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# $Mn^{2+}$–DNA interactions in aqueous systems: A Raman spectroscopic study

Cristina M. Muntean [a,*,**], Rolf Misselwitz [b], Lubomir Dostál [b] and Heinz Welfle [b]

[a] *National Institute of Research & Development for Isotopic and Molecular Technologies, PO 5, Box 700, R-400293 Cluj-Napoca, Romania*

[b] *AG Biopolymerspektroskopie, Max-Delbrück-Centrum für Molekulare Medizin Berlin-Buch, Robert-Rössle-Str. 10, D-13092 Berlin, Germany*

**Abstract.** Interaction of natural calf thymus DNA with $Mn^{2+}$ ions was studied by means of Raman spectroscopy. Spectra of DNA in 10 mM Na-cacodylate buffer, pH 6.2, 10 mM NaCl and in buffer containing $Mn^{2+}$ ions were measured at room temperature. $Mn^{2+}$ concentrations varied between 0 and 0.6 M. DNA backbone conformational changes and DNA denaturation were not observed in the concentration range 0 and 0.5 M, however, DNA condensation was observed at a critical concentration of 100 mM $Mn^{2+}$ that prevented the measurement of Raman spectra. Binding of $Mn^{2+}$ to the charged phosphate groups of DNA is indicated in the spectra. A high affinity of $Mn^{2+}$ for guanine N7 was obvious, and binding to adenine was barely suggested.

Keywords: $Mn^{2+}$–DNA interaction, Raman spectroscopy, difference spectra

## 1. Introduction

DNA–metal cation interactions and their effects on DNA structure have been investigated extensively by a variety of techniques [8,14,23], although the mechanisms of the cation effects on the structure and physical properties of DNA have not yet been completely clarified [10]. DNA distortions have been implicated in processes of fundamental biological importance as carcinogenesis and mutagenesis [3,14].

Manganese ions stabilize DNA structure at very low concentrations [3]. DNA becomes distorted and denatured at high $Mn^{2+}$ concentrations. Complex formation of DNA in aqueous solutions with $Mn^{2+}$ induced highly cooperative compaction and aggregation of DNA [2,8,21].

Raman spectroscopy has been employed to assess nucleic acid binding sites of alkaline earth ions and transition metal ions, including manganese cations [8,11,14,15,26], and to characterize and compare the perturbations of secondary and tertiary structures induced in DNA [8,26]. The interaction of DNA molecules with $Mn^{2+}$ ions was also studied by IR spectroscopy [1,3,9,10]. Structural effects of manganese ions on DNA-like structures were described by several electronic and vibrational circular dichroism studies [2,3,21]. Conformational transition towards the C-form of DNA was observed in solution in the

*Corresponding author: Dr. Cristina Muntean, National Institute of Research & Development for Isotopic and Molecular Technologies, PO 5, Box 700, R-400293 Cluj-Napoca, Romania. E-mail: cmuntean@s3.itim-cj.ro.

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presence of $Mn^{2+}$ ions [21]. A model for the interaction between manganese ions and DNA mediated by water was suggested, destabilizing the double helix and partially breaking the hydrogen bonds between the base pairs [21]. It has been shown by absorption and VCD spectroscopy, that $Mn^{2+}$ ions induce at room temperature a B–Z transition of the synthetic oligonucleotide $(dG–dC)_{20}$ [2]. The Z-form of DNA is believed to be important in life processes, and to play a significant role in gene regulation and DNA replication [2]. This notion was supported by the fact that left-handed sequences have been found in natural DNA [2]. It has also been shown 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 [21].

In an attempt to understand the molecular mechanism of DNA–$Mn^{2+}$ interaction, we present here a Raman spectroscopic study of structural changes induced in calf thymus DNA at room temperature, in the presence of divalent manganese ions, by variation of $Mn^{2+}$ concentration between 0 and 600 mM.

## 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. Aliquots of 200 $\mu$l DNA (about 20 mg/ml) were dialyzed at 4°C against appropriate buffers containing 10 mM NaCl, 10 mM Na-cacodylate, pH 6.2 $\pm$ 0.15, and varying $MnCl_2$ concentrations, using dialysis tubes with a molecular mass cut-off from 1000 Da (Roth, Karlsruhe, Germany). DNA concentrations were estimated spectrophotometrically at 260 nm using an extinction coefficient of $A^{0.1\%,1\ \mathrm{cm}} = 20$ [14]. All samples were centrifuged (14,000 rpm, 20 min, 4°C) before the Raman measurements.

Raman spectra were measured at 0, 5, 100, 200, 300, 400, 500 and 600 mM $MnCl_2$, respectively. The pH values were controlled in the dialysis buffers and in the DNA samples. A critical concentration has been found at 100 mM $Mn^{2+}$, where condensation of DNA occurred and no Raman spectrum was obtained.

