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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 ± 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 cm^{-1}, the B-Z transition of the synthetic oligonucleotide, $(dG\text{-}dC)_{20}$, 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 $Mg^{2+} > Mn^{2+} > Ca^{2+} > Ba^{2+} \gg Na^{+}$[9]. 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 manganase(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·$3H_2O$ (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 μl 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 $A^{0.1\%,\,1\,cm} = 20$ [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 μl 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 cm^{-1} the grating with 1800 grooves per mm was used. With this setting the CCD chip covers only 600 cm^{-1}, therefore two regions ranging from 590 to 1760 cm^{-1} were collected for the spectra [2,15]. The first region ranges from 590 cm^{-1} to 1260 cm^{-1}, and the second region ranges from 1130 cm^{-1} to 1760 cm^{-1}. The overlapping part of 130 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 cm^{-1} DNA band, assigned to the sugar moiety [4,5], in order to calculate the difference spectra. The band near 1014 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 ± 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 590–1750 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.

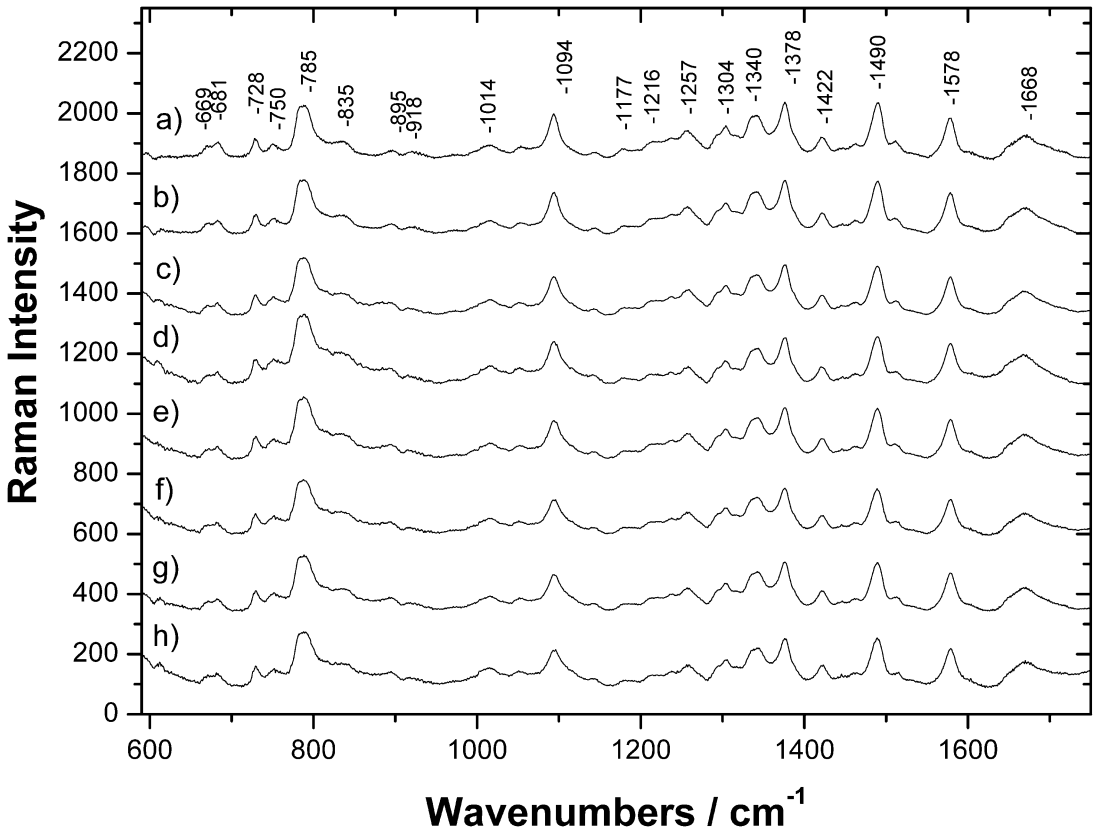

Fig. 1. Raman spectra of sonicated calf thymus DNA in the presence of 150 mM Na^{+}, at different Mn^{2+} concentrations: a) 0 mM, b) 5 mM, c) 100 mM, d) 200 mM, e) 300 mM, f) 400 mM, g) 500 mM, h) 600 mM. The spectra presented in the region 595–1750 cm^{-1} are background corrected. Peak positions of 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-

