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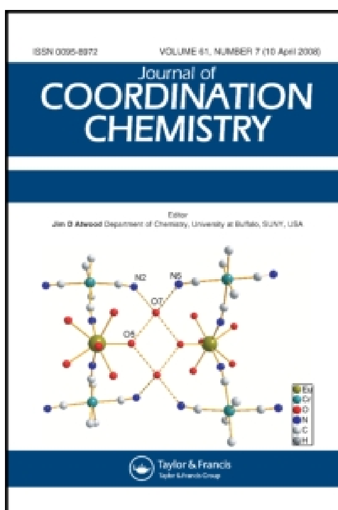

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DNA BINDING STUDIES OF MAGNESIUM(II), CALCIUM(II), BARIUM(II) AND ATP COMPLEXES

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DNA BINDING STUDIES OF MAGNESIUM(II), CALCIUM(II), BARIUM(II) AND ATP COMPLEXES

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We have investigated the ways by which magnesium(II), calcium(II) and barium(II) and their ATP complexes bind to B-DNA. Effects on native calf thymus DNA conformation were studied and compared with those involving the uncomplexed metal ions and the polymer. It was found that $MgATP^{-2}$, $CaATP^{-2}$ and $BaATP^{-2}$, interact with the phosphates of the polynucleotide; only $BaATP^{-2}$ appears to bind also through the bases. ATP complexes cause transitions mainly to the B family. The C form is induced by small amounts of $MgATP^{-2}$ or $CaATP^{-2}$. At high complex concentrations (r = 2.5 or 5) the polynucleotide adopts the ψ^- conformation, while $BaATP^{-2}$ induces this transition at r = 0.6. A contribution of the A form is observed in the case of CaATP-DNA at r = 0.6 and at the beginning of the reaction in the Ba-DNA system at 22°C, for r = 5. The alkaline earth metal ions induce a stabilization of the B conformation. Structural changes with magnesium(II) at r = 2.5 are interpreted in terms of a B to C transition. Calcium(II) at r = 5 produces the A form. In other cases the alkaline earth metal ions stabilize the B conformation. It was found that they all interact with the phosphates of the polynucleotide. The binding ratio, r, is an important factor in all structural changes.

Keywords: DNA; group IIA cations; ATP complexes; circular dichroism; thermal Denaturation

INTRODUCTION

As metal-containing species play a vital role in genetic information transfer [1a], in mutagenesis [1b] and in carcinogenesis [1c], considerable efforts have been devoted to understanding the structural nature of metal ion interactions with

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nucleic acids and their components [1d]. The binding of metal ions and of small as well as bulky ligands (drugs) to DNA has been the subject of a great number of studies, not only in physical chemistry, but also in molecular pharmacology [2a], medicinal chemistry and carcinogenesis [2b,c]. DNA has a number of types of sites in which a molecule might bind: between two bases (full intercalation), in the minor groove, in the major groove and on the outside of the helix [3]. The binding activity (and selectivity) varies with base-pair composition, binding ratio and ionic strength.

Magnesium is an important cofactor in cellular processes involving nucleic acids [4a]. In order to understand why magnesium ions are required, as opposed to other divalent cations, many researchers have studied the interactions of magnesium ions with double-stranded DNA. These studies have demonstrated that magnesium ions can have wide-ranging effects on DNA structure. At high concentration in aqueous solution, magnesium can change the structure of pyrimidine-purine polymers from the B to the Z form [4b]. Magnesium with the addition of a dehydrating solvent like ethanol, is able to condense genomic DNA into toroidal or rod-like structures [4c]. Recent work has demonstrated that at elevated temperatures divalent cations can aggregate DNA without the addition of a dehydrating agent [4d]. Studies using gel electrophoresis have shown that the magnesium ion concentration can affect the extent of curvature of bent DNA fragments and that the effect is sequence dependent. This suggests that magnesium ions can interact with DNA in a site-specific manner [4e]. From interpretation of ^{25}Mg NMR relaxation rates was suggested that the findings for a particular magnesium-coion-DNA system cannot be generalized to all magnesium-DNA interactions [4f].

