Binding of Mg^{2+}, Cd^{2+}, and Ni^{2+} to Liquid Crystalline NaDNA: Polarized Light Microscopy and NMR Investigations

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The interaction of the divalent metal ions Mg^{2+}, Cd^{2+}, and Ni^{2+} with liquid crystalline NaDNA solutions (molar ratios Me^{2+}/DNA-phosphate ≤ 0.050) was investigated by polarized light microscopy and multinuclear ^{31}P, ^{2}H, and ^{23}Na NMR. Our findings show that the state of the cholesteric NaDNA phase at equal $MgCl_2$, $CdCl_2$, or $NiCl_2$ concentration is affected in a different way and to a different extent by the nature of the divalent cation. Indeed, we found that the occurrence of an isotropic phase is markedly favored by Mg^{2+}, and to a lower extent by Cd^{2+}, and that the pitch of the cholesteric phase decreases in the presence of Cd^{2+} or Ni^{2+}. ^{23}Na NMR spectroscopy also shows differences between the binding behavior of Mg^{2+} and the transition metal ions in the counterion atmosphere around DNA. The results are discussed in terms of different binding modes of the metal ions.

Introduction

A variety of spectroscopic techniques such as UV−visible,[1,2] circular dichroism,[2,3] Raman,[4−6] X-ray diffraction,[7,8] and NMR[9−11] have shown that the binding mode of divalent cations to DNA depends strongly on the nature of the metal ion. Indeed, alkaline earth metal ions, known to stabilize B-DNA against thermal denaturation, are found to bind mainly to the negatively charged phosphate groups through electrostatic interactions. On the contrary, transition metal ions preferentially bind to the nucleic bases, disrupting hydrogen bonding between base pairs and destabilizing the B form of DNA. Comparing the metal ion effects on the B-DNA structure,[1,5] the following order of binding affinities to phosphates relative to the bases has been established: Mg^{2+}, $Ba^{2+} > Ca^{2+} > Fe^{2+} > Ni^{2+}$, $Co^{2+} > Mn^{2+} > Zn^{2+} > Cd^{2+} > Pb^{2+} > Cu^{2+} > Hg^{2+}$.

The influence of divalent metal ions on the structural stability and biochemical properties of the DNA double helix has been extensively investigated in dilute polyelectrolyte aqueous solutions. On the contrary, very little is known about the Me^{2+}−DNA interaction at high DNA concentrations (hundreds of mg mL^{-1}), where DNA rods spontaneously condense into ordered forms, i.e., liquid crystalline (lc) phases.[12,13] Since these molecular rearrangements are very similar to those observed in vivo,[14,15] lcDNA phases are interesting systems for investigating the main features of DNA aggregation in nature and their implication in the DNA activity, such as replication and transcription.[16] In this connection, investigating the interaction between divalent metal ions and lcDNA is particularly attractive for understanding the influence of the double helix (de)stabilization, following the metal ion binding, on the aggregation of DNA molecules. To this end, we have used the competitive binding of Mg^{2+}, Cd^{2+}, or Ni^{2+} with the physiological DNA counterion Na^{+} to investigate the effects of the Me^{2+}−DNA interaction on the liquid crystalline state of cholesteric DNA by polarized light microscopy and multinuclear ^{31}P, ^{2}H, and ^{23}Na NMR.

Materials and Methods

Reagents. Calf thymus DNA and PIPES [piperazine-*N*,*N*′-bis(ethanesulfonic acid)] were purchased from Sigma Chemical Company; $MgCl_2$, $CdCl_2$, and $NiCl_2$ were obtained from Carlo Erba and used without further purification; D_2O (99.9%) was purchased from Isotec Inc.

Sample Preparation. The preparation of DNA samples, described in detail elsewhere,[17] involved an ethanol precipitation to remove contamination from proteins, sonication, dialysis, and lyophilization of a stock NaDNA solution. The molecular weight distribution of the sonicated DNA was found to range from ca. 3.6×10^4 to 4.1×10^5 Da by agarose gel electrophoresis. The DNA concentration in nucleotide units (P) was determined on a Varian Cary 50 UV−visible spectrophotometer from the absorbance at 260 nm ($\epsilon_{260} = 6600$ mol L^{-1} cm^{-1}). The observed A_{260}/A_{280} ratio of the DNA solution was 1.9, consistent with low protein content. The sodium concentration was measured on the ^{23}Na NMR spectrum by comparison with the signal from a capillary reference containing aqueous NaCl at known concentration and the shift reagent $[Dy(P_3O_{10})_2]^{7-}$ to avoid spectral overlap. The ratio of the total concentrations of sodium ions and DNA phosphates was 1.4.

