

# The Influence of $Mn^{2+}$ on DNA structure in the presence of $Na^+$ ions: A Raman spectroscopic study

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**Abstract.** The influence of  $Mn^{2+}$  ions on the structure of natural calf thymus DNA was studied by Raman spectroscopy. Measurements were done at room temperature and  $pH\ 6.2 \pm 0.2$ , in the presence of the physiological concentration of 150 mM  $Na^+$  ions, and in the presence of  $Mn^{2+}$  concentrations that varied between 0 and 600 mM. No condensation of DNA was observed at any of the  $Mn^{2+}$  concentrations. At 5 mM  $Mn^{2+}$  and 150 mM  $Na^+$  no significant influence of  $Mn^{2+}$  ions on the DNA structure can be observed. Compared with our previous results obtained at 10 mM  $Na^+$  ions, binding of  $Mn^{2+}$  ions to charged phosphate groups and to DNA bases is inhibited in the presence of 150 mM  $Na^+$  ions. DNA backbone conformational changes were not observed in the whole concentration range of  $Mn^{2+}$  ions as judging from the Raman spectra. No evidence for DNA melting was identified. A high  $Mn^{2+}$  affinity for binding to guanine N7 and possibly, in a much lesser extent, to adenine have been found.

Keywords: Manganese(II) ions, sodium ions, DNA structure, Raman spectroscopy, difference spectra

## 1. Introduction

The mechanisms of cation effects on the structure and physical properties of DNA have not yet been completely clarified [1,2], although DNA-metal cation interactions and their influence on DNA structure have been investigated extensively by a variety of techniques [2–5].

Several different effects of manganese(II) ions on the DNA structure have been presented by us elsewhere [2,5]. It has been shown that manganese(II) ions stabilize DNA structure at very low concentrations [6]. At higher concentrations of these divalent ions DNA distortion and denaturation take place. Such distortions were connected with processes as carcinogenesis and mutagenesis [5,6].

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Besides, in the range of  $1800-800\text{ cm}^{-1}$ , the B-Z transition of the synthetic oligonucleotide, (dG-dC)<sub>20</sub>, induced by  $Mn^{2+}$  ions at room temperature, was investigated by absorption and Vibrational Circular Dichroism (VCD) spectroscopy. Metal ion concentration was varied from 0 to 0.73 M  $Mn^{2+}$  [7]. DNA complexed with  $Mn^{2+}$  ions in films was studied at different relative humidities and ion contents by IR spectroscopy [8]. It has been shown that DNA- $Mn^{2+}$  complexes are able to absorb more water molecules than DNA [8].

The effects of various cations, including manganese(II) on the properties of parallel pyrimidine motif DNA triplexes were intensively investigated and characterized by several different techniques [9]. It was established, that the interaction of metal ions with the triplexes clearly depended on the type and ionic strength of the cations, and the efficiency with which the cations stabilized the global triplex was in the order  $Mg^{2+} > Mn^{2+} > Ca^{2+} > Ba^{2+} \gg Na^+$ . It was shown that these observations would be useful for the design of triplex-forming oligonucleotides for antigene drugs and therapeutic purposes [9].

The thermodynamic parameters of an antiparallel G-quartet formation of d(G4T4G4) with 1 mM divalent cation ( $Mg^{2+}$ ,  $Ca^{2+}$ ,  $Mn^{2+}$ ,  $Co^{2+}$ , or  $Zn^{2+}$ ) were also obtained [10,11]. These parameters showed that the divalent cation destabilizes the antiparallel G-quartet of d(G4T4G4) [11]. It was found that a higher concentration of a divalent cation induced a transition from an antiparallel to a parallel G-quartet structure. These results indicate that the divalent cations are a good tool for regulating the G-quartet structures and their stability [10,11].

Other studies reveal that  $Mn^{2+}$  ions are able to change the enzymatic activity of some nuclear proteins. For example, DNase I cuts both DNA strands in the presence of  $Mn^{2+}$  ions [2,12]. Besides, the interactions of DNA with the nonhistone chromatin protein HMGB1 and histone H1 in the presence of manganese(II) ions were studied by using absorption and optical activity spectroscopy in the electronic and vibrational regions [13]. In the presence of  $Mn^{2+}$ , the protein-DNA interactions differ from those without the ions and cause prominent DNA compaction and formation of large intermolecular complexes [13]. By using circular dichroism (CD) technique, it has also been observed, that the interaction of manganese(II) ions with DNA has a marked influence on the local DNA structure changing the properties of protein-binding sites [14]. Such changes in the mode of the DNA-protein interactions occur at concentrations as small as 0.01 mM  $Mn^{2+}$  [14].

