Communications

Diamagnetic Metal Species That Induce Pronounced Changes in the ^{31}P NMR Spectrum of DNA

Sir:

Little direct information is available on the binding sites of diamagnetic metal species on native double helical DNA in solution. Metal species are, however, very important in influencing nucleic acid structure and stability[1] and are involved in virtually every aspect of genetic information transfer.[2] Likely binding sites can be inferred indirectly by studies of comparative effects of metal ions on DNA electronic spectra (UV, ORD–CD) and stability, by detailed studies of small molecules, etc.[3] NMR spectroscopy would undoubtedly be the most useful procedure for studying such interactions, but the large size and restricted molecular motion of such DNA has in the past restricted NMR studies of DNA mainly to denaturing conditions.[4] Recently it was demonstrated that extensive sonication can produce double helical DNA (approximately 200 base pairs in length), which has observable ^{31}P NMR spectra over a range of temperatures and in the presence of a variety of intercalating ligands.[5] In this report, we show that characteristic changes in the ^{31}P chemical shift, line width, and T_1 of DNA are observed for several classes of diamagnetic metal species generally believed to interact with DNA in different manners.[6] Evidence is presented supportive of site-specific binding of the biologically important Mg^{2+} and Ca^{2+} ions. Previously, good evidence for site-specific binding of Ca^{2+} was lacking.

Metal species bound to DNA can be divided into two classes: (1) territorially bound ions, which retain the original full inner sphere, and (2) site-bound species, which coordinate directly with a group on the nucleic acid and as a result lose one or more first coordination sphere ligands.[7] The alkali metal ions are examples of those believed to bind exclusively by territorial interactions.[8] Species such as those containing Hg(II), at least at low ratios of metal ion to DNA phosphate, bind almost exclusively at sites on the heterocyclic bases of DNA.[9] The biologically important alkaline earth metal ions present a complex intermediate situation. They definitely exhibit effects that can be explained by territorial interactions with negligible direct interactions at the DNA bases.[7] Recent ^{25}Mg NMR evidence, however, has suggested that magnesium can exhibit important site-binding interactions with DNA.[10] A determination of the sites for this specific magnesium interaction and whether other similar ions can also site bind to DNA is essential for understanding metal ion–DNA interactions and for developing theories for ionic effects on the physical properties of DNA. For example, alkaline earth ions appear to facilitate the B to Z form conversion of G,C polynucleotides

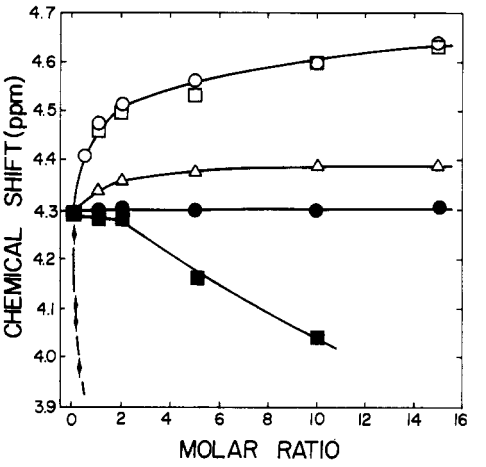

Figure 1. ^{31}P chemical shift of DNA, relative to that of internal trimethyl phosphate, plotted as a function of the molar ratio of added metal ion to DNA–P: Mg^{2+}, ○; Ca^{2+}, □; Sr^{2+}, △; Ba^{2+}, ●; Hg^{2+}, ■; platinum metallointercalator, ♦. Spectra were accumulated on a JEOL FX 60Q NMR spectrometer with quadrature detection at 24.15 MHz with 0.025 M DNA phosphate in NMR buffer at 30 °C using 10-mm NMR tubes. Samples were lyophilized and redissolved in an equivalent volume of 99.8% D_2O before accumulation, and metal ions were added as solid salts. Typically 1000 scans were obtained with fast Fourier transformation of 8192 time domain points, a 90° pulse, 13-s delay time, broad-band proton decoupling, and 0.5-Hz line broadening.

