

## Structural Analysis of DNA Interactions with Magnesium Ion Studied by Raman Spectroscopy

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**Abstract: Problem statement:** In the present study, FT Raman spectroscopy had been used to extend our knowledge about Magnesium ion - DNA interactions at various volume ratios (1:50, 1:20, 1:10 and 1:5). **Approach:** The analysis of FT Raman data supported the existence of structural specificities in the interaction and also the stability of DNA secondary structure. **Results:** Results from the Raman spectra clearly indicate that the interaction of Magnesium ion with DNA is mainly through the phosphate groups of DNA with negligible change of the B-conformation of DNA at all the volume ratios studied. For example, band at  $1079\text{ cm}^{-1}$  is assigned to the symmetrical stretching vibration of the nucleic acid phosphodioxy ( $\text{PO}_2$ ) group. This band in the order  $1079 \rightarrow 1075 \rightarrow 1070 \rightarrow 1066 \rightarrow 1063\text{ cm}^{-1}$  at all Magnesium ion DNA concentrations studied. Similarly, Raman band at  $845\text{ cm}^{-1}$  due to antisymmetrical phosphodiester (O-P-O) stretching of DNA. **Conclusion:** Magnesium ion interaction with the DNA phosphate is weak in comparison to interactions with the bases. On the other hand, the Raman signature of B-DNA is largely unperturbed by magnesium ion, suggesting much weaker interactions.

**Key words:** Stretching vibration, B conformation, FT Raman data, FT Raman spectroscopy, Raman spectra, volume ratios, nucleic acids, magnesium deficiency, stacking interactions

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### INTRODUCTION

Metal ions are essential for the biological activity of nucleic acids, as well as the characteristics that can prove harmful, result from the interaction of metals to nucleic acids. It is for this reason that studies of the effects of metal interaction in nucleic acids are very much important. Magnesium deficiency causes renal complications. The appearance of several diseases is related to its depletion in the human body (Anastassopoulou and Theophanides, 2002). The binding of metals by nucleosides and nucleotides has been investigated for a number of years. Because of recent observations that some metal ions cause chromosome damage and consequently is mutagenic, there is renewed interest in the binding of heavy metals to polynucleotide. Interestingly, binding of Magnesium ions to the

backbone phosphate groups may play role in enhancing the lifetime of the open state of the DNA base pairs, thus accelerating the regulatory process of genetic information transfer. The alkaline earth metals also play the role of stabilizing the double helix by neutralization of the negative charges on the phosphate groups. Remarkably, extreme stabilization by metal ions can lead to rather adverse result, i.e., the mis-pairing of bases. Since the correct propagation of the genetic code requires the correct complementary base pairing, mis-pairing can lead to error (Printz and Hippel, 1965).

In view of this, the objective of the present study is to characterize the structural changes that DNA undergoes in the presence of Magnesium ions. One of the methods of promise to provide information on binding sites, stacking interactions and conformation, is vibration spectroscopy, particularly FT Raman spectroscopy. Here the FT Raman spectroscopy has been used to

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analyze the effect of Magnesium ion on DNA at various volume ratios.

### MATERIALS AND METHODS

Highly polymerized calf-thymus DNA sodium salt (6.2% sodium content, 13% H<sub>2</sub>O content) and were purchased from Sigma Chemical Co. DNA was deproteinated by the addition of CHCl<sub>3</sub> and isoamyl alcohol in NaCl solution. Sodium-DNA was dissolved in 50mM NaCl (pH 7.20) at 5°C for 24 h with occasional stirring to ensure the formation of a homogeneous solution.

The appropriate amount of magnesium ion (50 mM) was prepared in distilled water and added drop wise to the DNA solution to attain the desired Magnesium ion - DNA (1:50, 1:20, 1:10 and 1:5), volume ratios. The pH of all the solutions was adjusted at 7.

**FT Raman measurements:** FT Raman spectra were recorded in a Bruker Equinox 55 FT spectrometer supplied with a Raman module. Spectra are applied at the spectral resolution of 2cm<sup>-1</sup>, using excitation radiation wave number at 1064 nm from Nd-YAG laser working at 500 mW. We used a standard quartz cell for liquids (1-cm section) where approximately 1 mL of the solutions was placed. Backscattering collection of the Raman radiation was performed using a mirror behind the cell and minimum of 2000 scans were accumulated in all case to enhance the signal-to-noise ratios. Raman spectra were recorded between 600 and 1800 cm<sup>-1</sup>. The Raman spectra of Magnesium ion-

DNA complexes at various volume ratios (1:50, 1:20, 1:10 and 1:5) are recorded.