In this study interaction of natural calf thymus DNA with  $Mn^{2+}$  ions, in the presence of 150 mM  $Na^+$  ions, was studied at room temperature by means of Raman spectroscopy.  $Mn^{2+}$  concentration varied between 0 and 600 mM. This is a continuation of our previous study referring to model  $Mn^{2+}$ -DNA complexes [2], in the presence of low concentrations of monovalent ions.

## 2. Experimental

### 2.1. Chemicals

Cacodylic acid-Na-salt·3H<sub>2</sub>O (research grade) and TRIS were from SERVA, Heidelberg, Germany. NaCl and  $MnCl_2 \cdot 2H_2O$  were from Merck, Darmstadt, Germany.

### 2.2. Preparation of DNA samples

Lyophilized fibrous DNA (Type I) from calf thymus (Sigma-Aldrich, Taufkirchen, Germany) was dissolved in 10 mM Tris/HCl, pH 7.0, 150 mM NaCl and sonicated under cooling in 1 min steps [2]. Aliquots of 200  $\mu$ l from the DNA stock solution (about 20 mg/ml) were dialyzed at 4°C against 10 mM

sodium cacodylate buffers, pH 6.0, 150 mM NaCl, containing 0 mM, 5 mM, 100 mM, 200 mM, 300 mM, 400 mM, 500 mM and 600 mM  $MnCl_2$ , respectively, using dialysis tubes with a 1000 Da molecular mass cut-off (Roth, Karlsruhe, Germany). After dialysis, the pH values were measured in the dialysis buffers and in the DNA samples and were between  $pH = 6.2 \pm 0.2$ . DNA concentrations were estimated spectrophotometrically at 260 nm using an extinction coefficient of  $A^{0.1\%, 1\text{ cm}} = 20$  [2,5]. All samples were centrifuged (14000 rpm, 20 min, 4°C) before the Raman measurements.

### 2.3. Raman spectroscopy

Sample solutions of approximately 15  $\mu\text{l}$  were sealed in homemade cuvettes consisting of cylindrical quartz bodies with quartz bottom windows and Teflon stoppers [15].

Raman spectra were excited with the 488-nm line of a Coherent Innova 90 argon laser. The excitation energy was approximately 100 mW at sample space. Spectra were collected at 22°C on the Raman spectrometer T64000 (Jobin Yvon, France) equipped with a liquid nitrogen-cooled charge-coupled-device (CCD) detector [16].

In standard measurements, to achieve a spectral resolution of  $0.5\text{ cm}^{-1}$  the grating with 1800 grooves per mm was used. With this setting the CCD chip covers only  $600\text{ cm}^{-1}$ , therefore two regions ranging from 590 to  $1760\text{ cm}^{-1}$  were collected for the spectra [2,15]. The first region ranges from  $590\text{ cm}^{-1}$  to  $1260\text{ cm}^{-1}$ , and the second region ranges from  $1130\text{ cm}^{-1}$  to  $1760\text{ cm}^{-1}$ . The overlapping part of  $130\text{ cm}^{-1}$  was used to enable the Software package to properly connect the two parts of the spectrum after the spectral treatment [2,15].

6 spectra of 300 s each were accumulated and averaged. To avoid any possible drifts of the wavenumber scale during the measurements, a calibration spectrum was collected after each 300 s accumulation step of sample spectra. The description of the calibration procedure is given elsewhere [17].

Software packages LabSpec (Jobin Yvon, France) and GRAMS (Thermo Galactic, USA) were used to perform Raman data analyses. Solution spectra were corrected by subtraction of the averaged buffer spectrum and fluorescence background that was approximated by a polynomial curve [5,17]. Raman spectra of DNA samples were scaled to have equal intensity in the  $1014\text{ cm}^{-1}$  DNA band, assigned to the sugar moiety [4,5], in order to calculate the difference spectra. The band near  $1014\text{ cm}^{-1}$  is one of the least sensitive to DNA melting and divalent metal binding [2,4].