Magnesium activates a large number of enzymes, especially kinases and carboxylases. Kinases transfer a phosphate group from ATP to the substrate [5]. Calcium performs a wide variety of biological functions. Its important roles may be classified into three categories. The first is a structural role, the ability to stabilize the conformation of proteins. Ca(II) in α-amylase, DNAase, and microbial proteinase seems to play this role. The second is its role in forming solid skeletal material, such as bones and shells, for various organisms; the bulk of the skeletal material consists of inorganic Ca(II) compounds. The third and most important is its ability to trigger certain physiological activities, such as muscle contraction and the release of hormones [5]. Calcium and magnesium coordinate preferably to the phosphate group of ATP or other mono- or polynucleotides. Thus they participate in enzymatic reactions involving ATP or other phosphate-containing compounds, and they are believed to play a role in stabilizing polynucleotides [5]. The study of DNA interaction with ATP

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complexes seems to be biologically interesting since they are present in physiological systems.

As part of our investigations on the interaction of metal ions with DNA [6a-c] and in view of the great interest in this field, we report conformational changes of DNA induced by Mg-ATP, Ca-ATP and Ba-ATP and also, for reasons of comparison, the aquo complexes $[M(H_2O)_6{}^{2+}]$, M = Mg, Ca, Ba.

RESULTS AND DISCUSSION

Circular Dichroism Studies of Mg(II), Ca(II) and Ba(II)

Strong evidence for binding and conformational changes in the double helical structure of DNA upon interaction with metal ions was obtained by circular dichroism measurements. The CD spectrum of DNA alone after 4 h incubation at 22°C with [DNA-P] = 5×10^{-5} M, exhibits one positive and one negative band at 275 and 243 nm having $\Delta\epsilon$ values +1.4 and -2.4 M^{-1} cm^{-1} (throughout) respectively (Figure 1). This spectrum remains unchanged at 37°C for all incubation times (1-96 h).

Binding of $[Mg(H_2O)_6]^{2+}$ to the DNA molecule, (Figure 2) results in a decrease of the negative band intensity, which ranges between 0.8 and 0.6, while

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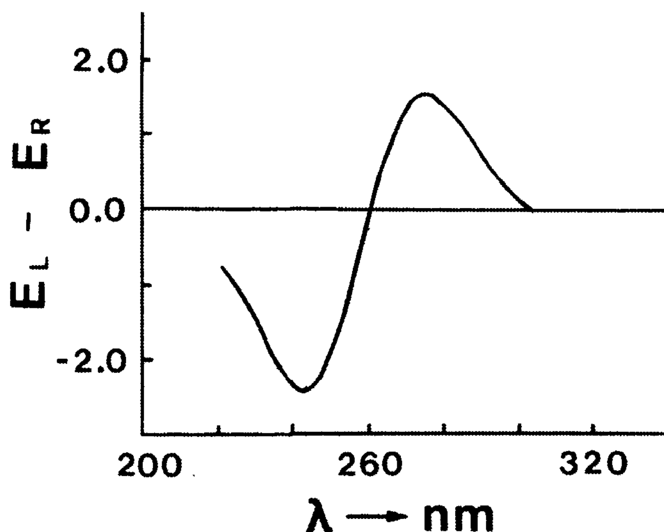

FIGURE 1 CD spectrum of native CT DNA (5×10^{-5} M) in KBr (1.5×10^{-3} M), pH = 7.0, at 22°C after 1h of incubation. It remains the same for all incubation times at both 22 and 37°C.

the positive peak retains its intensity. These changes are observed at 22°C as well as at 37°C for all interaction times and molar ratios r = 0.6 and r = 1. When r = 2.5 the positive peak loses half its intensity while the decrease of the negative ellipticity is greater. At the greatest molar ratio studied the Mg-DNA CD spectrum becomes almost the same as that of pure DNA. The temperature or the incubation time seem to provoke changes to the CD peaks ranging between 7% and 14%. The data suggest that, for r = 0.5, r = 1 and mainly for r = 5, the B conformation of the DNA chain remains unaltered [7a]. Theoretical calculations carried out by Moore and Wagner [7b] have showed that this kind of trend to a non-conservative positive or negative spectrum result from a change of base pairs distance from the diad helix axis. The negatively charged phosphate backbone of DNA interacts with the positively charged Mg(II) ion through electrostatic forces either through the phosphate oxygen atom, reducing thus the helix charge and

