# Interaction of $MgATP^{2-}$ with DNA: Assessment of Metal Binding Sites and DNA Conformations by Spectroscopic and Thermal Denaturation Studies

R. G. BHATTACHARYYA*, K. K. NAYAK

*Department of Chemistry, Jadavpur University, Calcutta 700 032, India*

and A. N. CHAKRABARTY

*Department of Medical Microbiology, Dr. B. C. Roy Post-graduate Institute of Basic Medical Sciences, University of Calcutta, Calcutta 700 020, India*

(Received January 29, 1988)

## Abstract

Spectroscopic (IR, UV, CD and fluorescence) and thermal denaturation studies of native calf thymus DNA, DNA–$MgATP^{2-}$ and DNA–$Mg^{2+}$ have been carried out in aqueous KBr medium (introduced by the present authors as a very effective solvent for DNA). The IR data recorded for the systems indicate that $MgATP^{2-}$ binds to the $N_7$ and $C_6$–O of the guanine residue of DNA forming a five-membered chelate ring. The data also sugggest that despite binding to the guanine bases, $Mg^{2+}$ binds more strongly to the phosphate moiety of DNA. Solution CD spectra of DNA, DNA–$MgATP^{2-}$ and DNA–$Mg^{2+}$ indicate that in each case DNA exists in the B conformation. Thin-film CD studies reveal that irrespective of the relative humidity conditions, pure DNA as well as that after interaction with $Mg^{2+}$ show a structural transition B → C, conformationally, although belonging to the B family. A similar study shows that DNA on interaction with $MgATP^{2-}$ assumes a more packed conformation $(B)_n$ giving rise to a $\psi^-$ spectrum. Steady-state as well as dynamic fluorimetric studies clearly indicate that $MgATP^{2-}$ does not intercalate between CG–GC base pairs. The thermal denaturation studies support the IR data with respect to the metal binding sites and the mode of binding in both cases.

## Introduction

The binding of metal ions and small as well as bulky ligands (drugs) to DNA has attracted wide attention among scientists [1–7]. Studies on the interaction of metal complexes with DNA are comparatively rather scanty [8–11]. However, such studies are urgently needed in view of the fact that some Pt(II) complexes have varied degrees of anti-cancer effects [2, 6, 12] and that it is recognised [13] that virtually all the clinical and experimental anti-tumour drugs act via the disruption of nucleic acid metabolism at some level. It is now well known that A or B conformational stabilization of DNA is dependent on the relative humidity (RH) [14–16] and salt environment [17], but the literature on the effects of the metal ion on conformational stabilization of native [18, 19] or synthetic [16, 20, 21] DNA is very much on the increase. Since the existence of metal complexes in physiological systems is widespread, a study of the interaction of metal complexes on DNA is also relevant in determining the genetics and its expression by such modified cells.

In the present paper we report the interaction of a magnesium complex with adenosine triphosphate (ATP) [22], $MgATP^{2-}$, with native DNA, with a view to assessing the metal binding sites and conformational changes of DNA. Also, a comparative study on the interaction of aquo $Mg^{2+}$ ions with DNA, made side by side under an identical experimental set-up, is reported here to emphasize any change in reactivity pattern between complexed or uncomplexed (rather, aquo complexed) $Mg^{2+}$ towards the said nucleic acid. This study is thought to be biologically highly significant since ATP and its $Mg^{2+}$ complex control the bioenergetics of physiological systems and about one-sixth of all enzyme systems need metallated ATP or a related adenine cofactor for their reactivity [23].

## Experimental

### *Reagents*

Calf thymus (CT) DNA (type I, highly polymerised), ethidium bromide (EB) and dialysis bags were obtained from Sigma Chemical Company, U.S.A. The disodium salt of 5′-ATP (extra pure) and potassium bromide (specpure) were obtained

*Author to whom correspondence should be addressed.

0020-1693/88/$3.50

from E. Merck, F.R.G. All other chemicals were either of AR (BDH, India) or GR (E. Merck, India) grades.

$Na_2MgATP$ (henceforth abbreviated as MgATP) was synthesized following the literature method [22] and was found to give a single spot on a TLC plate [22]. Triply distilled (all glass) water was used in each experiment.

*Instrumentation*

Infrared spectral measurements were carried out using a Perkin-Elmer 597 IR spectrophotometer after the deposition of the respective compounds as thin films on Irtran-2 (ZnS) plates. UV spectra were measured on a Pye-Unicam Model SP 8-400 UV–Vis spectrophotometer using a pair of matched quartz cells. Circular dichroism (CD) spectra in solution, or thin films on quartz plates, were measured in the range 220 to 320 nm with the help of a JASCO J 500 C spectropolarimeter. Steady-state fluorescence intensities were measured using a Perkin-Elmer MPH 44B spectrofluorimeter, exciting the molecules at the wavelengths 510 and 337 nm. In both cases emission maxima were found to occur at 610 nm. The slit widths for excitation and emission were 4 and 2.5 nm, respectively. Fluorescence life-time measurements were made with a nanosecond fluorimeter 199 system from Edinburgh Instruments, U.K., selecting 337 nm as the excitation wavelength, and measurements were made at 610 nm. Thermal denaturation (Tm) studies of DNA were carried out by measuring its absorbance at 260 nm with the help of a Zeiss VSU-2 spectrophotometer fitted with a U1 type thermostatically controlled bath. Temperature lag between the bath and the cuvette was corrected by a thermocouple. Concentrations of magnesium and potassium in the DNA solution after dialysis were estimated by a Perkin-Elmer Model 2380 atomic absorption spectrometer and a Foto-flame (India) flamephotometer, respectively.

