effects in the dodecamer's one-dimensional ^{1}H NMR spectrum, while diamagnetic-shift effects are observed during a Zn(II) titration. The ^{1}H NMR spectrum has been assign by 2D-NMR (NOESY). We are taking advantage of the fact that the chosen dodecamer has been studied extensively both in the crystalline state by X-ray crystallography[19] and in the solution[12,13,20,21] and solid state[22] by NMR-methods. Recently the interaction with Ca(II) has been investigated by means of ^{43}Ca NMR.[23]

The oligomer contains the sequence d(G–A–A–T–T–C), which is recognized by the restriction enzyme EcoRI. The cleavage site is between the guanosine and adenosine residues. It is important to realize that both strands are cut at sites related by twofold rotational symmetry, which also reflects the concomitant symmetry of the enzyme. The structure of the DNA–EcoRI complex has been studied by X-ray crystallography.[24]

Materials and methods

The DNA dodecamer d(C–G–C–G–A–A–T–T–C–G–C–G)$_2$ was obtained from Dr. B. R. Reid (University of Washington, Seattle, WA). It was synthesized using solid-phase phosphoroamidite techniques,[20] purified by chromatography on Sephadex G-25 and finally lyophilized to dryness.[25] The palindrome dodecamer readily forms a duplex. The annealing step normally used for non-palindrome oligomers is thus unnecessary. The high purity of the material was confirmed by the the absence of resonances from minor components (shorter oligomers from the synthesis) in the ^{1}H NMR spectra. The purity was estimated to the better than 95 %.

The samples used for the Mn(II) and Zn(II) titrations contained the same amount of the DNA dodecamer, i.e. about 125 OD_{260} units. This corresponds approximately to 1.5 mM of DNA duplex, using a calculated value for the extinction coefficient (1.107×10^5 M^{-1} cm).[26] The samples were dissolved in 400 µl of D_2O, which also contained 10 mM of sodium phosphate buffer adjusted to pH 7, and were then transferred to 5 mm NMR tubes. No internal chemical shift standard was added. The Mn(II) solution was made from a 0.02 M stock solution of $MnCl_2$ in H_2O. The final concentration of $MnCl_2$ was 2.0×10^{-5} M after dilution in D_2O. A 0.10 M solution of $ZnCl_2$ in D_2O was used for the Zn(II) titration. All salts were Baker Analyzed reagents. No attempt was made to remove the residual HDO from the metal solutions. $MnCl_2$ and $ZnCl_2$, were added to the DNA samples directly into the NMR tubes with a micropipette. During the course of the titrations the two DNA samples contained from 0.1×10^{-6} to 4.0×10^{-6} M Mn(II) and from 0.25×10^{-3} to 12.5×10^{-3} M Zn(II), respectively. The sample used for the two-dimensional experiment contained 500 OD_{260} units (ca. 6 mM) of the dodecamer. The concentration of the buffer, the pH and the sample volume were identical to those of the 125 OD_{260} samples.

All one-dimensional ^{1}H NMR spectra were collected on a Bruker WM-500 spectrometer. 8192 data points were acquired in quadrature detection mode, using a spectral width of 5000 Hz, a 14 µs pulse width and a recycling delay of 2 s. Typically, 32 transients were averaged. During the the recycling delay the water resonance was suppressed by a very weak irradiation field (ca. 20 Hz). The experiments were made with the probe temperature held at ca. 305 K.

The two-dimensional experiment was performed on a Bruker AM-400 WB instrument. A NOESY[27,28] spectrum was collected in the pure-phase absorption mode with quadrature detection into 1024 complex points for 640 t_1 values, using the TPPI method.[29,30] 32 transients were averaged for each t_1 increment. The spectral width was 3378.4 Hz. A recycle delay of 1 s was used, during which time the residual HDO signal was subjected to a very weak irradiation. The mixing time was 300 ms and was randomly varied (±10 %) in order to suppress zero-quantum J cross peaks.[28]

The NMR data were processed on a Micro Vax II computer using the program FTNMR.[31] The one-dimensional FIDs were multiplied by an exponential window prior to the Fourier transformation. Typically, 2 Hz was added to the linewidths. The baselines of the spectra were corrected by a third-order polynomial fit. The spectra were referenced to the water resonance set to 4.68 ppm at 305 K.