### *2.3. Raman spectroscopy*

Sample solutions of approximately 15 $\mu$l were filled in a homemade cuvette consisting of cylindrical quartz bodies with quartz bottom windows [6]. Then, the cuvette was centrifuged for 1 min to remove air bubbles from the solution. After that, the cuvette was tightly closed with a Teflon stopper and positioned in the macrochamber of the Raman spectrometer T64000 (Jobin Yvon, France) that was equipped with a liquid nitrogen-cooled charge-coupled-device (CCD) detector [5,6].

Raman spectra were excited with the 488-nm line of a Coherent Innova 90 argon ion laser, and the radiant power was approximately 100 mW at the sample space. Spectra were collected at 22°C. In standard measurements, the grating with 1800 grooves per mm was used to achieve a spectral resolution of approximately 0.5 $cm^{-1}$. With this setting the CCD chip covers only 600 $cm^{-1}$, therefore two regions were collected for the spectra ranging from 590 to 1760 $cm^{-1}$ [6]. The first region ranges from 590 to

1260 $cm^{-1}$ and the second region from 1130 to 1760 $cm^{-1}$. The overlapping part of 130 $cm^{-1}$ was used to enable the Software package to properly connect the two spectra after the data treatment [6].

A total of 6 spectra of 300 s each were accumulated and averaged. To exclude all possible drifts of the wavenumber scale during the measurements, a calibration spectrum was collected after each 300 s accumulation step of sample spectra. The calibration procedure is described elsewhere [4].

Raman data were analyzed with the software packages LabSpec (Jobin Yvon, France) and GRAMS (Thermo Galactic, USA). Solution spectra were corrected by subtraction of the averaged buffer spectrum and fluorescence background that was approximated by a polynomial curve [4,14]. Difference spectra were calculated using Raman spectra that were measured at the indicated manganese (II) ions and scaled to have equal intensity in the 1014 $cm^{-1}$ DNA band, assigned to the sugar moiety [8,14]. The band near 1014 $cm^{-1}$ is one of the least sensitive to DNA melting and divalent metal binding [8].

Difference bands were considered as significant when the following criteria are fulfilled: (i) the intensity of the difference band is at least 2 times higher than the signal-to-noise ratio, and (ii) the difference bands reflect intensity changes of at least 5% of there parent band [7,14].

## 3. Results and discussion

Raman spectra of sonicated calf-thymus DNA are shown in Fig. 1. The spectra were obtained at selected $Mn^{2+}$ concentrations between 0 and 0.6 M $Mn^{2+}$ and cover the spectral region 620–1750 $cm^{-1}$. A critical concentration has been found by us at 100 mM $Mn^{2+}$, at which condensation of DNA occurred. No Raman spectrum was obtained at this $Mn^{2+}$ ions concentration.

The Raman signature of dissolved calf-thymus DNA is given in Fig. 1a. Wavenumber positions of the major peaks are in accordance with those previously reported in the literature [4,27,28]. In the 600–1150 $cm^{-1}$ region, Raman markers that contain information about deoxynucleoside conformation, DNA backbone geometry and $PO_2^-$ interaction are located [7,8,17,20,26–28], whereas electronic structures of the bases and base pairing influence Raman bands in the wavenumber region 1150–1720 $cm^{-1}$ [8, 20,27,28]. The nucleoside conformation markers at 681 $cm^{-1}$ (dG) [22,24,26], 728 $cm^{-1}$ (dA) [16,22], 750 $cm^{-1}$ (dT) [16,22] and 785 $cm^{-1}$ (dC) [22,24,26] identify the C2′-*endo*-anti conformers [7,22,26]. These bands do respond to the unstacking of bases [19]. The intensity of the guanine band decreases upon unstacking during DNA melting [22] whereas the Raman intensity of other bands increases [14]. The band centered around 835 $cm^{-1}$ [16,22,24,26] is a marker of B-form DNA backbone and C2′-endo sugar conformations [22,24]. The band near 1092 $cm^{-1}$ is sensitive to the electrostatic environment of the $PO_2^-$ group [22,26].

The Raman spectra correspond to those of B-form DNA as demonstrated by the band centered around 835 $cm^{-1}$ [16,22,24,26] (Fig. 1). This is in accordance with studies on DNA–$Mn^{2+}$ interaction in aqueous system, carried out by means of IR absorption and vibrational circular dichroism (VCD) spectroscopy [1].

Figure 2 shows Raman difference spectra 1, 2, 3, 4, 5, and 6 that were obtained by subtraction of the spectrum of aqueous DNA (spectrum (a) in Fig. 1) from the spectrum in buffer containing 5, 200, 300, 400, 500 and 600 mM $Mn^{2+}$, respectively. In the presence of $Mn^{2+}$, the DNA Raman signature is perturbed extensively. The negative (troughs) and positive (peaks) features of the difference spectra indicate $Mn^{2+}$ dependent changes in the DNA structure.

*681 $cm^{-1}$*: No spectral change is observed at the 681 $cm^{-1}$ guanine nucleoside marker band (Fig. 2).

*728 $cm^{-1}$*: Weak difference features are present at adenine residues marker around 728 $cm^{-1}$, at concentrations equal or higher than 400 mM $Mn^{2+}$ (Fig. 2, spectra 4 and 6).