*General Methods*

*Preparation of solution and thin films of DNA, DNA–$Mg^{2+}$ and DNA–MgATP*

*(a) DNA solution for infrared spectral studies without dialysis.* An appropriate amount (0.8–1.2 mg) of native CT DNA was dissolved in 0.2 ml of 0.015 M KBr solution and occasionally shaken mildly to obtain a clear solution within 6–8 h (see also Results section). The pH of the solution was maintained at 6.8–7.0 by adding a few droplets of aqueous KOH. The molar concentration of CT DNA was estimated spectrophotometrically using the relation $\epsilon = 6600\ l\ mol^{-1}\ cm^{-1}$ at 260 nm.

*(b) DNA–MgATP solution for infrared spectral studies after dialysis.* Native CT DNA (2.0–2.4 mg) was dissolved by being mildly shaken with a 0.2-ml portion of the above KBr solution. To the above solution, 0.08–0.1 ml of MgATP in aqueous KBr containing 4–5 mg of the nucleotide complex was added, whereby the molar proportion of DNA: MgATP became 1:1 in the resulting solution. The latter was incubated for 4 h at 37 °C in a closed glass tube and then dialysed against pure water for 6 h. The $Mg^{2+}$ and $K^+$ content of the dialysed product was estimated as stated above. The molar concentration of DNA:MgATP was found to be 1:0.6 and that of KBr to be 0.004 M.

*(c) Preparation of DNA–$Mg^{2+}$ (henceforth abbreviated as DNA–Mg) solution for IR studies.* The same procedure as in (a) was repeated and an appropriate amount of $MgCl_2$ was added to the solution to give a molar concentration ratio of DNA:$Mg^{2+}$ equal to 1:2.5. The resulting solution was incubated for 4 h at 37 °C. In the present case dialysis was not necessary since $MgCl_2$ does not interfere in IR measurements up to 700 $cm^{-1}$.

*(d) Preparation of thin films for infared measuremetns.* A few drops of the solutions as in (a), (b) or (c) were spread over different Irtran-2 plates and gently dried (37 °C) in an incubator to obtain thin films. All the films were equilibrated for about 24–40 h in the humidity chambers, where relative humidities were maintained at 75, 84 and 95%, using the recommended saturated salt solutions [24]. For the sake of comparison, a DNA solution was made in NaCl medium as described by previous authors [25] and thin films over Irtran-2 plates were prepared as described above. Also, a thin film of $Na_2MgATP$ was prepared similarly over such a plate from its aqueous solution having a concentration commensurate with that of $MgATP^{2-}$ present in the DNA–MgATP solution from which the films of the latter were prepared.

*(e) Preparation of the solution and thence the thin films of DNA, DNA–Mg and DNA–MgATP for CD measurements.* A solution of CT DNA was prepared (2 mg/ml of 0.015 M KBr) so as to give a specific concentration in optical density units (for instance, 40 OD units) at 260 nm. For studying as thin films, the above DNA solution was sheered following the literature method [26] to avoid any unwanted orientation problems. An appropriate amount of $Mg^{2+}$ or MgATP was added to the known amount of DNA solution to give the molar concentration ratio of DNA:Mg = 1:2.5 and that of DNA: MgATP = 1:0.60. The mixed solutions were incubated for 4 h at 37 °C and 50 μl of the incubated solution were spread over a quartz plate of 1

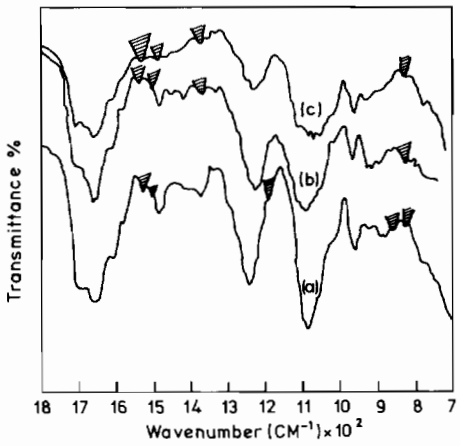


Fig. 1. Thin-film IR spectra: (a) pure CT DNA; (b) DNA–MgATP; (c) DNA–Mg, at 75% RH.

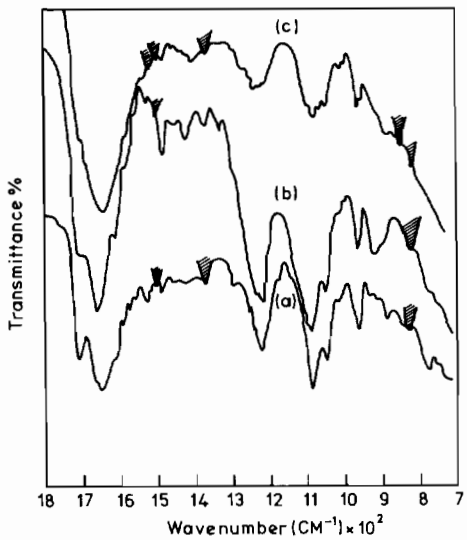


Fig. 2. Thin-film IR spectra: (a) pure CT DNA; (b) DNA–MgATP; (c) DNA–Mg, at 84% RH.

cm X 2 cm dimensions. These quartz plates were taken in Petri dishes and gently dried at 37 °C. The process was carried out very cautiously and slowly to avoid formation of any 'skin' or stretching of the films in some unwanted direction and orientation. The orientation was checked by placing the films at different angles with respect to the incident light beam and observing that the spectral characteristics of the films were in agreement with those of the unoriented ones [26]. As soon as the films were obtained they were equilibrated against saturated salt solutions for 24–40 h.