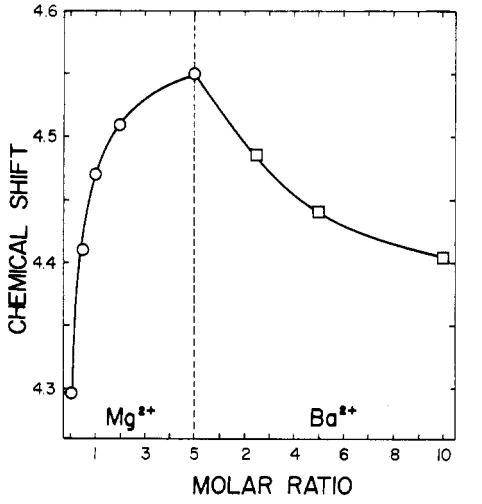

Figure 2. DNA ^{31}P chemical shifts (ppm) determined as in Figure 1. Mg^{2+} up to a molar ratio of 5/1 Mg^{2+}/P was added as in Figure 1. At that point a titration with Ba^{2+} was begun and continued up to a molar ratio of 10/1 Ba^{2+}/P. The final chemical shift change induced by Mg^{2+} in Figure 1 is approximately 0.3 ppm. When the Ba^{2+}/Mg^{2+} ratio is 1, the shift is approximately 0.15 ppm, and when the ratio is 2, the shift is approximately 0.1 ppm.

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with significant differences among the metal species in some cases.[11]

Some of the data from our studies are presented in Figure 1. The differential effects of the alkaline earth metal ions could be due to differences in (a) affinity for the DNA, (b) conformation of DNA as a function of metal ions, or (c) shifts caused by the expected different electron-perturbing properties of the metal centers. All of the alkaline earth metal ions appear to have similar affinity for the DNA. First, ^{31}P competition experiments between Ba^{2+} and Mg^{2+} clearly demonstrate direct competition with approximately equal affinity for binding sites as shown in Figure 2. Second, all four alkaline earth ions increase the T_m of the DNA by approximately the same amount.[12] We are not aware of previous melting studies with the two larger ions. Differential conformational effects are also unlikely to be significant since the spin–lattice relaxation time of the DNA (2.2 s at 30 °C) is unaffected by both Mg^{2+} and Ba^{2+} and since these ions have similar effects on the CD of DNA in this concentration and ionic strength range. This leaves only differential effects caused by the differences in the perturbing properties of the metals or differences in the degree of site-specific vs. non-site-specific binding as explanations for the differential shifts of Figure 1. We favor the explanation that the differences arise from differences in perturbing properties of the site-bound metal ions,[13a-c] since it is difficult to conceive of how territorial binding of these metal ions could lead to differential shifts. Furthermore, ^{25}Mg NMR studies have been interpreted as clearly involving site-specific binding.[10] If this explanation is accepted, then Ca^{2+} may also be binding at specific sites on DNA since both Mg^{2+} and Ca^{2+} shift the ^{31}P NMR signal to higher field in an almost identical manner (Figure 1). Although site-spectific binding is consistent with our results, there is presently insufficient information available about the effects of metal ions on ^{31}P chemical shifts to rule out completely territorial binding contributions to the shifts.

To explore further the utility of this NMR technique, we examined the effects of some soft metal species. In Figure 1, we show that $HgCl_2$ causes *downfield* shift of this ^{31}P signal, but only at higher ratios of $HgCl_2/P$. However, since Cl^- is present in the solution, the effect of $HgCl_2$ may be underestimated. Nevertheless, the shift for this typical base-binding metal[9] is opposite to that of the "phosphate-binding" alkaline earth metal ions. Considerable broadening accompanies the shifts, again in contrast to our observations with the hard metal ions, and T_1 is decreased to $\sim 1 \pm 0.1$ s^{-1} at Hg/P = 5/1.

Finally, we have examined a metallointercalating agent, (2-mercaptoethanolato-*S*)(terpyridine)platinum(II). This species is known to intercalate with an unwinding angle of approximately 20°.[14] Studies on the effects of organic intercalating agents on ^{31}P NMR shifts show similar results;[5b,c] on the basis of such studies the NMR method gives an unwinding angle of 18 ± 2° for the Pt agent, in agreement with the literature.[14]

In summary, we have shown that the ^{31}P NMR technique is a most promising method for gaining insight into the binding of diamagnetic metal species to DNA. We have found evidence for site-specific binding of Ca^{2+} as well as Mg^{2+}. Different patterns of shifts and broadening characterize the different types of metal species used in these preliminary studies.[15] We are currently extending these studies to other metal species, particularly more Pt(II) derivatives and paramagnetic species.

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Registry No. Mg, 7439-95-4; Ca, 7440-70-2; Sr, 7440-24-6; Ba, 7440-39-3; Hg, 7439-97-6; (2-mercaptoethanolato-*S*)(terpyridine)platinum(II), 54637-64-8.

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(15) Preliminary work reveals that the antitumor agent *cis*-dichlorodiammineplatinum(II) results in a downfield shoulder on the main DNA ^{31}P NMR signal.

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