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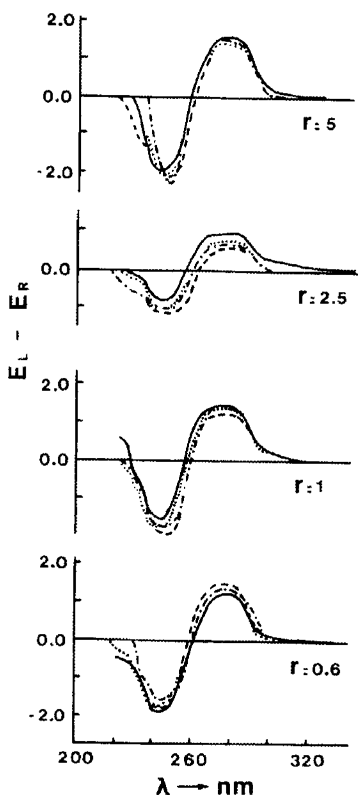

FIGURE 2 CD spectra of the Mg-DNA system at 37°C for different times of interaction: (—) 1 h, (····) 4 h, (---) 96 h and (-----) 1 h at 22°C.

TABLE I Differential molar absorptivities CD studies of Ca-DNA (M^{-1} cm^{-1}).

r = 0.6				r = 1				r = 2.5				r = 5			
λ (nm)	Δε	θ (°C)	t (h)	λ (nm)	Δε	θ (°C)	t (h)	λ (nm)	Δε	θ (°C)	t (h)	λ (nm)	Δε	θ (°C)	t (h)
272	+1.0	22	1	274	+1.0	1	22	274	+0.9	22	1	274	+0.9	22	1
240	−1.5			242	−1.5	242	−1.4	242	−1.4						
274	+0.9	37	1	273	+1.0	1	37	271	+1.2	37	1	274	+1.0	37	1
243	−1.4			241	−1.6	240	−1.1	240	−1.3						
272	+1.0	37	4	274	+0.9	4	37	272	+1.0	37	4	276	+1.2	37	4
243	−1.5			242	−1.7	241	−1.8	242	−2.0						
272	+0.9	37	96	275	+1.0	96	37	272	+0.9	37	96	272	+0.9	37	96
241	−2.0			242	−1.7	242	−1.6	241	−1.4						

stabilizing the B conformation or an energetically favourable location in the major groove [7a]. In these cases the spectrum retains its conservative character (the sum of the rotational strengths for the polymer is equal to zero).

The conservative character of the spectrum is not maintained when r = 2.5. A considerable decrease in intensity and a small red shift in the position of both bands was observed. These changes have been interpreted in terms of a B to C transition with unwinding of the helix, decrease of the pitch and a decrease in the groove size [7c] (C belongs to the B family). This type of transition was previously found in the presence of divalent metal ions, such as Mn(II), Zn(II) and Co(II) [8a] or Ca(II), Sr(II) and Mn(II) at 0.005 M and 0.001 M NaCl [8b]. Platinum(II) compounds (*trans* and *cis*-dichlorobis(cyclohexylamine)) [8c] cause local changes in the DNA conformation which are characteristic of a B to C transition. This type of packing has been observed also in the case of DNA in nucleoproteins when treated with magnesium [7d], and in calf thymus DNA when treated with $RhCl_3(H_2O)_3$ and $RhCl_2(H_2O)_4$ [6c]. The process is thermodynamically favourable, since it has been suggested that the B to C transition requires only a very small enthalpy change ($\Delta H^0 = 41.9$ kJ mol^{-1}) and a positive entropy change [8d].