Difference bands were considered as significant when the intensity of the difference band is at least 2 times higher than the signal-to-noise ratio [2,5,18].

## 3. Results and discussion

Raman spectra of calf thymus DNA were measured at  $pH 6.2 \pm 0.2$ , at a constant salt concentration of 150 mM NaCl and  $MnCl_2$  concentrations between 0 and 600 mM.

Figure 1 shows the Raman spectra of sonicated calf-thymus DNA obtained at 0 mM, 5 mM, 100 mM, 200 mM, 300 mM, 400 mM, 500 mM and 600 mM  $MnCl_2$ , in the region  $590\text{--}1750\text{ cm}^{-1}$ .

Wavenumber positions of the major peaks are given in Figure 1 and are in accordance with those given previously in the literature [2,17,19,20].

Figure 2 shows Raman difference spectra 1 to 7 that were obtained by subtraction of the spectrum of aqueous DNA from the corresponding spectra obtained for samples containing  $Mn^{2+}$  ions. The negative (troughs) and positive (peaks) bands of the difference spectra indicate manganese(II) dependent changes in the DNA structure, in the presence of 150 mM  $Na^+$  ions.

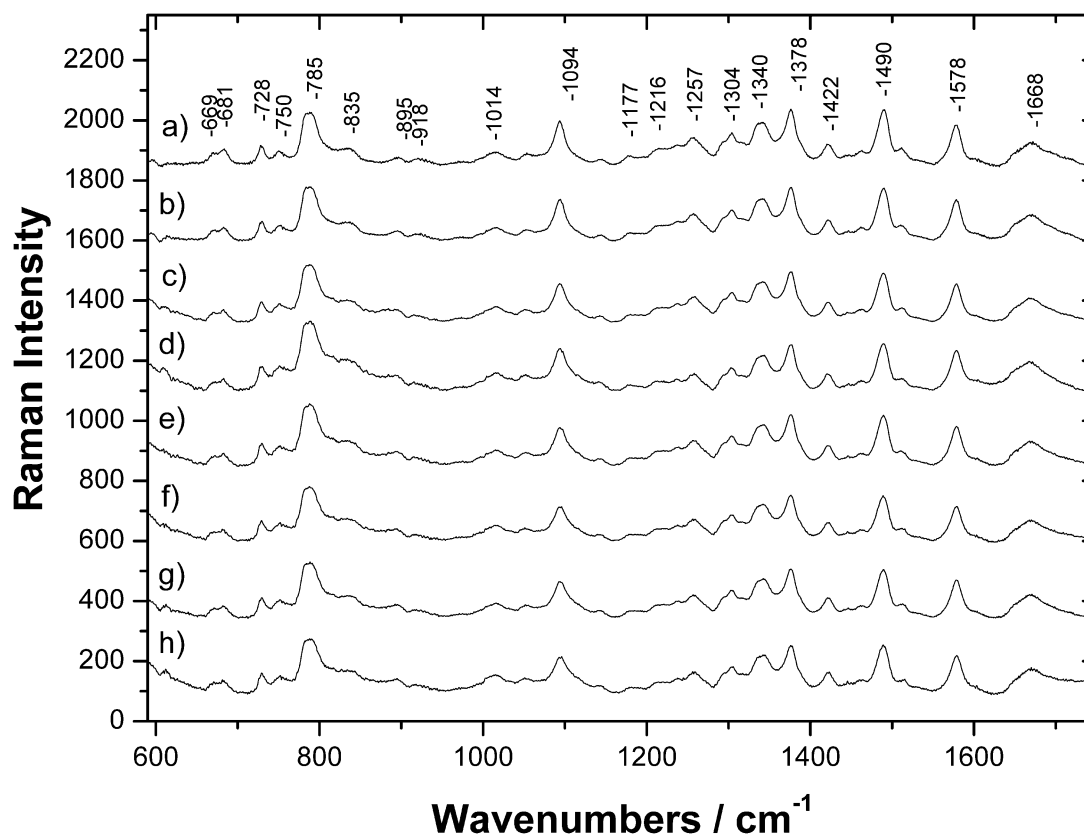


Fig. 1. Raman spectra of sonicated calf thymus DNA in the presence of 150 mM  $Na^+$ , at different  $Mn^{2+}$  concentrations: a) 0 mM, b) 5 mM, c) 100 mM, d) 200 mM, e) 300 mM, f) 400 mM, g) 500 mM, h) 600 mM. The spectra presented in the region 595–1750  $cm^{-1}$  are background corrected. Peak positions of prominent Raman bands are labeled. The DNA concentration is approximately 20 mg/ml. The spectra were scaled to have equal intensity in the 1014  $cm^{-1}$  DNA band assigned to the sugar moiety [20]. For all measurements, the laser power at the sample space was 100 mW. 6 measurements of 300 sec were averaged for each spectrum.