### RESULTS AND DISCUSSION

**Analysis of FT Raman spectroscopic data:** The FT Raman spectra of calf-thymus DNA and Magnesium ion - DNA complexes (1:50, 1:20, 1:10 and 1:5 volume ratios) are displayed in Fig. 1 and Table 2 shows the relevant wave numbers for the complete range of concentrations studied.

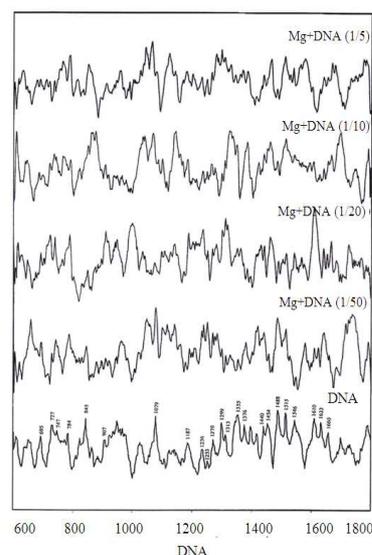


Fig. 1: FT Raman spectrum of calf thymus DNA and Magnesium ion – DNA complexes (1:50, 1:20, 1:10 and 1:5 volume ratios)

Table 1: Wave numbers for the complete range of concentrations studied

DNA	Mg+DNA (1:50)	Mg+DNA (1:20)	Mg+DNA (1:10)	Mg+DNA (1:05)	Assignments
695	693	690	687	688	Guanine [C1' -N9 stretching]
727	727	723	722	722	dA[C1' -N9 stretching]
747	740	738	736	736	dT[N-H out- of- plane bending]
784	786	789	790	790	dC, dT, O-P-O sym.str mode
845	845	848	848	850	O-P-O asym.str mode
907	908	908	909	909	Deoxyribose
1079	1075	1070	1066	1063	PO <sub>2</sub> sym. str
1187	1187	1185	1183	1178	dT[C5-CH <sub>3</sub> bond and in-plane ring stretching]
1236	1236	1235	1233	1230	dC [in plane ring str]
1253	1250	1250	1248	1247	dC[in-plane ring stretching]
1270	1267	1265	1262	1264	dC[in-plane str. mode]
1299	1298	1298	1297	1296	dC,dT[ring stretching]
1313	1310	1310	1308	1306	dG[imidazole ring coupled with(c8-N9) str]
1355	1352	1349	1347	1343	dG[C2 = N3-C4 = C5-N7 = C8 triene moiety]
1376	1379	1382	1382	1384	dT[C5-CH <sub>3</sub> str,C6-H in-plane bending], dA
1440	1440	1440	1438	1436	dT[C5'-H <sub>2</sub> deformation]
1454	1455	1459	1460	1463	dT[C5-CH <sub>3</sub> def., N3-H in-plane bending]
1488	1486	1486	1485	1483	dG, dA[N7-C8 stretching]
1515	1513	1513	1512	1510	dA [ring str]
1546	1546	1543	1541	1538	dG [imidazole ring and C6 moiety]
1610	1608	1608	1607	1603	dC [ring str]
1633	1636	1638	1640	1640	dC [Carbonyl C2=Ostr mode]
1660	1660	1665	1666	1668	dT [in-phase stretching]

The FT Raman spectra of the Magnesium ion - DNA at various volume ratios show wave number shifts, indicating that interactions are present without condensation.

**Interpretation of FT Raman spectra of solutions in water:** Raman bands in the 600-800  $\text{cm}^{-1}$  regions occur due to C-C and C-N bonds of the base residues, which stretch and contract in phase. Such a ring breathing vibrations invariably give often the most intense Raman bands in the spectrum (Thomas, 1970).

The Raman band observed at 695  $\text{cm}^{-1}$  of calf thymus DNA is assigned to breathing motion of the guanine coupled through the C1'-N9 glycoside bond to a deoxyribose vibration (Benevides *et al.*, 1984). The 695  $\text{cm}^{-1}$  band also characterizes the C2'-endo/anti conformation of B-DNA in aqueous solution (Kiefer, 2007). In Magnesium ion - DNA spectrum the 695  $\text{cm}^{-1}$  band of metal free DNA shifts in the order 695 $\rightarrow$ 693 $\rightarrow$ 690 $\rightarrow$ 687 $\rightarrow$ 688  $\text{cm}^{-1}$  at all the concentrations studied. This result indicate that in the presence of Magnesium ions the B-form structure is melted and the population of dG conformers changes from predominantly C2'-endo/anti to a mixture of C2'-endo/anti and C2'-endo/syn conformers. Duguid *et al.* (1993) observed similar spectral changes in the study of Raman spectroscopy of DNA - metal complexes.