The results obtained from the binding of $[Ca(H_2O)_6]^{2+}$ to CT DNA are summarized in Table I. We observe a diminution in the intensities of both DNA CD bands after the addition of the cation. The decrease of the negative band intensity ranges between 0.4 and 1.0, and the positive band ranges between 0.2 and 0.5. These changes are nearly the same for all molar ratio values. They do not seem to depend directly on temperature or incubation time. Peak positions remain about the same and the spectra retain their conservative character. The reduction of the intensity suggests a decrease in the helicity of the DNA; however there is no evidence for large distortions from the native helix [9a]. It is concluded, as in the case of magnesium ions, that stabilization of the B

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TABLE II Differential molar absorptivities for CD studies of Ba-DNA ($M^{-1}cm^{-1}$).

r = 0.6				r = 1				r = 2.5				r = 5			
λ (nm)	Δε	θ (°C)	t (h)	λ (nm)	Δε	θ (°C)	t (h)	λ (nm)	Δε	θ (°C)	t (h)	λ (nm)	Δε	θ (°C)	t (h)
273	+1.2	22	1	272	+1.0	1	22	274	+1.0	22	1	274	+1.7	22	1
242	−2.0			240	−1.7	238	−2.0	243	−1.0						
272	+1.2	37	1	273	+1.0	1	37	272	+1.2	37	1	270	+1.3	37	1
242	−1.6			243	−1.5	240	−1.5	241	−1.6						
274	+1.3	37	4	275	+1.0	96	4	272	+1.1	37	4	272	+1.2	37	4
241	−1.6			242	−1.7	241	−1.8	242	−1.7						

conformation occurs. Consequently the conformational change of the polymer and the binding mode of the metal ions to the DNA chain are the same.

The changes that Ba(II) bring to the CD spectrum of CT DNA are listed in Table II. They are comparable to those caused by Ca(II). The only difference observed is at 22°C and r = 5, where a slight increase in the positive peak occurs, indicating a contribution of the A DNA conformation to this spectrum [9c]. In the other cases the same stabilization of the B conformation is found. Normal DNA can be very easily converted to an A form by addition of ethanol, guanidine, isopropanol or dioxane [9b,c]. The main feature that characterizes the members of the A family is the inclination of the base pairs to the helix axis which is far from the base pair centre of gravity [9c]. This transition was not seen in the cases of Mg(II) or Ca(II) ions, proving that these cations stabilize the B conformation of the polynucleotide.

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Circular Dichroism Studies of Mg-ATP, Ca-ATP and Ba-ATP

Upon interaction of Mg-ATP with DNA (Figure 3) a decrease in the intensity of the polynucleotide positive ellipticity is observed which ranges between 0.3 and 0.7. At r = 0.6 a decrease in the intensity of the negative peak is observed, which diminishes with progressive addition of complex. For r = 1 at 22°C it almost reaches the value exhibited by pure DNA, while for r = 2.5 it is 46% greater. When r reaches 5 it is not possible to record the negative peak as the solution now absorbs strongly in this region. The positive peak at the beginning of the reaction when r = 0.6 has an intensity which is 57% of that of the free polynucleotide. Four days later it is greater by 0.3 but still remains less than that of free DNA. When the ATP complex concentration equals the DNA concentration at 22°C the positive peak of the DNA-MgATP system is half that of the uncomplexed DNA. By raising the temperature this peak shows a small increase compared to that at 22°C and reaches a value of 1.1 after 4h incubation. It remains the same after 4 days. At higher complex concentrations changes in the positive CD band upon

incubation time or temperature vary between +0.1 and −0.1. The position of both peaks is shifted toward longer wavelengths in all cases.