The 640 t_1-incremented FIDs of the NOESY experiment were multiplied by an exponential window (3 Hz). After Fourier transformation along t_2, a careful phase correction and a third-order polynomial baseline correction were carried out. Prior to Fourier transformation along t_1 the first increment (F_2 spectrum) was multiplied by 0.5 to suppress t_1 noise.[32] In the t_1-dimension the data were apodized with a squared and skewed (0.7) phase-shifted (75°) sine-bell function. Partially owing to inherent properties of the TPPI method, the subsequent phase corrections in F_1 produce baseline distortions[33] which badly effect the baseplane close to strong peaks near the edges of the spectrum. Therefore, a third-order polynomial baseline correction was also applied to the data along F_1.

Results and discussion

Shown in Fig. 1 is the normal ^1H spectrum of the DNA dodecamer d(C–G–C–G–A–A–T–T–C–G–C–G)$_2$. The resonance assignments rely partly on the work of Hare *et al.*[20] However, the temperature is not stated in that study, and the one-dimensional spectrum appears to be slightly different from those obtained in the present study. Therefore we have performed a 300 ms NOESY experiment to obtain the correct assignment, corresponding to our choice of experimental conditions. The standard procedure for performing a complete assignment has been employed.[20,34,35] These results are not shown. Owing to a limit in our amount of DNA, this experiment was only performed without metals. Since no shift effects were observed for the manganese(II) titration, this should not be of any practical importance. On the other hand, the interpretation of the results from the zinc(II) titration is somewhat hampered by the lack of metal-dependent assignments.

The results from the titration of the dodecamer with manganese(II) are shown in Fig. 2. Only line-broadening effects are considered. These tend to be dominated by scalar paramagnetic relaxation, which in turn rules out the possibility of obtaining precise geometric information.[36,37] Nevertheless, the method is quite a powerful tool for pinpointing possible binding sites in a qualitative manner.[7,38] The most pronounced effects from paramagnetic line broadening are found in the aromatic region of the spectrum, and only this region, together with the H5/H1′ part of the spectrum, are shown. The other protons of the sugar moieties are barely affected, except from a general broadening through the whole range of resonances at rather high concentrations of manganese. The same applies to the methyl resonances (not shown). It should be noted, however, that the complexity of the ribose parts of the spectrum and the presence of an annoying HDO peak might very well preclude the observation of more subtle, but significant, effects.

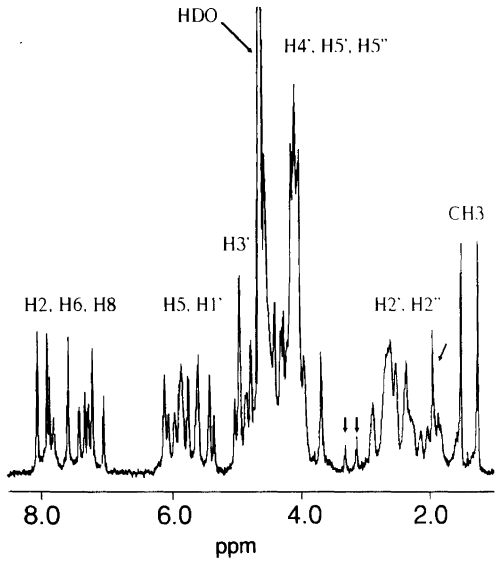

Fig. 1. ^1H NMR spectrum of the DNA dodecamer d(C–G–C–G–A–A–T–T–C–G–C–G)$_2$ (ca. 125 OD_{260} units, ca. 1.5 mM duplex) in D_2O solution containing 10 mM sodium phosphate buffer (pH 7.0). The temperature is ca. 305 K. The chemical shift of the residual HDO peak is set to 4.68 ppm. The chemical shifts for various types of ^1H resonances in the monomers are indicated. The weak resonances marked by small arrows are due to impurities.