*(f) Preparation of DNA, DNA–Mg and DNA–MgATP solutions for other spectroscopic measurements and Tm studies.* An appropriate amount of DNA was dissolved in a suitable volume of the above KBr (0.015 M) solution and the volume was made up to 25 or 50 ml, as the case may be, and the pH of the solution was adjusted to 6.8–7.0. Suitable aliquots were taken, then known amounts of MgATP or Mg were added and incubated for 4 h at 37 °C. The molar ratio of DNA:MgATP was maintained at 1:06 for all the measurements (CD, fluorescence and Tm) in solution. The molar ratio of DNA:$Mg^{2+}$ was, however, adjusted to 1:2.5 in all the cases studied.

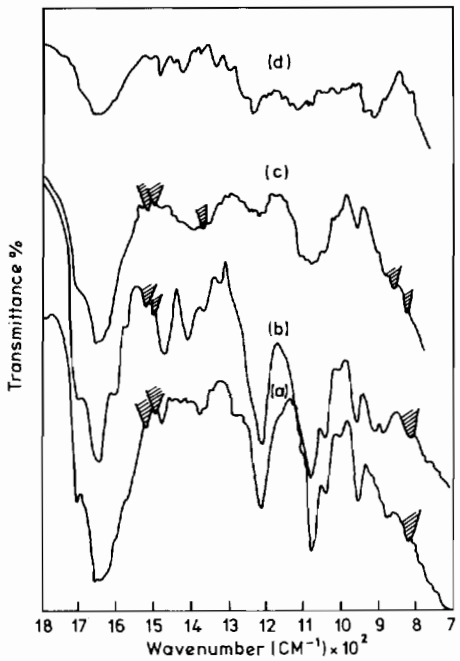


Fig. 3. Thin-film IR spectra: (a) pure CT DNA; (b) DNA–MgATP; (c) DNA–Mg at 95% RH; (d) pure $Na_2MgATP \cdot 2H_2O$.

## Results and Discussion

*Dissolution of DNA*

We observed that CT DNA dissolves quite quickly (6–8 h) in KBr solution (see General Methods) while NaCl requires days to furnish a solution of DNA. Also, there is no difference in the IR band positions and profiles of DNA taken either in NaCl or in KBr solution, the bands being even slightly better defined in the latter. Moreover, in both media, the UV spectra are identical. So, it may be acclaimed that the discovery that aqueous KBr is a better solvent for studying DNA solutions is a significant improvement in the methodology of nucleic acid research.

*Infrared Studies*

The involvement of the base moieties of DNA in binding with MgATP is apparent from the modifications of the infrared bands of pure DNA in the region 1710–1690 $cm^{-1}$ after interaction with Mg-ATP (Figs. 1–3) at all three relative humidities (75, 84 and 95%) studied here. It is noteworthy that an

otherwise sharp (at RH 84 and 95%) or a shoulder-like (RH 75%) band at 1710 $cm^{-1}$ in the native DNA loses its relative absorbance significantly, or is flattened (at RH 95%) after complexation with MgATP. Figure 3(d) indicates that such a change cannot take place by the mere presence of MgATP in the system. This implies that MgATP binds to nucleobases via guanine or thymine or both [19, 27], in so far as it is known that the bands at 1710–1690 $cm^{-1}$ in pure DNA arise because of the absorption of the $C_2$–$NH_2$ and $C_6$–O of guanine and the $C_2$–O of thymine [27]. However, it has been reported [27, 28] that involvement of the thymine base in metal binding causes the ring vibrational mode to appear at 1545 $cm^{-1}$, but such a band is not observed in DNA–MgATP in either of the three relative humidities studied. Hence, it appears that MgATP does not bind to thymine. The band at 1660 $cm^{-1}$ in pure DNA (at all three RHs) is unperturbed after interaction with MgATP [which itself does not have a band of such prominence in that region; compare Fig. 3(d)], indicating that the $NH_2$ groups of adenine and/or cytosine are not involved [27] in binding with MgATP. At all three RH values, there appear two weak bands, one as a shoulder at 1500 and the other at 1530 $cm^{-1}$, in pure DNA. In DNA–MgATP these bands retain their position and intensity (except at RH 75%, when the intensity of the 1530 $cm^{-1}$ band is very much reduced), thereby indicating the non-involvement of the cytosine moiety [28, 29] of DNA in binding with MgATP.