Decrease in the positive band around 275 nm with a simultaneous position shift after the addition of metal ions is indicative of a B to C transition [10a,b]. The C form of DNA belongs to the B family. According to the literature, our experimental data in the cases where MgATP is present at r = 0.6 and 1 suggest a B to C transition of the polynucleotide. When r = 2.5, a great increase in the negative peak appears, together with a band shift; this is consistent with a transition from B to ψ^- conformation. This conversion is not fully accomplished as the positive peak did not disappear absolutely. Similar behaviour was observed in the case of DNA-MgATP thin films at relative humidities of 85 and 95% [10a]. Such particular structures have been previously found for DNA-poly(Lys70Ala30) [10c] and DNA-histone [10d]. The ψ^- spectrum of DNA

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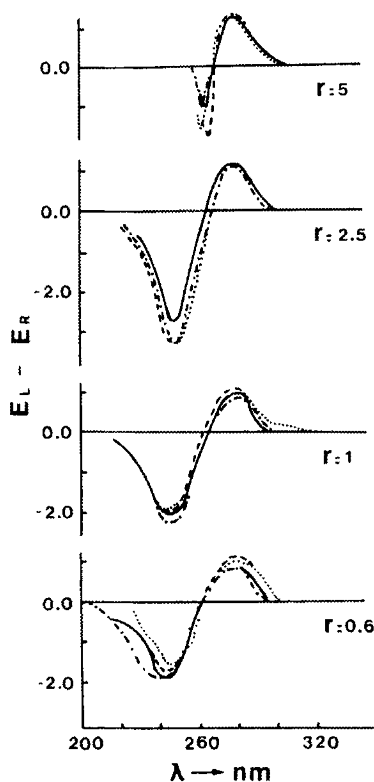

FIGURE 3 CD spectra of the MgATP-DNA system at 37°C for different times of incubation: (—) 1 h, (····) 4 h, (---) 96 h and (-·-·-) 1 h at 22°C.

arises from a compact form where the polynucleotide helices are anisotropically ordered in twisted, left-handed, oriented aggregates [10c]. It has been shown that DNA in the ψ^- state forms part of the B family [10e].

In the case of the CaATP-DNA system (Figure 4) r = 2.5 we observe the same phenomena that MgATP causes to DNA at an equal concentration. The CD spectra are indicative of an incomplete B to ψ^- transition. As the reaction begins at 37°C and at the lowest ratio studied, r = 0.6, a contribution of the A conformation is observed (enhanced positive and reduced negative ellipticity) [9c], but the DNA molecule regains its B conformation as the reaction proceeds. When the Ca complex concentration corresponds to that of the phosphate group, a small decrease in both bands is observed at the beginning of the reaction at

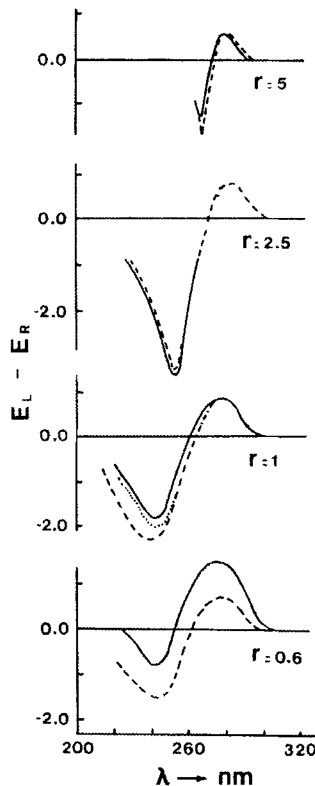

FIGURE 4 CD spectra of the CaATP-DNA system at 37°C for different times of incubation: (—) 1 h, (····) 4 h and (---) 96 h. The spectra obtained at 22°C are the same as that recorded at 37°C after 1 h of interaction. Those obtained at 37°C and 4 h of incubation and not depicted in the Figure, but are represented by the curves at 96 h.