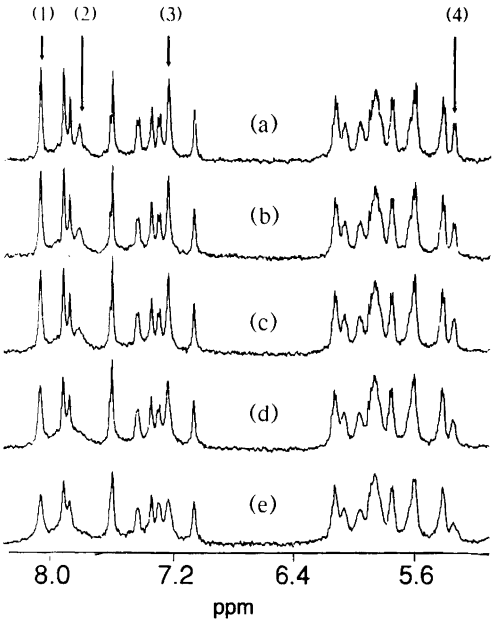

Fig. 2. ^1H spectra showing the effect of titrating a ca. 1.5 mM solution of the DNA dodecamer with $MnCl_2$. Only the aromatic and 5H/1H′ regions of the spectra are shown. The solutions contain (a) no Mn(II) added, (b) 0.3×10^{-6} M Mn(II), (c) 0.7×10^{-6} M Mn(II), (d) 1.8×10^{-6} M Mn(II) and (e) 3.3×10^{-6} M. Arrow (1) shows the overlapping A5/H8 and A6/H8 resonances, arrow (2) shows the G4/H8 resonance, arrow (3) shows the overlapping A5/H2 and C3/H6 resonances and arrow (4) shows the C3/H5 resonance.

As demonstrated in Fig. 2, the dramatic effect on the H8 resonance of the G4 residue at 8.10 ppm is by far the most prominent, even at an extremely low manganese concentration (3×10^{-7} M). This result immediately suggests a selective binding of manganese to a specific site on a guanosine residue, i.e. in the proximity of the H8 resonance, which in turn strongly indicates a possible N7 binding on the base itself (although not necessarily with N7 in the inner coordination sphere of manganese). A comparison of the large effect on G4 and the nearly unaffected H8 protons on G2, G10 and G12 at 7.92–7.96 ppm (Fig. 3) has an even more important implication, as it shows that the manganese binding not only is selective towards a specific site on a certain kind of nucleotide, but also seems to be selective towards the position of that nucleotide in the DNA sequence.

Careful inspection of the spectra (Fig. 2) reveals some broadening effects of moderate strength on certain other resonances. These are not observable at low manganese concentrations where the huge G4 effect first appears, but

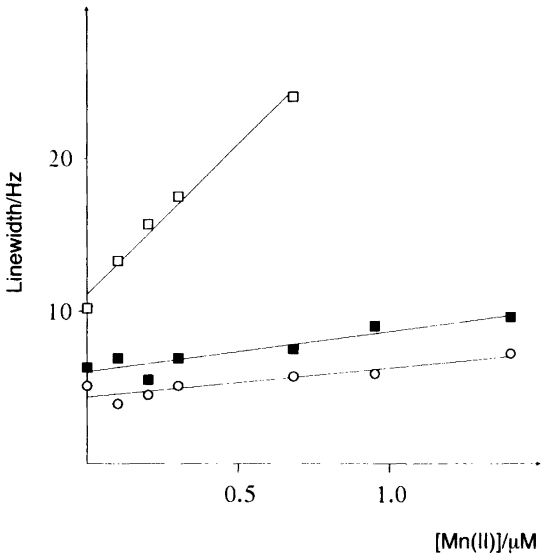

Fig. 3. The linewidths of the different guanosine H8 resonances plotted against manganese(II) concentration. The open squares indicate G4, the filled squares G10 and the open circles the overlapping H8 signals from G2 and G12.

they are observable well in advance of the general blurring of the spectrum at high manganese concentrations. Most noticable, there are broadening effects on the overlapping A5/H2 and C3/H6 at 7.28 ppm and also on the H5 of the C3 residue at 5.39 ppm. The effect on C3/H5 suggests that C3/H6 rather than A5/H2 is mainly responsible for the broadening of their overlapping peaks. A slight broadening of the overlapping H8 resonances of A5 and A6 at 8.10 ppm also seems to be significant. The inherent limitations of the paramagnetic broadening approach in obtaining precise geometric information should not impede us from suggesting, at least, a plausible qualitative model for the binding of manganese to DNA. An examination of the proposed three-dimensional structure of the dodecamer in solution[13] does show that the observations are consistent with a model which suggests Mn(II) binding near N7 on G4. Secondly, it does not seem to be necessary to incorporate conformational changes to make the data fit. Interestingly, the solution structure of the dodecamer shows some very characteristic features,[13] including a kink between the C3–G10 and G4–C9 base-pairs.