Interestingly, it has been found that at RH 84 and 95% the band at 1485 $cm^{-1}$ of pure DNA, which arises because of the $\nu_{CN}$ vibrational mode involving the $N_7$ of guanine [18], shifts to 1478 and 1480 $cm^{-1}$, respectively, with a considerable increase in intensity in the DNA–MgATP complex. However, at 75% RH the same red shift occurs with only a small increase in intensity. This red shift is an indication that the $N_7$ of guanine is involved in binding [18] with MgATP. So, from the above results it appears that MgATP binds to the $N_7$ and $C_6$–O or $C_2NH_2$ of the guanine residue [18, 27]. The involvement of the $N_7$ of guanine is plausible since the latter happens to be more nucleophilic [30] and sterically more accessible [31] than the other ring nitrogen atoms in the nucleobase. However, arguments as forwarded below imply that the MgATP complex binds to the $C_6$–O instead of the $C_2$–$NH_2$ of guanine. Since the $NH_2$ group is situated in the $C_2$ position of guanine, which in turn is situated in-between two ring N atoms (one of which is $sp^2$-hybridized and more acidic), the $C_2$ position assumes a more positive character. The $NH_2$ group attached to this positive centre becomes less nucleophilic in nature, while $C_6$–O, being situated in between an $sp^3$-hybridized ring nitrogen NH(1) and a carbon atom at the fused ring, will have a significantly high nucleophilicity. Moreover, it has been shown that the $C_6$–O position is more electron-rich than the $C_2NH_2$ position [32]. Another argument in favour of the $C_6$–O being bound to the metal centre is that modelling [33] indicates that an involvement of the $N_7$ of guanine almost commits the participation of $C_6$–O also from the stereochemical standpoint. As such, the formation of a five-membered chelate ring involving the $C_6$–O and $N_7$ of guanine is quite favourable in terms of energy considerations. This type of involvement of the $C_6$–O and $N_7$ of guanine has also been suggested [34] or otherwise proposed from a solution study [35] which has subsequently been verified at the isolated nucleoside level by spectroscopic measurements [36].

A comparison of the infrared vibrational modes of phosphate groups of DNA [29] (1230, 1085 and 965 $cm^{-1}$ at 84 and 95% RH; 1235, 1060 and 960 $cm^{-1}$ in 75% RH) and DNA–MgATP shows that the bands remain essentially unmodified (Figs. 1–3), indicating thereby the non-involvement of the phosphate moiety of the nucleic acid in binding with MgATP [21], which can be explained by considering the fact that $Mg^{2+}$ is already thoroughly bound through the ATP phosphate residue [22] and hence it does not prefer to bind with other phosphate centres.

To assess whether aquo $Mg^{2+}$ reacts in a different mode (than MgATP) with DNA or not, a study of the interaction of the aquo $Mg^{2+}$ ion with DNA has been undertaken under the same experimental conditions. At the DNA:$Mg^{2+}$ concentration mentioned in the Experimental section, and at all three relative humidities studied, a modification of the bands at 1710–1690 and 1485 $cm^{-1}$ occurs leaving the 1660 $cm^{-1}$ band unperturbed. Applying the same reasoning as forwarded in the case of MgATP, it may be inferred that there is an indication that $Mg^{2+}$ possibly binds with the $C_6$–O and $N_7$ of guanine, and not via the $C_2$–$NH_2$ of the latter. However, contrary to the case of the DNA–MgATP system, in DNA–Mg the bands in the phosphate region are significantly perturbed (Figs. 1–3), indicating that the $Mg^{2+}$ ion binds to the phosphate moiety also. Since the degree of modification observed for the phosphate bands are greater than that of the base bands, we also subscribe to the accepted view [37] that $Mg^{2+}$ binds more strongly to the phosphates than to the bases of DNA. However, the modification of both the $N_7$ and $C_6$–O bands of DNA after interaction with $Mg^{2+}$ is not so pronounced as in the case of the corresponding ATP complex. This implies that the $Mg^{2+}$ ions rather weakly interact with guanine bases and perhaps the detailed nature of the interaction is slightly different here compared to MgATP. Perhaps $Mg^{2+}$ primarily binds to the $N_7$ of guanine and in doing so the hydrogen atoms of water molecules, bound to $Mg^{2+}$, find occasion to form H-bonds with the $C_6$–O

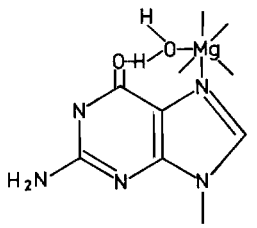

Fig. 4. Proposed model of Mg–nucleobase interaction in DNA–Mg.

of guanine [34, 38] (Fig. 4). This weak bonding, however, cannot cause the disruption of H-bonds responsible for maintaining the double helix structure. This is substantiated from Tm studies, which show that $Mg^{2+}$ (as usual) stabilizes the double helix (*vide infra*). Regarding the availability of the $N_7$ site for metal binding, it may be argued that the thermal agitation through DNA respiration can locally modify the guanine conformation into a 'syn' geometry in solution without breaking the Watson–Crick base pairing [18, 39], thereby making the $N_7$ site of guanine available for metal binding. Although the life-time of this configuration is short, it allows a sufficient time-interval for the metal ions to gain access to the $N_7$ site of guanine.