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All lcNaDNA samples were prepared into 5-mm NMR tubes at the concentration of 275 mg of DNA/mL of solvent. The divalent metal free lcNaDNA solution was prepared by dissolving lyophilized NaDNA (137.5 mg) in 1 mM PIPES buffer (500 μL, 50% D_2O, pH 7). Samples of lcNaDNA containing $MgCl_2$, $CdCl_2$, or $NiCl_2$ were prepared at $[Me^{2+}]$/[P] molar ratios of 0.025 and 0.050 by dissolving lyophilized NaDNA (137.5 mg) in a 1 mM PIPES buffer (500 μL, 50% D_2O, pH 7) containing the proper amount of $MeCl_2$. All samples were mixed by repeated heating and centrifugation and allowed to equilibrate for at least 15 days before NMR experiments and microscopic examination.

Polarized Light Microscopy. For each sample, a droplet of solution was deposited between a microscope slide and coverslip, sealed, and allowed to equilibrate for 7 days before observation through crossed polarizers with a Leica DMR microscope equipped with 10×, 63×, and 100× (oil immersion) objectives at ambient temperature. The cholesteric pitch was measured with a 100× objective, after achieving a macroscopic alignment of the samples through exposition to a 4.7 T NMR magnet for 64 h with the field oriented parallel to the microscope slide. Effects of the alignment procedure on the pitch measurement were not observed.

NMR Spectroscopy. ^{2}H,^{23}Na, and ^{31}P NMR spectra were recorded on a Varian Unity-Inova 400 spectrometer at the resonance frequency of 61, 106, and 162 MHz, respectively. The temperature was controlled at 298 K. ^{2}H NMR spectra were acquired using a quadrupolar echo sequence[18] with a 90° pulse of 35 μs, a spectral width of 2000 Hz and 64 transients. The recycle time delay was always at least five times the spin−lattice relaxation time. ^{23}Na NMR spectra were recorded using a simple one pulse sequence with a 90° pulse of 21 μs, a spectral width of 10 kHz and 64 transients.^{31}P NMR measurements were performed with gated proton decoupling, a 90° pulse of 22 μs, 50 kHz of spectral width, and 7000 transients. The ^{31}P chemical shifts were referenced to 85% H_3PO_4. All samples were thermally equilibrated for about 1 h before running the NMR experiments. The spectra were taken on a nonspinning sample and recorded repeatedly during a period of 3−4 months during which no changes of the spectra were observed. The field homogeneity was adjusted on the proton NMR FID.

Results

^{31}P NMR spectra of lcNaDNA solutions are shown in Figure 1. In the absence of divalent metal ions, the spectrum consisted of a main broad resonance superimposed on a weak narrower downfield signal (Figure 1a).

This result, very similar to that reported for linear[19] and supercoiled[20] DNA liquid crystalline solutions, agrees with the coexistence of an anisotropic phase and a residual isotropic one, the former corresponding to the broad signal and the latter to the sharp one. The fractions of DNA molecules in the two phases were estimated by deconvoluting the ^{31}P NMR spectrum with a combination of a Lorentzian and a Gaussian function.[21] The Lorentzian line shape, typically observed in pure isotropic phases, originates from the rapid tumbling motions of DNA molecules which average

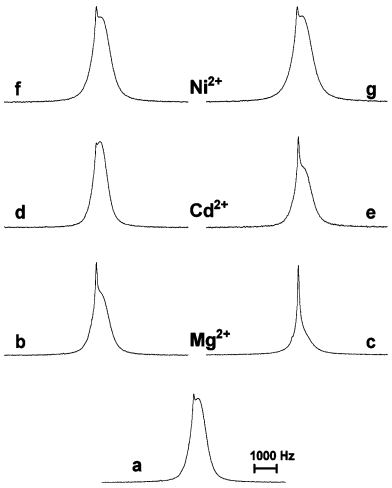

Figure 1. ^{31}P NMR spectra of 275 mg/mL lcNaDNA samples in the absence (a) and in the presence of Mg^{2+} (b,c), Cd^{2+} (d,e), and Ni^{2+} (f,g) at the $[Me^{2+}]$/[P] molar ratios of 0.025 (left) and 0.050 (right).