Our results, which strongly suggest sequence-specific Mn (II) binding to the DNA dodecamer, clearly demonstrate the great importance of studying larger and more realistic model systems on the way towards an understanding of the nature of the interaction between metals and nucleic acids. Phenomena which hardly can be predicted from extrapolations from monomer studies have been observed. The clear preference for G4 in the binding of manganese to this DNA sequence is not easily explained. One could speculate upon whether binding is specifically promoted by local features of the DNA near G4, or hindered around the other guanosine residues. The last proposal implies the presence of local differences in geometry along the sequence which might obstruct binding of manganese to its favoured site by steric hindrance. This is because the intrinsic electronic properties of a certain potential binding site are unlikely to differ much between the various residues, except from differences due to base stacking effects. Since the G2, G10 and G12 residues with their unaffected H8 protons are close to the ends of the dodecamer, it is unlikely that steric hindrance should reduce the binding strength considerably compared to that of the momomer. This is especially true for the terminal residue G12. Hence it is more likely that the local geometry in the neighbourhood of the G4 residue enhances its affinity towards manganese, and thus makes its N7 a very attractive binding site. This could, for instance, be due to a favourable orientation of the phosphate group to promote simultaneous binding to the base and phosphate, directly or through bridging water molecules. This does not rule out a concomitant electronic effect from the base stacking, which might promote metal binding.

Since only a few protons are affected, and we have virtually no information about the backbone, a detailed interpretation of the geometry around manganese is impossible. Is it possible that the guanosine binding is more or less a general property of the DNA/manganese complex, and that the lack of H8 broadening for all guanosines except G4 is due to end effects? This is not very probable,

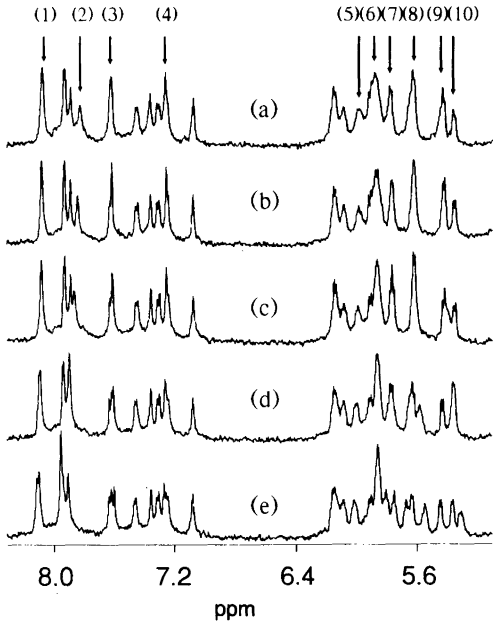

Fig. 4. ^{1}H spectra showing the effect of titrating a ca. 1.5 mM solution of the DNA dodecamer with Zn(II). Only the aromatic and H5/H1′ regions of the spectra are shown. The solutions contain: (a) no metal added, (b) 2.0×10^{-3} M Zn, (c) 2.9×10^{-3} M Zn, (d) 3.8×10^{-3} M Zn and (e) 5.7×10^{-3} M Zn. Shift effects are observed where indicated by the arrows. In the aromatic region: (1) A5/H8 and A6/H8, (2) G4/H8, (3) C1/H6 and A6/H2, (4) C3/H6 and A5/H2. In the H5/H1′ region: (5) A5/H1′, (6) C1/H5, G2/H1′, T7/H1′ and G10/H1′, (7) C1/H1′ and C11/H1′, (8) C3/H1′, C9/H1′ and C9/H5, (9) G4/H1′ C11/H5, (10) C3/H5.

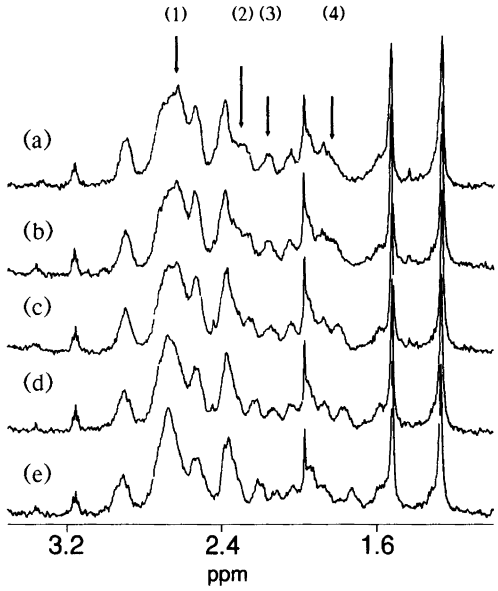

Fig. 5. As Fig. 4, but only the H2′/H2″ regions of the spectra are shown. The arrows indicate the residues for which shift effects are observed: (1) see text, (2) C3/H2″, (3) T8/H2′ and (4) C3/H2′.

however, as in that case we should expect a gradually decreasing paramagnetic effect from the strongly affected G4 nucleotide to a barely affected G12. This is not the case. Even G10 (neighbour to the G4–C9 base-pair) is as unaffected as G12.