The bands at 1375 and 835 $cm^{-1}$ (weak) appear in pure DNA as well as in DNA–MgATP at 84 and 95% RH, which indicates that at the above RHs DNA and its MgATP complex both remain in the B family of conformation [40]. However, pure DNA at 75% RH shows bands at 1375(w), 835(w), 1185(sh) and 860(sh) $cm^{-1}$ (all shoulders shaded), indicating either a coexistence of both A and B forms [21] or a change in the sugar phosphate geometry [21] within the B framework. That the latter proposition is reasonably correct is evident from the CD spectrum (*vide infra*). However, in DNA–MgATP at 75% RH the last two bands are not observed, which suggests that the above complex remains exclusively in the physiologically stable B family [21]. It is noteworthy that at all three relative humidities studied, DNA–Mg does show the 860(sh) $cm^{-1}$ band, implying thereby that a similar type of change as occurred in free DNA at 75% RH is occurring in the case of DNA–Mg, irrespective of the RH conditions.

*Thermal Denaturation Studies*

The Tm value of pure DNA, DNA–MgATP and DNA–Mg are found to be 73.5, 61.0 and 80.0 °C, respectively. The decrease in Tm when MgATP interacts with DNA indicates that the latter binds with the DNA bases [41], as has been inferred from IR studies also. In so far as the Tm of the DNA–Mg system is higher than that of free DNA, it becomes obvious that $Mg^{2+}$ ions bind more strongly to the phosphates than to the bases [31].

*Fluorimetric Studies*

The fluorescence intensity of ethidium bromide (EB), DNA–EB and DNA–EB–MgATP were compared with one another. As is typical [42], the intensity of DNA–EB was much enhanced compared to pure EB. However, even when an excess of MgATP was added to the DNA–EB solution and incubated for 4 h, the fluorescence intensity did not change significantly. For further verification of the results obtained above via steady-state fluorimetric studies, the fluorescence life-times of free EB, DNA–EB and DNA–EB–MgATP at two different concentration ratios with respect to MgATP–DNA (one being higher, other being lower; as usual, 4 h incubation) were measured and found to be 2.1, 22.6, 22.8 and 22.6 ns (nano-second), respectively. Both studies are consistent with the fact that no displacement of EB by MgATP from CG–GC pairs of DNA occurs via intercalation [43]. This is also substantiated from the Tm studies where there occurs a decrease (of 12.5°) in Tm.

*UV Spectral Studies in Thin Films*

The UV absorptions of the DNA films (in the range 220–320 nm) at all three relative humidities studied show higher absorptivity than that of the corresponding DNA–Mg film. This suggests that after complexation with $Mg^{2+}$, the DNA double helix is further stabilized and hence hypochromicity results. An outright comparison of absorptivity of DNA–MgATP, however, is not possible since ATP also absorbs strongly in this region.

*Circular Dichroism Spectral Studies*

The solution CD spectra of DNA–MgATP, DNA–$Mg^{2+}$ (maintaining the same concentration as in the case of IR studies) and that of pure DNA recorded after 4 h of incubation [Fig. 5(a)] show that the positive band of pure DNA at 271 nm as well as the negative band at 245 nm retain their positions and intensities after interaction with MgATP. It may be pointed out that though a negative band of very moderate intensity having maxima at around 260 nm appears in the CD spectrum of MgATP at a concentration of *ca.* $1 \times 10^{-4}$ M, this band will hardly affect the DNA–MgATP spectrum, since the total concentration of MgATP (not to speak of free MgATP) in the latter preparation is too low to be significant (see also Experimental section) both in solution and in thin film conditions. Hence, it indicates that like pure DNA, DNA–Mg and DNA–MgATP all remain in the B family of conformation in the solution phase [44].

To elucidate the function of different RH conditions towards the conformation of CT DNA, DNA–MgATP and DNA–Mg, the CD spectra of thin films were also measured (under the same conditions as in the case of IR studies). However, to avoid orienta-

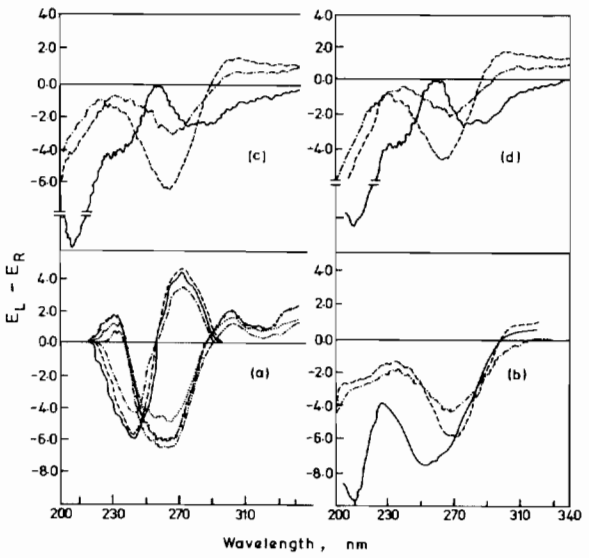


Fig. 5. CD spectra: (a) native CT DNA (- - - -), native DNA–MgATP (——), native DNA–Mg (– · –), sheered CT DNA (– X –), sheered DNA–MgATP (– ·· –) and sheered DNA–Mg (·····), in solution; (b) sheered CT DNA (– – –), DNA–MgATP (——) and DNA–Mg (– · –) in thin films at 75% RH; (c) sheered CT DNA (– – –), DNA–MgATP (——) and DNA–Mg (– · –), in thin films at 84% RH; (d) sheered CT DNA (– – –), sheered DNA–MgATP (——) and sheered DNA–Mg (– · –), in thin films at 95% RH.