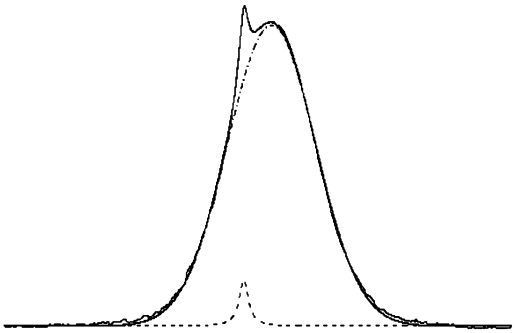

Figure 2. ^{31}P experimental (solid curve) and deconvoluted (Lorentzian: dashed curve; Gaussian: dashed−dot curve) NMR spectra of the 275 mg/mL lcNaDNA sample.

out the ^{31}P chemical shift anisotropy. The Gaussian line shape, which characterizes the distribution of resonances in an anisotropic phase, is due to restricted molecular motions only partially averaged out. Figure 2 shows the good agreement between the ^{31}P experimental and simulated NMR spectra of the 275 mg/mL lcNaDNA sample. From the integrated areas of the resonances, which are proportional to the fraction of molecules in the pertinent phase, it was found that the fraction of DNA in the isotropic domain was less than 0.02.

The morphology of the anisotropic phase in divalent ion free lcNaDNA was characterized by microscopic examination of the sample through crossed polarizers. The solution exhibited a strong birefringence and no phase separation. A regular fingerprint-like pattern was observed (Figure 3a), indicating unambiguously the presence of a cholesteric phase. The average cholesteric pitch, measured as twice the distance between the fringes after macroscopic alignment of the preparation slide in a 4.7 T magnetic field, was 2.6 ± 0.2 μm.

Following the addition of $MgCl_2$, the ^{31}P NMR spectrum of lcNaDNA changed notably (Figure 1b,c). The marked enhancement of the relative intensity of the downfield resonance at increasing Mg^{2+} concentrations was taken as evidence of the notable growth of the isotropic phase at the expense of the anisotropic one.[21] This conclusion was

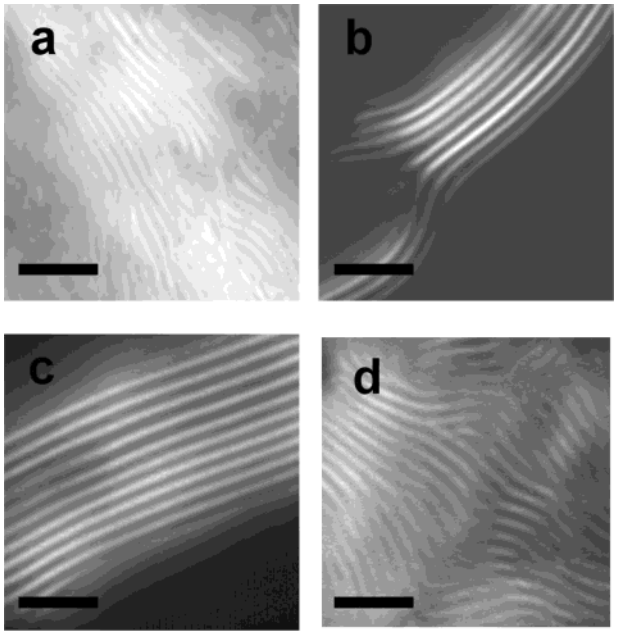

Figure 3. Polarized light microscopic textures of 275 mg/mL lcNaDNA solutions in the absence (a) and in the presence of Mg^{2+} (b), Cd^{2+} (c), and Ni^{2+} (d) at the binding molar ratio of 0.050 (original magnification 100×; bars = 10 μm).

reinforced by the observation of small isotropic dark domains adjacent to the cholesteric ones in the microscopic textures of the lcNaDNA/Mg^{2+} samples (Figure 3b), where the cholesteric pitch did not differ from that of the divalent ion free sample. By the above-mentioned deconvolution procedure, we found that the fractions of isotropic DNA were 0.1 and 0.3 at [Mg^{2+}]/[P] = 0.025 and 0.050, respectively.

Adding Cd^{2+} produced no significant changes on the ^{31}P NMR spectrum at the binding molar ratio of 0.025 (Figure 1d), the sample remaining fully cholesteric (polarized light microscopy textures not shown). At [Cd^{2+}]/[P] = 0.050 an isotropic phase coexisted with the anisotropic one, as was clearly shown either by ^{31}P NMR spectroscopy (Figure 1e) and polarized light microscopy (Figure 3c); the fraction of DNA rods in the isotropic phase was found equal to 0.1 and the average cholesteric pitch to 2.1 μm.