The power of the paramagnetic approach in understanding the nature of the interaction between divalent metal ions and DNA lies in its great sensitivity. The medium in which DNA is dissolved is hardly affected. The system is nearly the same chemically, since a minute amount of manganese "tickles" many DNA molecules to achieve pronounced effects on the NMR spectra (DNA duplex to manganese ratio 15 000/380). The situation is fundamentally different for a diamagnetic system, e.g. a DNA solution containing zinc(II). This is because diamagnetic shift effects are far less sensitive than paramagnetic induced broadening. In order to obtain reliable shift effects, the zinc concentration has to be in the same range as that of the ligand.[39,40] In this study the ratio of DNA duplex to zinc varied between 6 and 0.12.

Several problems arise, mainly for two reasons. First, the effect of a relatively large amount of zinc on the ionic medium could very well produce some less specific effects on the structure of the DNA. Secondly, the shift parameter itself is influenced by a range of effects in a rather more complex way than relaxation parameters. Therefore, a comparison of two such different approaches would be of interest.

The results of the zinc titration are presented in Figs. 4 and 5. Even though no reliable chemical shift standard is present, several zinc-dependent changes in the relative chemical shifts are visible. These can be classified according to their magnitude. Most pronounced are the downfield shift of H8 on the G4 residue at 7.86 ppm, the upfield shifts of H1′ of G4 at 5.47 ppm and one of the overlapping H1s of C3 or C9 at 5.68 ppm (Fig. 4). Since overlapping signals sometimes are split during the course of titration, it is not possible to rely solely on the assignments obtained for the zinc(II)-free solution. Some of the ambiguities which thus arise were resolved by simple homodecoupling experiments. In addition to the larger shift effects there are also some minor changes. There is a splitting of the two overlapping H8s of A5 and A6 at 8.10 ppm, a slight upfield shift of H2 of A6 at 7.65 ppm, a splitting of the overlapping H6 of C3 and H2 of A5 at 7.28 ppm, a slight downfield shift of H1′ of A5 at 6.01 ppm, and a splitting of the overlapping H1′ of C1 and C11 at 5.81 ppm. There is also an upfield shift of H1′ of G2 or T7, but unfortunately it was not possible to resolve this ambiguity by simple homodecoupling.

The H2′/H2″ part of the spectrum is shown in Fig. 5 and reveals the following changes. There is a very clear upfield shift of the H2′ proton of C3 at 1.86 ppm, and a comparable effect is present for C3's H2″ proton at 2.29 ppm. The spectra also show a small upfield shift of the H2′ proton of T8 at 2.19 ppm. There are also some changes at about 2.7 ppm, but no serious attempt was made to determine their precise identity, although H2′ and H2″ of G4 are strong candidates for being responsible for the observed changes.

The major shift effects can easily be interpreted in terms of selective zinc binding, although the abovementioned reservation regarding diamagnetic shifts should be kept in mind. Interestingly, again the G4 residue and its neigh-