tion problems, a sheered DNA solution was used [26] here for preparing the thin films, contrary to native DNA being used in other studies. For the sake of comparison, the solution CD spectrum [Fig. 5(a)] of identically sheered DNA was also recorded. It appears that the solution CD spectra of native and sheered DNA are not identical; the latter gives a positive band at 295 nm as against 272 nm in native form, with a reduced intensity, and a negative band of comparable intensity at 260 nm [native DNA at 242 nm, see Fig. 5(a)]. This pattern of change is indicative of a pure B → C (also belonging to the B family) [45] conformational transition due to sheering. This transition may be due to the change in the packing of the DNA bases [46] which arises out of the increase in the winding angle of DNA and a consequent decrease in the number of base pairs per turn [46], without essentially changing the helical pattern (C belongs to the B family). This type of packing has also been observed in the case of DNA existing in nucleoproteins, especially in chromatin, when treated with $Mg^{2+}$ [46]. The process is also favourable thermodynamically, since it has been suggested that the B → C transition requires only a very small enthalpy change ($\Delta H^\circ \approx$ 10 kcal) [47] and a positive entropy change.

The CD spectra of sheered DNA, DNA–MgATP and DNA–Mg in thin films at the relative humidities 75, 84 and 95% are shown in Fig. 5(b)–(d). At 75% RH the DNA film shows only a negative band at 267 nm; in DNA–Mg the band position is retained but the intensity is reduced. At 84% RH, besides the negative bands at the same region as observed above, a very weak positive band shows up at 300 nm in both DNA and DNA–Mg. This suggests that with the increase in hydration both DNA and its $Mg^{2+}$ complex show a tendency towards attaining the same conformation as found in the solution spectrum of sheered DNA. That the increase in hydration causes the DNA and DNA–Mg structure to attain a more and more similar conformation to that which exists in solution is perfectly exhibited in the thin-film spectra at 95% RH. Here, after 24 h of equilibration, DNA and DNA–Mg show the negative bands at 262 and 267 nm, respectively, with the positive band intensities increasing more than with the 84% case.

The above spectral behaviour indicates that at a lower degree of hydration (75%) both DNA and DNA–Mg attain the C conformation, although in solutions the structures are more akin to the normal B conformation. This transition from B → C may be due to a change in the packing pattern in DNA, either alone or in the presence of $Mg^{2+}$ at lower hydration in the films. At the said RH, DNA in the presence of the $Na^+$ counter-ion shows a CD spectrum characteristic of the A conformer [24], but the present work indicates that $K^+$ counter-ions cause a change in the packing in DNA and hence stabilize the B family of conformers in thin films. However, the $K^+$ counter-ion may not be an all important proposition since at higher degrees of hydration DNA and DNA–Mg try to attain a conformation close to B. We do not find indication of the existence of the A conformation in DNA or in DNA–Mg at any of the relative humidities studied, including the 75% RH [24]. It may be recalled here that growth of the 860(sh) and 1185(sh) $cm^{-1}$ IR bands in DNA, and that of the 860(sh) $cm^{-1}$ in DNA–Mg, is due, as also suggested earlier, to the change in sugar phosphate geometry only [19].

The CD spectra of DNA–MgATP films deserve special mention. The spectrum at 75% RH shows a much enhanced negative band at 260 nm and a long tail at 210 nm with no absorption at the positive side [Fig. 5(b)]. As the RH is increased to 84 and 95%, the profile of the spectra remains largely the same with a shift of the 260 nm band to 280 nm in both cases with a decrease in intensity. These [Figs. 5(c)–(d)] can be regarded as $\psi^-$ spectra [48] and it is observed that at all the three RH values studied, DNA gives $\psi^-$ spectra under the influence of MgATP, in thin films. However, the solution CD spectrum of sheered DNA–MgATP is very much like the solution spectrum of sheered DNA itself. It may be mentioned that the MgATP film prepared from an aqueous MgATP solution of identical con-

centration present in the DNA–MgATP solution from which film of the latter is prepared, shows no characteristic CD spectrum. Obviously, the above $\psi^-$ type of spectra is exhibited by DNA alone under the influence of MgATP. It has been found that DNA produces a $\psi^-$ spectrum when the latter binds to histone (H1 and H4) [49] and polylysine [50], as evidenced, of course, from solution studies. It has also been suggested that DNA in chromatin generates this type of spectrum [51].

Some authors have proposed the B form [52] and a structure between the B and C forms [53] of DNA for a $\psi^-$ spectrum. According to another group of authors [54], this $\psi^-$ spectrum is generated by the more compact $(B)_n$ type of structure. Mandel and Fasman [55] proposed that the CD changes that occur when $(Lys)_n$ binds to DNA do not arise from a B → C transition in DNA but arise out of a DNA-$(Lys)_n$ asymmetric aggregate. From a CD study in DNA and DNA–$(Lys)_n$ in ethanolic buffer, it has been proposed that $\psi^-$ and $\psi^+$ spectra arise because of the change in the left and right coiling of the tertiary structure and have nothing to do with the secondary structure [56]. Further, from linear infared dichroic studies, some authors have suggested [57] that DNA in DNA–$(Lys)_n$ at high humidity assumes a structure B*, where there is only a change in the relative orientation of the phosphate groups with respect to the B form. From Raman spectral studies, another group [58] has proposed that binding of $(Lys)_n$ to DNA does not change the DNA backbone, it only modifies the interaction between the bases.