In the presence of Ni^{2+}, the ^{31}P NMR spectra at [Ni^{2+}]/[P] molar ratios of 0.025 and 0.050 were equal to that of lcNaDNA (Figure 1f,g), thus indicating similar phase composition in the samples. Moreover, no isotropic domains emerged from the polarized light microscopic textures of the complexes (Figure 3d), pointing out a reduction of the cholesteric pitch to 2.1 μm, as for Cd^{2+}.

^{2}H NMR was used to obtain complementary information about the phase composition of the samples (Figure 4). The ^{2}H NMR spectrum of water in divalent ion free lcNaDNA consisted of a symmetrical doublet (Figure 4a), originated from the anisotropic interaction of the ^{2}H quadrupole moment with the intramolecular electric field gradient of the water molecule.[22] The quadrupolar splitting, 47 Hz, compared favorably with those reported in the literature for similar cholesteric NaDNA solutions.[23,24]

The addition of divalent metal ions did not affect the quadrupolar splitting, thus indicating that no changes in the orientational order of water molecules occurred in the

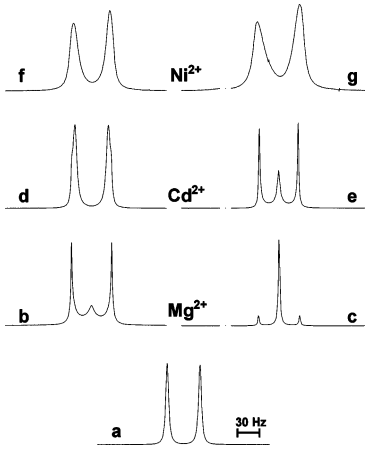

Figure 4. ^{2}H water NMR spectra of 275 mg/mL lcNaDNA samples in the absence (a) and in the presence of Mg^{2+} (b,c), Cd^{2+} (d,e), and Ni^{2+} (f,g) at the [Me^{2+}]/[P] molar ratios of 0.025 (left) and 0.050 (right).

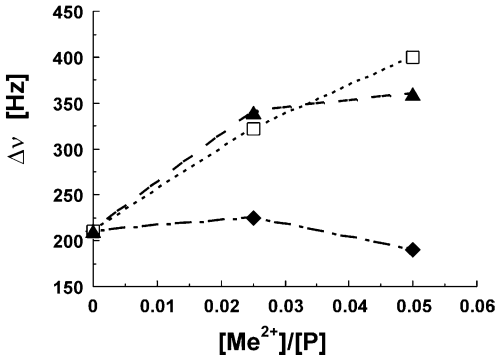

Figure 5. ^{23}Na NMR quadrupolar splitting of 275 mg/mL lcNaDNA as a function of the [Me^{2+}]/[P] molar ratio: Mg^{2+} (◆), Cd^{2+} (▲), and Ni^{2+} (□).

anisotropic domain.[22] Moreover, the spectra showed the existence of two phases in the lcNaDNA/Mg^{2+} samples (Figure 4b,c), and in lcNaDNA/Cd^{2+} at [Cd^{2+}]/[P] = 0.050 (Figure 4d,e). The resonance appearing at the center of the doublet was due to the exchange of water molecules between isotropic and anisotropic domains with a rate smaller than the inverse of the splitting. The slight asymmetry of each doublet component (cf. inner and outer edges) is likely ascribable to a Gaussian distribution of the orientation of the cholesteric director about the applied magnetic field.[23] In the case of Ni^{2+}, the doublet components broadened asymmetrically and progressively with the amount of ions (Figure 4f,g), this marked differential broadening arising from cross correlation between quadrupolar and dipolar (paramagnetic) fluctuations.[25]

Differences upon addition of Mg^{2+}, Cd^{2+}, or Ni^{2+} to DNA also emerged in the ^{23}Na NMR spectra: the quadrupolar splitting ($\Delta\nu$) of the ^{23}Na triplet, which in lcNaDNA was 210 Hz, increased in the presence of Cd^{2+} or Ni^{2+}, whereas it decreased slightly by adding Mg^{2+} (Figure 5).