The Raman spectra were analyzed in the wavenumber region 600–1150  $cm^{-1}$  that contains information about nucleoside conformation, backbone geometry and  $PO_2^-$  interaction [4,18–23]. The  $C2'$ -endo-anti nucleoside conformers [2,18,21,24] are identified by the conformation markers at 681  $cm^{-1}$  (dG) [21,24,25], 728  $cm^{-1}$  (dA) [24,26], 750  $cm^{-1}$  (dT) [24,26] and 785  $cm^{-1}$  (dC) [21,24,25]. These bands do respond to the unstacking of bases [27]. During DNA melting the intensity of the guanine band decreases upon unstacking [24], whereas the Raman intensity of other bands increases [2,5]. Such an effect was not observed in any of our Raman spectra. The marker band of B-form DNA backbone and  $C2'$ -endo sugar conformations [24,25] is centered around 835  $cm^{-1}$  [3,24–26]. The band near 1094  $cm^{-1}$  is sensitive to the electrostatic environment of the  $PO_2^-$  group [2,21,24].

Raman bands in the wavenumber region 1150  $cm^{-1}$ –1720  $cm^{-1}$  are influenced by the electronic structures of the bases and base pairing [4,19,20,23].

The DNA Raman signature is extensively perturbed in the presence of divalent transition metals [4]. Raman difference spectra of DNA for the selected manganese(II) ions concentrations are given in Fig. 2.

The Raman spectra at 22°C correspond to those of B-form DNA as demonstrated by the band centered around 835  $cm^{-1}$  [21,24–26] (Fig. 1). This is in accordance with studies on DNA- $Mn^{2+}$  inter-