We believe that in the present case, DNA under the influence of MgATP remains in the B family of conformation with a more compact $(B)_n$ type of structure to produce a $\psi^-$ spectrum, as also suggested by Shin and Eichorn [54] in a different situation. Moreover, as observed from the thin-film IR studies of DNA–MgATP at various RH values, DNA in the presence of MgATP prefers to remain essentially in the B family of conformation, irrespective of RH values; *i.e.*, indicating no serious change in the DNA backbone. So, CD and IR studies of films convey mainly the same information towards the DNA structure existing in films of DNA-interacting molecules studied here. To be more precise, MgATP helps DNA to remain in the B family of conformation with a minor change in the packing pattern.

In solution we do not find the presence of any $\psi^-$ type of spectrum for DNA–MgATP. Considering the fact that in a cell the situation is not as relaxed as is found in solution of DNA, in so far as the solution phase gives more freedom to DNA than what it can achieve in the compact cellular system. Hence, the findings in thin films are, perhaps, more relevant to the situation prevalent in the cellular domain. As mentioned earlier, the suggested $\psi^-$ type of spectrum for DNA in chromatin [51] also supports our hypothesis.

## Conclusions

From the foregoing facts and discussions it becomes apparent that MgATP binds to the $N_7$ and $C_6$–O of guanine of DNA but not to the phosphates, while $Mg^{2+}$ binds primarily to phosphate and interacts rather weakly with the $N_7$ of guanine, and the $C_6$–O of the said base is involved in hydrogen bonding only. One conspicuous finding is that a more compact DNA structure is imparted, at least in the thin films, especially under the influence of MgATP. From a thin-film circular dichroism study it is revealed that DNA–MgATP always remains in a conformation [*i.e.* $(B)_n$] compatible with the $\psi^-$ spectrum normally found in nucleoproteins.

## Acknowledgements

Our sincere thanks are due to the Alexander Von Humboldt Foundation for the donation of the PE 597 IR spectrophotometer, including Irtran-2 cells, used for this work. The authors are grateful to Prof. S. G. Dastidar, The Department of Pharmacy of this university, to the Director, IICB, Calcutta, and to Dr. M. Maiti of the same institute for their interest in the work and for providing some instrumental facilities. The authors extend their thanks to Mr. S. M. Roy of the above institute for providing assistance in measuring Tm values. The CD spectra were measured by Mrs. T. Roy of the IACS, Calcutta 700032, with the facilities provided by the DST project SERC 2B (IP-2/8-STP-2). Our thanks are also due to the U.G.C., New Delhi for providing one of the authors (K. K. N.) with a teacher fellowship.

## References

1 H. M. Sobell, C.-C. Tsai, S. C. Jain and S. G. Gilbert, *J. Mol. Biol., 114*, 333 (1977).
2 B. Rosenberg, J. VanCamp, J. Trosk and V. H. Mansour, *Nature (London), 222*, 385 (1969).
3 W. A. Denny, C. J. Atwell, B. C. Baguley and B. F. Cain, *J. Med. Chem., 22*, 134 (1979).
4 M. J. Waring, *Annu. Rev. Biochem., 50*, 159 (1981).
5 E. Sage and M. Leng, *Proc. Natl. Acad. Sci. U.S.A., 77*, 4597 (1980).
6 M. Maeda, N. Abiko, H. Uchida and H. Sasaki, *J. Med. Chem., 27*, 444 (1984).
7 M. B. Jones, U. Holstein and F. S. Allen, *Biopolymers, 26*, 121 (1987).
8 J. M. Kelley, A. B. Tossi, D. J. McConnel and C. Ohugin, *Nucleic Acids Res., 13*, 6017 (1985).
9 R. C. Srivastava, J. Frochlich and G. L. Eichorn, *Biochimie, 60*, 879 (1978).