Discussion

The overall results from NMR spectroscopy and polarized light microscopy indicated unambiguously that the state of cholesteric NaDNA is altered in the presence of $MgCl_2$, $CdCl_2$, or $NiCl_2$ at the same concentration in a different way

and to a different extent. In particular, the addition of Mg^{2+} promoted notably the formation of an isotropic phase from the cholesteric one, and the presence of Ni^{2+} affected the arrangement of the cholesteric phase mainly by reducing the pitch, whereas effects strongly reminding those of either Mg^{2+} and Ni^{2+} were observed in the presence of Cd^{2+}.

To evaluate possible ionic strength effects[26,27] on phase behavior of the lcNaDNA/Me^{2+} samples, two additional divalent ion free lcNaDNA solutions, containing excess NaCl, were investigated at the same DNA concentration and ionic strength as in lcNaDNA/Me^{2+}. No differences in the NMR spectra of the three Me^{2+}-free lcNaDNA solutions were observed (data not shown), and thus, the influence of the ionic strength was ruled out.

Since the primary targets of magnesium ions on the DNA binding sites are the phosphate groups,[1,5,6] the formation of the isotropic phase promoted by Mg^{2+} stresses the importance of the electrostatic $Mg^{2+}-PO_4^-$ interactions in bringing down the preexisting liquid crystalline phase. This finding agrees pretty well with previous optical microscopy observations on nonsonicated calf thymus lcDNA solutions containing Mg^{2+};[28] however, it differs from the observations made in liquid crystalline solutions obtained from nucleosome core DNA fragments (ca 150 base pairs), where no phase separation occurred.[29] From the results of these investigations and ours, the question of whether the observed partial phase transition in the presence of Mg^{2+} is length dependent or not cannot be answered. However, we point out that, though the partitioning of differently long DNA filaments between isotropic and anisotropic phase cannot be excluded,[30,31] this phenomenon is expected to be of a minor entity because of the low molecular binding ratios used in this work.

Comparing lcNaDNA/Cd^{2+} or lcNaDNA/Ni^{2+} with lcNaDNA/Mg^{2+} shows that transition metal ions have a lower efficiency in promoting the isotropic phase and, thus, bind to phosphates to a much lower extent than Mg^{2+}, this binding mode being almost negligible in the case of Ni^{2+}. These results are not unexpected if one remembers that, compared to Mg^{2+}, both Cd^{2+} and Ni^{2+} exhibit a stronger affinity for the bases than for phosphates.[1,5,6]

Unlikely Mg^{2+}, binding of transition metal ions to DNA reduces the pitch of the cholesteric phase. Thus, following the above discussion on the different binding modes of Mg^{2+}, Cd^{2+}, and Ni^{2+} to DNA, we reasonably infer that the observed changes of the cholesteric pitch cannot be attributed to sole electrostatic effects. In this regard, it is well-known that Cd^{2+} and Ni^{2+} induce substantial structural perturbations on DNA, such as base unstacking and/or changes in the nucleoside conformation.[5] Since these effects come into play in the packaging of the rods,[32] a complex and subtle interplay of these overall factors must be involved in the observed modifications of the cholesteric pitch.

The different nature of the complexing divalent metal ions is also evidenced by the changes of the ^{23}Na quadrupolar splitting upon DNA binding (Figure 5). Comparing the data obtained by ^{23}Na, ^{31}P, and ^{2}H NMR, an apparent disagreement seems to exist. However, it is worth reminding that the three nuclei probe different properties of DNA; in particular, the ^{23}Na quadrupolar splitting arises from contributions of bound counterions exchanging among DNA sites differing either in order parameters and/or quadrupolar coupling constants.[33] Due to difficulties in analyzing the ^{23}Na splitting via a manifold of different binding sites, the observed $\Delta\nu$ is commonly rationalized in terms of two average sites: the phosphate groups and the bases.[34] According to this simple model and assuming a fast exchange regime, the splitting is given by a population average of contributions possibly differing in magnitude and/or sign, depending on the orientation of the electric field gradient experienced by sodium ions at the binding sites.[34]

Since Cd^{2+} and Ni^{2+} modify significantly the local structure of DNA, contrariwise to Mg^{2+}, it is likely that electric field gradients are sensibly affected by binding to DNA and change the ^{23}Na quadrupolar splitting. This might not be the case upon binding of Mg^{2+}. However, it must be stressed that, due to the competitive exchange of Na^+ and the divalent ions at the binding sites, a redistribution of the sodium ions between phosphates and bases in the presence of Mg^{2+}, Cd^{2+}, or Ni^{2+} can make the above picture more complex.

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