The sugar coupled adenine vibration observed at 727  $\text{cm}^{-1}$  in metal free DNA is observed at 727, 723 and 722  $\text{cm}^{-1}$  in Magnesium ion -DNA complexes, which may be due to strong interaction of the bases with one another or with the metal ions. Harada and Takeuchi, (1986) observed similar results in the Raman spectra of tryptophan and related compounds. The N-H out-of plane bending vibration of thymine near 747  $\text{cm}^{-1}$  of metal free DNA identifies the C2' endow/anti conformers of dT (Rush and Peticolas, 1995). In Magnesium ion-DNA complexes, the 747  $\text{cm}^{-1}$  band of DNA shifts to lower frequency in the order 747 $\rightarrow$ 740 $\rightarrow$ 738 $\rightarrow$ 736  $\text{cm}^{-1}$ , which indicates that the tensional rotations about the glycosyl bond become more flexible in Magnesium ion - DNA complexes. Duguid *et al.* (1996) have observed similar results.

The 784  $\text{cm}^{-1}$  band is the composite of two major bands, one due to the cytosine ring breathing mode (Tajmir-Riahi *et al.*, 1988) and the other due to phosphodiester symmetric stretching vibration (Dam *et al.*, 2002). The cytosine vibration is sensitive to deputation exocyclic substitution while the phosphodiester vibration is less sensitive to deputation. The thymidine vibration, which

involves both significant contribution from deoxyribose ring and dominant contribution from the thymine vibration, is sensitive to C2' deuteration (Tsuboi *et al.*, 1997). The appearance of the cytosine vibration at higher frequency at 790  $\text{cm}^{-1}$  in Magnesium ion -DNA (higher concentration) spectrum indicates that Magnesium ions react readily with the N3 atom of cytosine.

Raman bands in the 750-850  $\text{cm}^{-1}$  spectral intervals have been assigned to vibrations which involve a large contribution from oxygen-phosphorous stretching of the phosphodiester (O-P-O) linkages (Ruiz-Chica *et al.*, 2001) and oxygen-phosphorous stretching of the phosphodioxy (PO<sub>2</sub>) group (Nafisi *et al.*, 2008). Because of obvious coupling between sugar and phosphate groups, these bands are extraordinarily sensitive to the nucleic acid backbone conformation and provide a firm empirical basis for DNA conformational analysis (Nafisi *et al.*, 2008). FT-Raman spectrum of calf thymus DNA exhibits a band at 845  $\text{cm}^{-1}$  due to ant symmetrical phosphodiester (O-P-O) stretching (Ruiz-Chica *et al.*, 2001). This band is considered as diagnostic B conformation (Nafisi *et al.*, 2008). The 845  $\text{cm}^{-1}$  frequency originates from vibrations associated with the 3' 5' - phosphodiester network (C-O-P-O-C). The 845  $\text{cm}^{-1}$  band is shifted in the order 845 $\rightarrow$ 848 $\rightarrow$ 850  $\text{cm}^{-1}$  at all the Magnesium ion - DNA complex ratios studied. This is an indicative of a wide distribution of torsion angles in the backbones of Magnesium ion - DNA. Thomas III Rush and Warner, 1995 pragmatic show the matching results in an ab initio transform calculation of resonance Raman spectra of uracil, 1-methyluracil and 5-methyluracil.

Raman band at 907 $\text{cm}^{-1}$  have been assigned to stretching vibrations of the deoxyribose rings for DNA (Prescott *et al.*, 1984). Their shifts upon Magnesium ion complication indicate contribution of the sugar moieties in the interaction. Also the Raman band assigned to the symmetrical stretching vibration of the nucleic acid phosphodioxy (PO<sub>2</sub>) group (Guan and Thomas Jr., 1996) is observed at 1079  $\text{cm}^{-1}$  in H<sub>2</sub>O solution. The ant symmetrical stretching vibration of the phosphodioxy group (PO<sub>2</sub>) is expected near 1200  $\text{cm}^{-1}$ . The Raman scattering of ant symmetrical stretching vibration is characteristically very weak, although it generates intense infrared absorption (Aubrey *et al.*, 1992). At all Magnesium ion- DNA concentrations studied, the 1079  $\text{cm}^{-1}$  band of metal free DNA shifts to lower frequency in the order 1079 $\rightarrow$