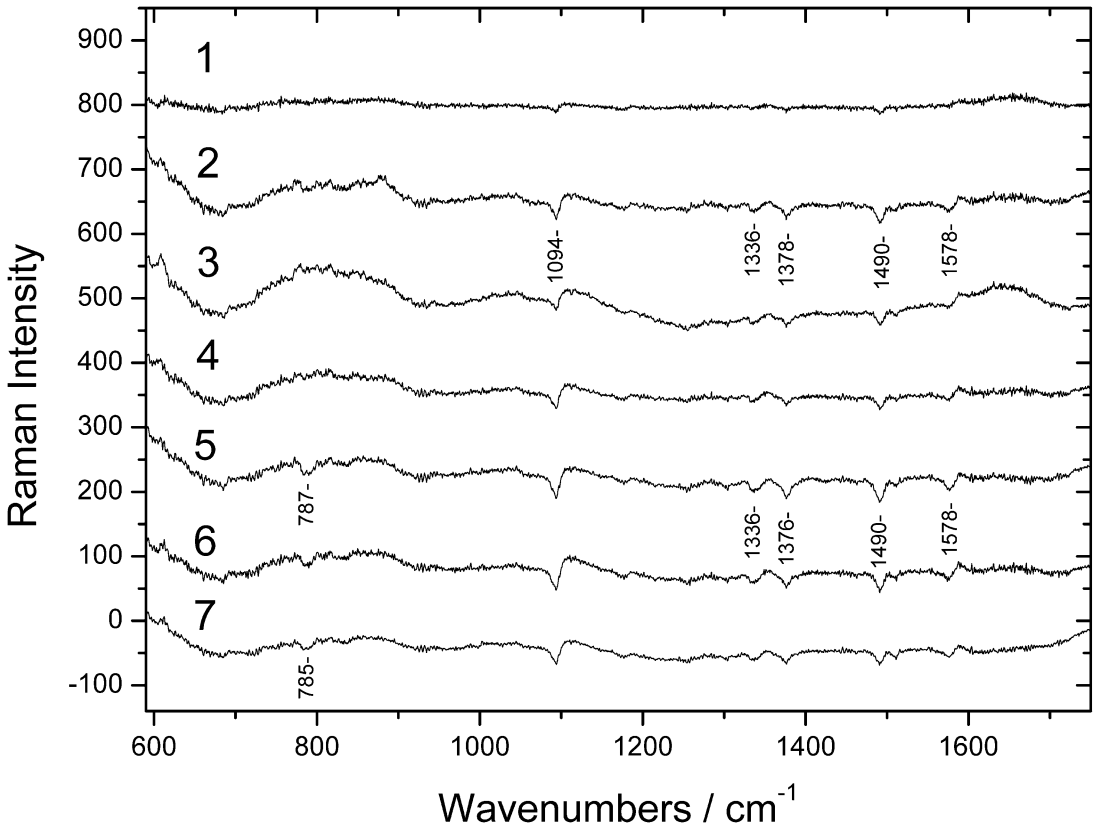

Fig. 2. Raman difference spectra obtained by subtracting the spectrum of aqueous DNA from the corresponding spectrum in the presence of $MnCl_2$, at the following Mn^{2+} ions concentrations: 5 mM (line 1), 100 mM (line 2), 200 mM (line 3), 300 mM (line 4), 400 mM (line 5), 500 mM (line 6) and 600 mM (line 7). All spectra were scaled to have equal intensity on the 1014 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 cm^{-1}, the guanine nucleoside marker, at adenine residues marker around 728 cm^{-1} and at the band near 750 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 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 cm^{-1} to 787 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 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 appearence 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 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 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 cm^{-1}.

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