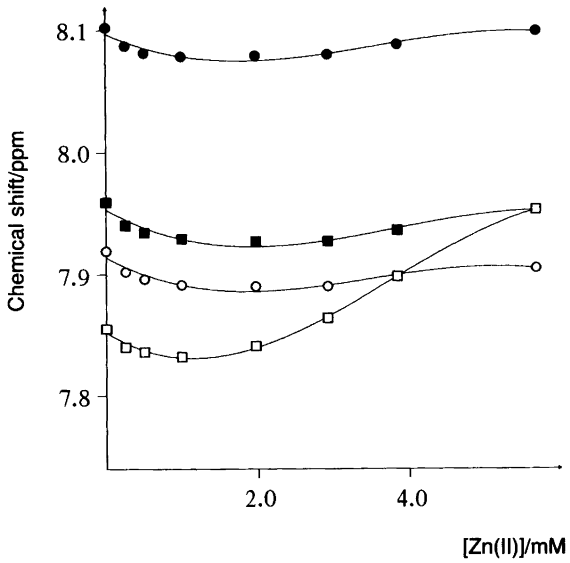

Fig. 6. The chemical shifts of the H8 resonances of the different guanosine residues and the overlapping H8 protons of the two adenosines plotted against the zinc(II) concentration. The filled circles indicate A5/A6, the filled squares G2/G12, the open circles G10 and the open squares G4. Since we do not refer to an internal shift standard, the absolute chemical shifts are not to be taken seriously: It is the relative shifts that matter.

bourhood seem to be the main site of attack from the metal ions. To stress the sequence specificity among the guanosine monomers, a comparison between the response of the four guanosine H8 protons to zinc addition is shown in Fig. 6. This is the only base proton which is largely affected. The anomeric proton of G4 is also sensitive to zinc addition. Likewise, the anomeric proton of C3 or C9 and H2′ and H2″ of C3 show rather marked effects.

The similarities between the results of the manganese and zinc titrations are rather remarkable, when considering the great difference in metal concentrations, the different means of monitoring the changes (paramagnetic relaxation vs. diamagnetic shifts) and differences in the inherent properties of the metals themselves. It should be noted, however, that both manganese and zinc appear to display similar behaviour with respect to the metal-dependent melting of DNA.[8] On the other hand, divalent manganese and zinc show slight differences in their ability to promote a B–Z helix transition in certain synthetic DNA polymers as studied by CD spectroscopy.[9] They also show some differences in their tendencies to invert/enhance ψ-effects in quarternary structures of tightly packed DNA.[41] In X-ray studies manganese – nucleic acid constituent complexes invariably display octahedral coordination geometry, while zinc (II) nearly always is found in a tetrahedral environment.[5,6] Interestingly, in two cases of complexes with methylated 6-oxopurine nucleotides, zinc(II) appears in an octahedral environment,[42] as normally found for manganese(II).[5,6]

It should be noted that there are certain indications of other minor binding sites, both for zinc and manganese. In particular we may notice small, but significant, broadening and shift effects on the overlapping H8 resonances of A5 and A6 (Figs. 2 and 4). The data do not allow a quantitative determination of the relative amount of secondary binding, but it may be estimated roughly as less than 10 %.

Finally, we should mention that the interaction between d(C–G–C–G–A–A–T–T–C–G)$_2$ and calcium has been studied by means of ^{43}Ca NMR.[23] These results also indicate some kind of site-specific interaction between DNA and the (presumably) phosphate binding metal at low metal/phosphate ratios. However, these results are difficult to compare directly with ours, since the metal itself is used as the NMR probe, and consequently it is difficult to discuss any kind of sequence specificity.