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TABLE III Differential molar absorptivities for CD studies of BaATP-DNA (M^{-1} cm^{-1}).

r = 0.6				r = 1				r = 2.5				r = 5			
λ (nm)	Δε	θ (°C)	t (h)	λ (nm)	Δε	θ (°C)	t (h)	λ (nm)	Δε	θ (°C)	t (h)	λ (nm)	Δε	θ (°C)	t (h)
280	+1.2	22	1	282	+0.6	1	22	281	+0.8	22	1	282	+1.2	22	1
250	−3.0			266	−2.9										
282	+0.9	37	1	282	+0.9	1	37	282	+0.8	37	1	284	+1.1	37	1
254	−3.6														
280	+1.3	37	4	282	+1.0	96	4	272	+0.9	37	4	283	+1.0	37	4
248	−3.3														

TABLE IV Melting temperatures, Tm (°C), of complex ion-DNA solutions in KBr (1.5×10^{-3} M), pH = 7.0, [DNA-P] = 5×10^{-5} M for interaction time of 1h.

	$MgNO_3$	$CaCl_2$	$BaCl_2$	MgATP	CaATP	BaATP
0.6	78.1	74.8	72.1	72.2	74.0	63.5
1	79.3	76.6	75.8	74.9	75.0	58.8

37°C; the spectrum is characteristic of B DNA. As time passes the negative peak increases and the positive band is red shifted, behaviour evoked by a B to C transition [8b]. At the highest concentration studied the positive peak was shifted to 282 nm while the negative band was so intense that no record of the CD of this peak was possible.

Table III presents CD data for CT DNA at different ratios of BaATP complex to polymer residues. A decrease of the positive band and an increase of the negative peak is observed at ratio r = 0.6, together with a shift of both bands to longer wavelengths, thus implicating a ψ^- transition [8c, 10c,d]. The CD spectrum remains that of the ψ^- form at 22°C when r = 1. If the temperature or the metal concentration is raised, the positive peak remains red shifted at about 282 nm with slightly reduced intensity, but the negative band is markedly enhanced that no record of its intensity was possible.

It should be noted here, that in all cases where the ATP complex is present, corrections have been made to the CD spectra by taking into account the negative CD of the ATP complex in the region around 260 nm. For the magnesium and calcium ATP complexes at concentrations of the order of 10^{-3} M at neutral pH, a considerable percentage of undissociated complex exists [11a].

Thermal Denaturation and UV Studies

The Tm value of pure DNA under the experimental conditions used is 59.0°C. All Tm results obtained are shown in Table IV. Since ATP absorbs strongly in

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the region, it was not possible to obtain thermal denaturation results for ratios greater than 1 for the ATP complexes.

The addition of MgATP ions after 1 h of incubation significantly augments the Tm value of native CT DNA ($\Delta\Theta$ = 13.2°C, for r = 0.6 and $\Delta\Theta$ = 15.9°C for r = 1). $[Mg(H_2O)_6]^{2+}$ ions cause an even greater increase ($\Delta\Theta$ = 19.1°C for r = 0.6 and $\Delta\Theta$ = 20.3°C for r = 1). It is obvious that both magnesium compounds interact with the phosphate groups of the DNA chain [11b]. These results contradict the findings of Bhattacharya *et al.* [10a] who gave the Tm values 73.5, 61.0 and 80.0°C for DNA, DNA-MgATP and DNA-Mg respectively. They used 0.015 M KBr as solvent and the molar ratio DNA:MgATP was 1:0.6, while that of DNA:Mg(II) was 1:2.5. We performed experiments using the same ionic strength but no difference in Tm values was observed upon addition of the magnesium compounds. The K^+ ion concentration proved to be too high. It stabilizes the double helix of DNA to such a point that the further addition of other metal ions at concentrations about 300 times less give no effect. Therefore we used 1.5×10^{-3} M KBr and the work was expanded to include the two other alkaline earth ions.

CaATP ions were also found to increase the Tm value of DNA ($\Delta\Theta$ = 15.0°C for r = 0.6 and $\Delta\Theta$ = 16.0°C for r = 1) to the same extent as Ca(II) ions ($\Delta\Theta$ = 15.8°C for r = 0.6 and $\Delta\Theta$ = 17.6°C for r = 1). From these data, it is concluded that they both stabilize the polynucleotide double helix [11b].