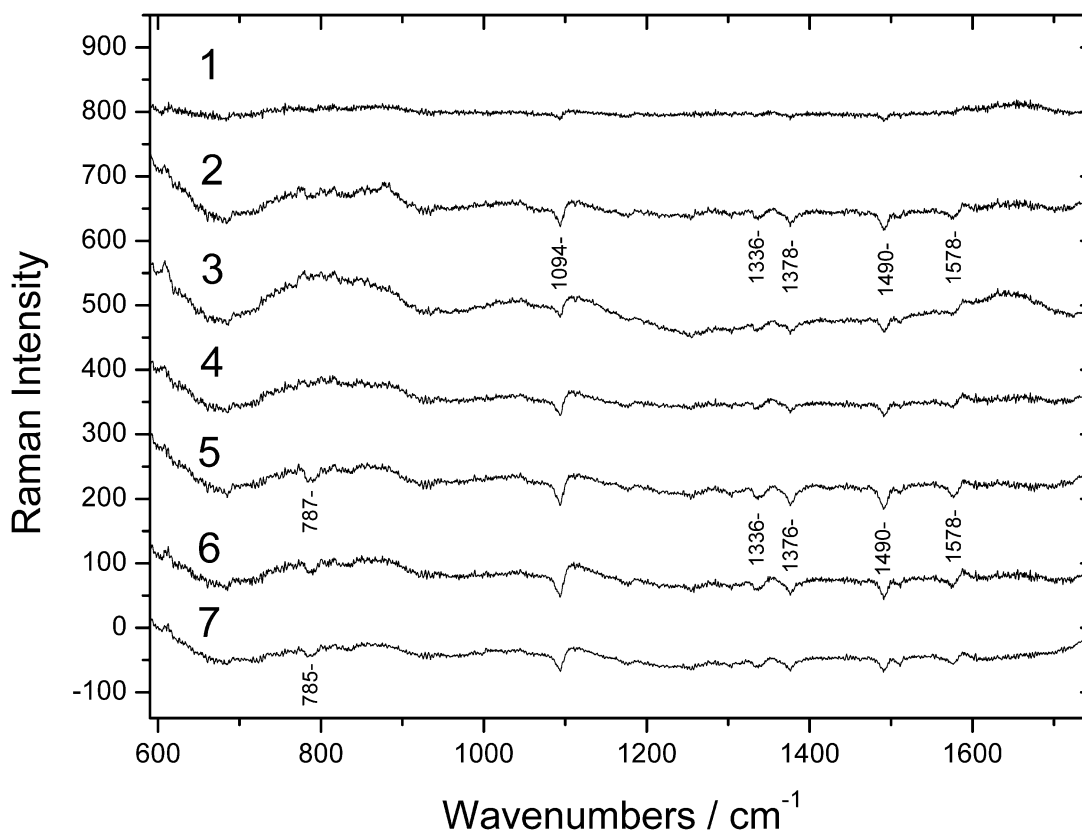


Fig. 2. Raman difference spectra obtained by subtracting the spectrum of aqueous DNA from the corresponding spectrum in the presence of  $MnCl_2$ , at the following  $Mn^{2+}$  ions concentrations: 5 mM (line 1), 100 mM (line 2), 200 mM (line 3), 300 mM (line 4), 400 mM (line 5), 500 mM (line 6) and 600 mM (line 7). All spectra were scaled to have equal intensity on the  $1014\text{ cm}^{-1}$  DNA band before subtraction.

action in aqueous solutions, carried out by means of IR absorption and vibrational circular dichroism spectroscopy [28].

Difference spectrum 1 (Fig. 2) doesn't undergo significant spectral features, showing that almost no  $Mn^{2+}$ -DNA interaction takes place at this divalent metal concentration (5 mM), in the presence of 150 mM  $Na^+$ . This result is different to that obtained by us for 5 mM  $Mn^{2+}$  and 10 mM  $Na^+$  ions [2].

No spectral change is observed in Fig. 2 at  $681\text{ cm}^{-1}$ , the guanine nucleoside marker, at adenine residues marker around  $728\text{ cm}^{-1}$  and at the band near  $750\text{ cm}^{-1}$  which is indicative for the  $C2'$ -endo/anti conformers of dT [5,26], suggesting that in the presence of 150 mM  $Na^+$  ions no specific interactions of the  $Mn^{2+}$  ions with these residues take place.

The band near  $785\text{ cm}^{-1}$  [5,21,24,25], characterizing the  $C2'$ -endo-anti conformation marker of dC, exhibits a decrease in intensity in several of our spectra, suggesting altered nucleoside conformations in dC residues (Fig. 2, spectra 5-7). A shift of the Raman band from  $785\text{ cm}^{-1}$  to  $787\text{ cm}^{-1}$  can be observed at 400 mM and 500 mM  $Mn^{2+}$ .

In our spectra, binding of  $Mn^{2+}$  ions to DNA phosphate groups ( $1094\text{ cm}^{-1}$ ) is indicated. The presence of 100 to 600 mM  $Mn^{2+}$  ions is accompanied by a decrease in the intensity of the  $PO_2^-$  symmetric vibration (difference spectra 2-7 in Fig. 2). Electrostatic interactions of the negatively charged phosphate

groups with the  $Mn^{2+}$  ions, stabilizing the double-helical DNA structure, are probably connected with these changes [2,28]. Other possible interpretations of these effects might be considered, as e.g. a much more direct type of interaction of the divalent metal ion (covalent binding) with one particular oxygen atom of the phosphate group [2,29].

Conformational transition towards the C-form of DNA was observed in solution in the presence of  $Mn^{2+}$  ions [12]. No such a drastic change in DNA structure is supported in our spectra published recently [2].

Bands in the 1200–1600  $cm^{-1}$  region, assigned to purine and pyrimidine ring vibrations, are sensitive indicators of ring electronic structures; they are expected to exhibit perturbations upon metal binding to DNA or upon base unstacking [4,5,21]. Loss of stacking represents loss of the regularly ordered arrangement among the nucleobases and among sugar-phosphate residues of the backbone [28].