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References

1. Eichhorn, G. L. and Marzilli, L. G. *Advances in Inorganic Biochemistry,* Elsevier/North-Holland, New York 1981, Vol. 3.
2. Sherman, S. E. and Lippard, S. J. *Chem. Rev. 87* (1987) 1153.
3. Reedijk, J. *Pure Appl. Chem. 59* (1987) 181.
4. Pezzano, H. and Podo, F. *Chem. Rev. 80* (1980) 365.
5. Swaminathan, V. and Sundaralingam, M. *C.R.C. Crit. Rev. Biochem. 6* (1979) 245.
6. Gellert, R. W. and Bau, R. In: Siegel, H., Ed., *Metal Ions in Biological Systems,* Marcel Decker, New York 1979, Vol. 8, Chap. 1.
7. Marzilli, L. G. In: Lippard, S. J., Ed., *Progress in Inorganic Chemistry,* J. Wiley and Sons, New York 1977, Vol. 23, Chap. 1.
8. Eichhorn, G. L. and Shin, Y. A. *J. Am. Chem. Soc. 90* (1968) 7323.
9. Narasimhan, V. and Bryan, A. M. *Inorg. Chim. Acta 91* (1984) L39.
10. Hare, D. R. and Reid, B. R. *Biochemistry 25* (1986) 5341.
11. Nerdal, W., Hare, D. R. and Reid, B. R. *J. Mol. Biol. 201* (1988) 717.
12. Reid, B. R., Banks, K. M., Flynn, P. F. and Nerdal, W. *Biochemistry 28* (1989) 1001.
13. Nerdal, W., Hare, D. R. and Reid, B. R. *Biochemistry 28* (1989) 1008.
14. Fouts, C. S., Marzilli, L. G., Byrd, R. A., Summers, M. F., Zon, G. and Shinozuka, K. *Inorg. Chem. 27* (1988) 366.
15. Lepre, C. A., Chassot, L., Costello, C. E. and Lippard, S. J. *Biochemistry 29* (1990) 811.
16. van der Veer, J. L., van der Marel, G. A., van der Elst, H. and Reedijk, J. *Inorg. Chem. 26* (1987) 2272.
17. Pelton, J. G. and Wemmer, D. E. *J. Am. Chem. Soc. 112* (1990) 1393.
18. Scott, E. V., Zon, G., Marzilli, L. G. and Wilson, W. D. *Biochemistry 27* (1988) 7940.
19. Dickerson, R. E., Drew, H. R., Conner, B. N., Wing, R. M., Fratini, A. V. and Kopka, M. L. *Science 216* (1982) 475.
20. Hare, D. R., Wemmer, D. E., Chou, S.-H., Drobny, G. and Reid, B. R. *J. Mol. Biol. 171* (1983) 319.
21. Patel, D. J., Ikuta, S., Kozlowski, S. and Itakura, K. *Proc. Natl. Acad. Sci. USA 80* (1983) 2184.
22. Kintanar, A., Huang, W.-C., Schindele, D. C., Wemmer, D. E. and Drobny, G. *Biochemistry 28* (1989) 282.
23. Braunlin, W. H., Nordenskiöld, L. and Drakenberg, T. *Biopolymers 28* (1989) 1339.
24. McClarin, J. A., Frederick, C. A., Wang, B.-C., Green, P., Boyer, H. W., Grable, J. and Rosenberg, J. M. *Science 234* (1986) 1526.
25. Kintanar, A., Klevit, R. E. and Reid, B. R. *Nucleic Acids Res. 15* (1987) 5845.
26. Borer, P. N. In: Fasman, G. D., Ed., *Handbook of Biochemistry and Molecular Biology,* 3rd ed., CRC, Cleveland, OH 1975, p. 589.
27. Jeener, J., Meier, B. H., Bachmann, P. and Ernst, R. R. *J. Chem. Phys. 71* (1979) 4546.
28. Macura, S., Huang, Y., Suter, D. and Ernst, R. R. *J. Magn. Reson. 43* (1981) 259.
29. Redfield, A. G. and Kunz, S. D. *J. Magn. Reson. 19* (1975) 250.
30. Bodenhausen, G., Kogler, H. and Ernst, R. R. *J. Magn. Reson. 58* (1984) 370.
31. Hare Research, Inc., Woodinville, WA, USA. *Unpublished work.*
32. Otting, G., Widmer, H., Wagner, G. and Wüthrich, K. *J. Magn. Reson. 66* (1986) 187.
33. Marion, D. and Bax, A. *J. Magn. Reson. 79* (1988) 352.

34. Scheek, R. M., Russo, N., Boelens, R., Kaptein, R. and van Boom, J. H. *J. Am. Chem. Soc. 105* (1983) 2914.
35. Scheek, R. M., Boelens, R., Russo, N., van Boom, J. H. and Kaptein, R. *Biochemistry 23* (1984) 1371.
36. Espersen, W. G. and Martin, R. B. *J. Phys. Chem. 80* (1976) 161.
37. Kowalewski, J., Nordenskiöld, L., Benetis, N. and Westlund, P.-O. *Progr. NMR Spectrosc. 17* (1985) 141.
38. Berger, N. A. and Eichhorn, G. L. *J. Am. Chem. Soc. 93* (1971) 7062.
39. Lönnberg, H. and Arpalahti, J. *Inorg. Chim. Acta 55* (1980) 39.
40. Arpalahti, J. and Lönnberg, H. *Inorg. Chim. Acta 78* (1983) 63.
41. Eichhorn, G. L., Tarien, E., Elgavish, G. A., Butzow, J. J. and Shin, Y. A. In: Sarma, R. H., Ed., *Biomolecular Stereodynamics,* Adenine Press, New York 1981, Vol. 2, pp. 185–195.
42. Miller, S. K., VanDerveer, D. G. and Marzilli, L. G. *J. Am. Chem. Soc. 107* (1985) 1048.

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