10 M. J. Clarke, B. Jansen, K. A. Mark and R. Kruger, *Inorg. Chim. Acta, 124*, 13 (1986).
11 S. Cauci, E. Alessio, G. Mestroni and F. Quadrifoglio, *Inorg. Chim. Acta, 137*, 19 (1987).
12 B. Rosenberg, *Biochimie, 60*, 859 (1978).
13 J. A. C. Montgomery, *Methods Cancer Res., 21*, 1 (1979).
14 M. Falk, K. A. Hartman, Jr. and R. C. Lord, *J. Am. Chem. Soc., 85*, 387 (1963).
15 M. Falk, K. A. Hartman, Jr. and R. C. Lord, *J. Am. Chem. Soc., 85*, 391 (1963).
16 W. Pohle and H. Fritzsche, *Nucleic Acids Res., 8*, 2527 (1980).
17 B. Wolf, S. Berman and S. Hanlon, *Biochemistry, 16*, 3655 (1977).
18 J. A. Taboury, P. Bourtayre, J. Liquier and E. Taillandier, *Nucleic Acids Res., 12*, 4247 (1984).
19 D. E. Dirico, Jr., P. B. Keller and K. A. Hartman, Jr., *Nucleic Acids Res., 13*, 251 (1985).
20 J. A. Taboury, S. Adam, E. Taillandier, J. M. Neuman, S. Tran-Dinh, T. Hynh-Dinh, B. Langlois d'Estaintot, M. Conti and J. Igolen, *Nucleic Acids Res., 12*, 6291 (1984).
21 A. H.-J. Wang, G. J. Quigley, F. J. Kolpak, G. Van der Marel, J. H. Van Boom and A. Rich, *Sciences, 211*, 171 (1981).
22 R. G. Bhattacharyya and I. Bhaduri, *J. Inorg. Nucl. Chem., 40*, 733 (1978).
23 N. J. Leonard, *Acc. Chem. Res., 15*, 128 (1982).
24 M. F. Maestre, *J. Mol. Biol., 52*, 543 (1970).
25 E. Taillandier, J. Taboury, J. Liquier, P. Sautire and M. Couppez, *Biochimie, 63*, 695 (1981).
26 M. J. B. Tunis-Schneider and M. F. Maestre, *J. Mol. Biol., 52*, 521 (1970).
27 M. Tsuboi, *Appl. Spectrosc. Rev., 3*, 45 (1970).
28 K. A. Hartman, Jr., *Biochim. Biophys. Acta, 138*, 192 (1967).
29 G. B. M. Sutherland and M. Tsuboi, *Proc. R. Soc. London, Ser. A, 239*, 446 (1957).
30 B. Pullman and A. Pullman, *Biochim. Biophys. Acta, 36*, 343 (1959).
31 B. Reiner and S. Zamenhof, *J. Biol. Chem., 228*, 475 (1957).
32 H. T. Miles, F. B. Howard and J. Frazier, *Science, 142*, 1458 (1963); N. K. Kochetkov, E. I. Budovaskii, E. D. Sverdlov, N. A. Simukova, M. F. Truchinskii and V. N. Shibaev, in N. K. Kochetkov and E. I. Budovskii (eds.), B. High (Engl. Trans.), L. Todd and D. M. Brown (eds.), 'Organic Chemistry of Nucleic Acids, Part A', Plenum Press, London, 1971, p. 121.
33 'Framework of Molecular Models', Prentice-Hall, Englewood Cliffs, U.S.A.
34 D. M. L. Goodgame, I. Jeeves, F. L. Phillips and A. C. Skapski, *Biochim. Biophys. Acta, 378*, 153 (1975).
35 N. Datta Gupta and D. M. Crothers, *Nucleic Acids Res., 9*, 2971 (1981).
36 J. Dehand and J. Jordanov, *J. Chem. Soc., Chem. Commun.*, 598 (1976).
37 G. L. Eichorn, *Nature (London), 194*, 474 (1962).
38 A. H.-J. Wang, G. J. Quigley, F. J. Kolpak, J. L. Crawford, J. H. Van Boom, G. Van der Marel and A. Rich, *Nature (London), 282*, 680 (1979).
39 M. Sundaralingam, *Biopolymers, 7*, 821 (1969), and refs. therein.
40 P. Champeil, T. P. L. Tran and J. Brahms, *Biochem. Biophys. Res. Commun., 55*, 881 (1973).
41 G. L. Eichorn and Y. A. Shin, *J. Am. Chem. Soc., 90*, 7325 (1968).
42 J. Olmsted III and D. R. Kearns, *Biochemistry, 16*, 3647 (1977).
43 R. V. Kastraup, M. A. Young and T. R. Krugh, *Biochemistry, 17*, 4835 (1978).
44 I. Tinoco, Jr., C. Bustamante and M. F. Maestre, *Annu. Rev. Biophys. Bioeng., 9*, 107 (1980).
45 B. J. Jenkins, R. M. Watwell and J. L. Alderfer, *Biopolymers, 25*, 823 (1986).
46 K. Watanabe and K. Iso, *Biochemistry, 23*, 1376 (1984).
47 S. Brahms, J. Brahms and K. E. VanHolde, *Proc. Natl. Acad. Sci., U.S.A., 73*, 3453 (1976).
48 C. F. Jordan, L. S. Lerman and J. H. Venable, Jr., *Nature (New Biol.), 263*, 67 (1972).
49 G. D. Fasman, B. Schaffhausen, L. Goldsmith and A. Alder, *Biochemistry, 9*, 2814 (1970).
50 D. Carrol, *Biochemistry, 11*, 421 (1972).
51 M. K. Cowman and G. D. Fasman, *Biochemistry, 19*, 532 (1980).
52 M. K. Cowman and G. D. Fasman, *Proc. Natl. Acad. Sci. U.S.A., 75*, 848 (1978).
53 M. Weiskopf and H. J. Li, *Biopolymers, 16*, 669 (1977).
54 Y. A. Shin and G. L. Eichorn, *Biopolymers, 16*, 225 (1977).
55 R. Mandel and G. D. Fasman, *Biochemistry, 15*, 3122 (1976).
56 M. F. Maestre and C. Rich, *Biochemistry, 19*, 5214 (1980).
57 J. Liquier, M. Pinot-Lafaix, E. Taillandier and J. Brahms, *Biochemistry, 14*, 4191 (1975).
58 B. Prescott, C. H. Chou and G. J. Thomas, Jr., *J. Phys. Chem., 80*, 1164 (1976).