1075→ 1070→ 1066→ 1063  $\text{cm}^{-1}$ . This effect is due to interaction of Magnesium ions with the ionized PO<sub>2</sub> groups through water molecules (outer-space interactions or, more generally, formation of water-separated in pairs). The effect of the metal action in this  $\text{Mg}^{2+}\cdots\text{O}(\text{H})\cdots\text{H}\cdots\text{O}_2\text{P}$  type of aggregate is to increase the acidity of the water molecules and to strengthen the hydrogen bond affecting the phosphate group, causing a decrease in frequency of the symmetric stretch PO<sub>2</sub> vibration (Toyama *et al.*, 1993).

Raman bands in 1180-1600  $\text{cm}^{-1}$  region of the Raman spectrum arises primarily from conjugated CC, CN and CO in-plane stretching modes of the urine and pyrimiding bases in which adjoining bonds stretch with 180° phase difference (Aida *et al.*, 1997). These Raman bands have the potential for diagnosis of nucleic acid secondary structure.

The FT-Raman spectrum of DNA in H<sub>2</sub>O solution exhibits bands at 1187 and 1236  $\text{cm}^{-1}$ . These two bands have been assigned to vibrations involving stretching of exocyclic C5-CH<sub>3</sub> bond of thymine and in-plane ring vibrations of cytosine (Rush and Peticolas, 1995). In the presence of Magnesium ions the 1187  $\text{cm}^{-1}$  thymine band of metal free DNA shifts in the order 1187→ 1185→1183→ 1178→ 1177  $\text{cm}^{-1}$ , the lower frequency shift is due to a decrease in electron delocalization in the thymine bases as DNA melts and the metal ion interaction with the melted structure. Duguid *et al.* (1993) have observed similar frequency shift during thermal denaturation of calf-thymus DNA. Similarly the 1236  $\text{cm}^{-1}$  band shifts in the order 1236→ 1235→ 1233→ 1230 in the presence of Magnesium ions. These effects are attributed to partial disruption of A-T base pairs and metal binding at N3 atom of thymine and cytosine.

Three bands are observed in the FT-Raman spectrum of DNA at 1253, 1270 and 1299  $\text{cm}^{-1}$ . The first two bands are due to ring stretching vibration of cytosine, while the 1299  $\text{cm}^{-1}$  band is due to ring stretching vibration of both cytosine and thymine (Spiker and Levin, 1975). All these bands shifted downwards to several  $\text{cm}^{-1}$  in presence of Magnesium ions indicating interactions are present. The FT-Raman spectrum of DNA shows a band 1313  $\text{cm}^{-1}$  due to guanine imidazole ring coupled with C8-N9 stretching vibration (Iyandurai and Sarojini, 2009). This band is shifted in the order 1313→ 1310→ 1308→ 1306  $\text{cm}^{-1}$  upon complex formation with Magnesium ions. Similarly, Raman band observed at 1355  $\text{cm}^{-1}$  band in H<sub>2</sub>O solution has been assigned to a guanine imidazole ring mode of the C2 = N3-C4 = C5-N7 = C8 triene moiety (Iyandurai and Sarojini,

2009) which shifts in the order 1355→ 1352→ 1349→ 1347→ 1343  $\text{cm}^{-1}$  at all the Magnesium ion -DNA complexes studied. The results suggest partial denaturation of DNA in the presence of metal ions and metal ion binding with the denatured structure. Duguid *et al.* have made similar observations in Raman spectra of calf-thymus DNA in the presence of divalent metal ions during thermal denaturation (Duguid *et al.*, 1993).

The intense band at 1376  $\text{cm}^{-1}$  is assigned to in-plane ring vibration (Iyandurai and Sarojini, 2009) and C6-H in plane bend vibration of thymine. This normal mode also involves the exocyclic C5-CH<sub>3</sub> group of thymine and is sensitive to the methyl group environment. Adenine also contributes to this intense band (Po *et al.*, 2007). This band shifts to higher frequency side in the presence of Magnesium ion indicating metal binding at N3 atom thymine and adenine. Raman band at 1440  $\text{cm}^{-1}$  has been assigned to C5'-H<sub>2</sub> deformation of thymine. This band is shifted to 1436  $\text{cm}^{-1}$  at higher Magnesium ion - DNA concentration. This shows minor C5'-H<sub>2</sub> deformation of thymine. The Raman band measured at 1454  $\text{cm}^{-1}$  in the Raman spectrum of DNA has been assigned to the deoxyribose moieties (Iyandurai and Sarojini, 2009). They correspond to ethylene bending modes, although they should also have some contributions from adenine vibrations. This band shifts in the order 1454→ 1455→ 1459→ 1460→ 1463  $\text{cm}^{-1}$  at all Magnesium ion -DNA complexes studied.