No spectral features are observed in our difference spectra at 1240  $cm^{-1}$ , a band assigned to dT (with a minor contribution from dC), and at 1257  $cm^{-1}$ , a band assigned to dC [2,5] indicating that unstacking of thymine and metal ion binding at N3 of cytosine do not take place at our experimental conditions.

Metal binding to N7 of guanine and to a lesser extent to N7 of adenine [4,28] seems to start at 100 mM  $Mn^{2+}$  ions as judging by the appearance of negative bands of the bases at 1336, 1376, 1490 and 1578  $cm^{-1}$  in the corresponding spectrum (Fig. 2). Changes in the Raman wavenumbers of some bands are also observed (from 1376 to 1378  $cm^{-1}$  and from 1488 to 1490  $cm^{-1}$ ).

The band at 1336  $cm^{-1}$  is attributed to adenine and guanine. Besides dA and dG also dT residues contribute to the band at 1376  $cm^{-1}$  [5,21].

Binding of divalent metal ions to the N7 acceptor of guanine [24–26] is indicated in the spectra by the loss in intensity of the guanine band at 1488  $cm^{-1}$ .

The band at 1578  $cm^{-1}$  is attributed to purine residues (dA, dG), but mostly due to guanine vibrations ([30] and references therein).

In our Raman spectra of the  $Mn^{2+}$ -DNA complexes, there is no intensity change of the band at 1668  $cm^{-1}$ , suggesting no change in their base pairing and no change induced in the structure of water by  $Mn^{2+}$  cations [2,4].

A nonlinear behaviour of Raman intensities of the main DNA vibrational markers as a function of  $Mn^{2+}$  concentration is observed at room temperature. This result is similar to that obtained by us in the previous study on  $Mn^{2+}$ -DNA interactions [2], and was observed also by other authors for some wavenumbers, using IR absorption [28]. The largest intensity changes observed in the 1200–1600  $cm^{-1}$  region were observed at 400 mM  $Mn^{2+}$  (difference spectrum 5).

A comparison of the results obtained here for DNA samples in buffers containing 150 mM  $Na^+$  ions with those described in our previous study [2] for DNA samples in buffers containing only 10 mM  $Na^+$ , suggests that higher concentrations of  $Na^+$  ions inhibit the binding of  $Mn^{2+}$  ions to phosphate groups and bases of DNA.

#### 4. Conclusions

Like in our previous study [2], the results obtained for  $Mn^{2+}$ -DNA systems, at room temperature, in the presence of  $Na^+$  ions proved the suitability of Raman spectroscopy to monitor in detail structural changes of metal-DNA complexes. We found that in DNA samples containing a higher concentration of  $Na^+$  ions the binding of  $Mn^{2+}$  ions to phosphate groups and to DNA bases is inhibited.

No meaningful  $Mn^{2+}$ -DNA interaction could be observed at 5 mM divalent metal concentration, in the presence 150 mM  $Na^+$ . This result is different to that obtained by us for 5 mM  $Mn^{2+}$  and 10 mM  $Na^+$  ions [2].

Within the studied concentration range of 0 to 600 mM  $Mn^{2+}$ , a critical concentration was observed previously by us at 100 mM  $Mn^{2+}$ , where DNA has undergone a structural transition into a compact form [2]. No such phenomenon was observed in the present study. The backbone conformation persisted in the manganese(II)-DNA complexes, in the presence of  $Na^+$  ions, since no important changes of the B-form marker at  $835\text{ cm}^{-1}$  were observed.

Binding of manganese(II) ions to the charged phosphate groups of DNA, stabilizing the double helical structure [2,30], is indicated in the spectra. As judged from the marker band of dC near  $785\text{ cm}^{-1}$ , altered nucleoside conformations in dC residues are supposed to occur, in the  $Mn^{2+}$  concentration range of 400–600 mM. DNA melting was not observed in our Raman data, in the range of metal ion concentration used in this study.

Binding of divalent ions to N7 of guanine [2,31–33] and, possibly, in a lesser extent to adenine was observed as judging from the Raman marker bands at 1336, 1376, 1490 and  $1578\text{ cm}^{-1}$ .

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