The third cation studied exhibits different behaviour. At r = 0.6, BaATP increases the DNA Tm value by only 4.5°C, whereas at r = 1.0 no change was observed ($\Delta\Theta$ = −0.2°C). In this case, binding to the phosphate groups is not very strong; the ATP complex interacts with the bases [11c]. On the contrary, Ba(II) ions bind more strongly to the phosphates [11b], as they raise the Tm value ($\Delta\Theta$ = 13.1°C for r = 0.6 and $\Delta\Theta$ = 16.8°C for r = 1).

The UV spectrum of DNA consists of one band having its maximum at 258 nm. It remained unaltered after addition of Mg(II), Ca(II) or Ba(II) ions, while their ATP complexes caused an expected increase in intensity as ATP absorbs at this wavelength; no shift in the position of the band was observed in any case. The above data suggest interaction of the metals with the phosphates of the DNA, as it was found that metal ions which bind preferentially to the phosphate moieties do not produce modifications in the ultraviolet spectrum [11d].

In conclusion, aquo complexes of Mg(II), Ca(II) and Ba(II) stabilize the B form of CT DNA, interacting mainly through the oxygen atoms of the phosphate groups. Magnesium(II) at r = 2.5 produces a B to C transition, while calcium(II) at r = 5 produces the A form. In the other cases the alkaline earth metal ions stabilize the B conformation. On the other hand, their complexes with ATP influence DNA conformation, inducing transitions that usually belong to the B

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family. The C form is usually induced by small amounts of MgATP or CaATP. At high complex concentrations (r = 2.5 or 5) the polynucleotide adopts the ψ^- conformation, while the BaATP system induces this kind of transition even at r = 0.6. A contribution of the A form is observed in the case of CaATP at r = 0.6 and at the beginning of the reaction as well as in the Ba-DNA system at 22°C, for r = 5. ATP complexes also interact with the phosphate moieties except for BaATP, which binds to the bases.

EXPERIMENTAL

Calf thymus (CT) DNA (type I, highly polymerised) was obtained from Sigma. Triply distilled water was used in each experiment. $Na_2MgATP.3H_2O$, $Na_2CaATP.2H_2O$ and $BaH_2ATP.2H_2O$ (abbreviated in the text as MgATP, CaATP, BaATP) were synthesized according to the literature [11e].

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Thermal Denaturation Studies

Stock solutions of 5×10^{-3} M [DNA-P] were prepared by adding 17 mg of native CT DNA to 10 cm^3 of a 0.015 M KBr solution (found to be a very effective solvent [10a]) and gently shaking the mixture at room temperature for a few hours. The pH of the solution was maintained neutral by the addition of 4 M KOH. Solutions were checked spectrophotometrically at 260 nm for DNA content and kept refrigerated. Working solutions were prepared before each experiment by 1:100 dilution of the stock solution. The pH was kept between 6.8 and 7.0. The amount of metal compound added to the DNA solution was expressed as r, the molar ratio of metal to phosphorus in DNA.

Melting curves were recorded on a 551S Perkin-Elmer spectrophotometer at various r ratios and at various interaction times. The rate of temperature increase was 1°C min^{-1} and measurements of absorbance and temperature values were carried out at a fixed wavelength of 260 nm.

Circular Dichroism Studies

CD measurements were carried out using an ISA Yvon Dichrographe III-S, in a 1 cm cell and in the 350-200 nm range. Molar absorptivities for absorption spectra ϵ and differential molar absorptivities were calculated on the basis of DNA concentration [11f]. Calibration of the instrument was checked with an aqueous solution of *d*-10-camphorsulfonic acid. Prior to use, the *d*-10-camphorsulfonic acid was purified by recrystallization from hot 95% ethanol and

dried to constant weight in vacuum at 40°C. CD spectra were recorded at two temperatures, 22°C and 37°C for all values of r. No significant changes in the CD spectra at 22°C were observed, even for prolonged times of incubation, so in this study only the initial values at 22°C are presented (incubation time 1 h). Ultraviolet spectra of complex ion solutions in absence as well as in presence of DNA were obtained on a Varian Cary 14 spectrophotometer. All measurements were performed twice.

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