The 1488  $\text{cm}^{-1}$  band of FT-Raman spectrum has been assigned to a vibration involving a large displacement of N7 and C8 atoms of urine (guanine and adenine) (Po *et al.*, 2007). The guanine contribution is greater, approximately threefold larger than that of adenine. The band has great diagnostic value, both as a monitor of deuterium exchange kinetics of the urine C8H group and as an indicator of urine N7 hydrogen bonding interactions. The present result shows metal ion binding at N7 position of guanine through water molecule. The effect of the metal ion in this  $\text{Mg}^{2+}\cdots\text{O}(\text{H})\cdots\text{H}\cdots\text{N7}$  type of aggregate is to increase the strength of the hydrogen bonds between the N7 of guanine and hydrogen of water, causing a decrease in frequency of the 1488  $\text{cm}^{-1}$  band.

The FT-Raman spectrum of DNA shows a band at 1515  $\text{cm}^{-1}$  due to adenine (Bruce, 2007) ring stretching vibration and the band observed at 1546  $\text{cm}^{-1}$  in H<sub>2</sub>O solution is due to guanine imidazole ring and C6 moiety (Kiefer and Beckmann, 1984). Upon higher Magnesium ion

concentration this band exhibits shift in the order  $1515 \rightarrow 1510$  and  $1546 \rightarrow 1538 \text{ cm}^{-1}$  indicating guanine contributes more than an adenine.

The region  $1600\text{-}1750 \text{ cm}^{-1}$  in Raman spectrum is often called the "double-bond region" contains bands due to stretching vibrations of double bonds including carbonyl groups of C, G and T. Also the bands in this region include contributions from in-plane deformations of NH groups of G (amino and amino), C (amino) and T (amino). The  $1610 \text{ cm}^{-1}$  band of metal free DNA due to ring stretching vibration of cytosine shifts to lower frequency in the order  $1610 \rightarrow 1608 \rightarrow 1607 \rightarrow 1603 \text{ cm}^{-1}$  in Magnesium ion -DNA complexes. This result indicates metal binding at N3 atom of cytosine. The  $1630 \text{ cm}^{-1}$  band of metal free DNA assigned to C2=O stretching and NH<sub>2</sub> scissoring vibration (Moreira *et al.*, 2008) of cytosine shifts to higher frequency near  $1640\text{cm}^{-1}$  in Magnesium ion -DNA (1:5 volume ratio) complex spectrum. Ordinarily, the frequency of a C = O stretching vibration increases with decreasing hydrogen-bonded strength (Tomlinson and Peticolas, 1970). The elevated C2 = O frequency in the complex spectrum reflects carbonyls less strongly hydrogen bonded in the Magnesium ion - DNA complex than in the metal-free DNA. The Raman band observed at  $1660 \text{ cm}^{-1}$  in metal free DNA, due to thymine in-phase stretching of C5-C6, C2 = O and C4 = O vibrations exhibits small shift in the order  $1660 \rightarrow 1665 \rightarrow 1666 \rightarrow 1668 \text{ cm}^{-1}$  in Magnesium ion-DNA complexes indicating unshackling of the A-T base pairs.

### CONCLUSION

The FT-Raman spectra of DNA with Magnesium ion interpret that the ion bind to the phosphate groups of DNA, with negligible change of the B-conformation of DNA. The ribose moieties of all Magnesium ion-DNA complexes have a mixture of C2'-endo and C3'endo sugar bucker. Magnesium ions interact (via H<sub>2</sub>O) with acceptor sites on the urine (N7) rings and make tensional rotations about the glycosyl bond more flexible. A notable exception is an extensive perturbation by magnesium ions on pyrimidine (N3) ring. Various bands assigned to urine and pyrimiding vibrations in the region  $1200\text{-}1600 \text{ cm}^{-1}$  are sensitive to base unshackling (elimination

of hypo chromic effect) in Magnesium ion - DNA complexes.

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