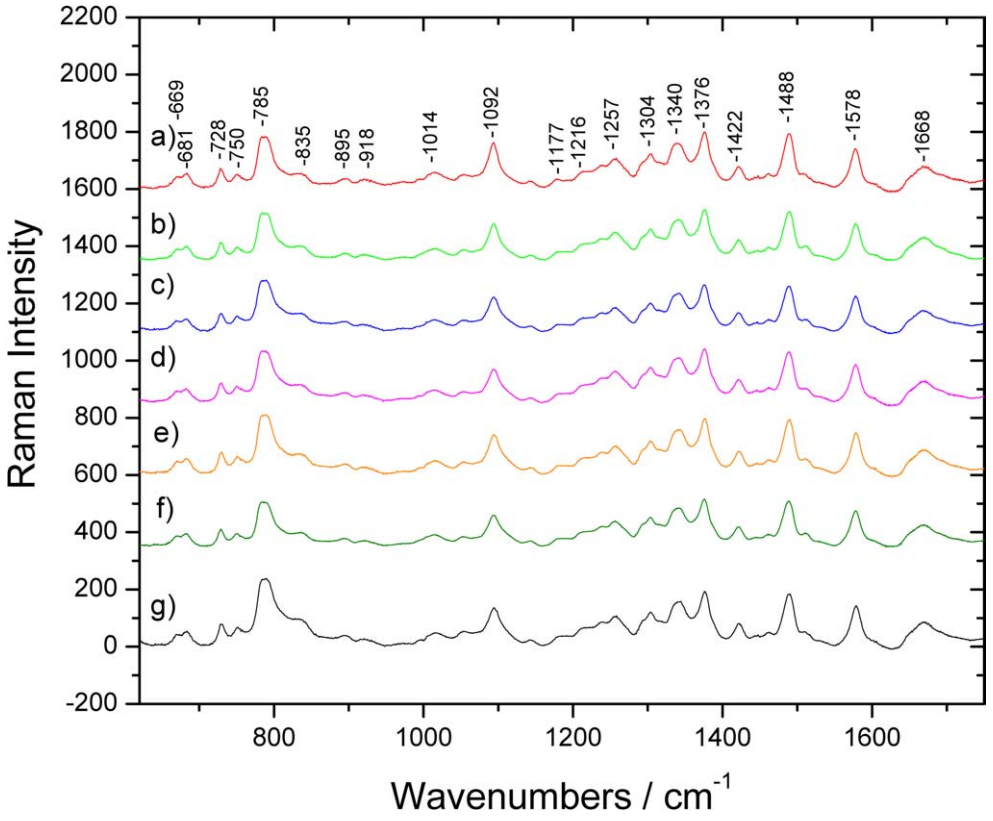


Fig. 1. Raman spectra of sonicated calf thymus DNA. DNA was in (a) 10 mM Na-cacodylate buffer, pH 6.2, 10 mM NaCl; and in buffer containing the following $Mn^{2+}$ concentrations: (b) 5 mM, (c) 200 mM, (d) 300 mM, (e) 400 mM, (f) 500 mM, (g) 600 mM. Background corrected spectra are presented in the region 620–1750 $cm^{-1}$. Peak positions of prominent Raman bands are labeled in spectrum (a). The DNA concentration was approximately 20 mg/ml. The spectra were scaled to have equal intensity in the 1014 $cm^{-1}$ DNA band assigned to the sugar moiety [8,14]. For all measurements, the laser power at the sample space was 100 mW. 6 measurements of 300 sec were averaged for each spectrum.

*750 $cm^{-1}$*: The band near 750 $cm^{-1}$ which is indicative for the C2-*endo/anti* conformers of dT [14,16] doesn't undergo any intensity change at 5, 200, 300 and 500 mM $Mn^{2+}$ ions, suggesting no specific interactions of the manganese(II) ions with dT residues.

*785 $cm^{-1}$*: The C2′-*endo*-anti conformation marker of dC near 785 $cm^{-1}$ [14,22,24,26] exhibits a decrease in intensity in several of our spectra, suggesting altered nucleoside conformations in dC residues (Fig. 2, spectra 1–3, 5).

*1092 $cm^{-1}$*: Binding of $Mn^{2+}$ ions to the phosphate groups is indicated in the difference spectra by the large trough at 1092 $cm^{-1}$. The presence of $Mn^{2+}$ ions in DNA samples, at all concentrations investigated is accompanied by a decrease in the intensity of the $PO_2^-$ interaction marker (difference spectra in Fig. 2). These changes are probably connected with electrostatic screening of the negatively charged phosphate groups by $Mn^{2+}$ ions, stabilizing the double-helical structure [1]. However, other study indicates that purely electrostatic interactions of the phosphate groups with the hydrated metal ions are not believed to modify appreciably the 1092 $cm^{-1}$ band of DNA [12]. In that study it is supposed that the observed intensity decrease in the Raman spectra is caused by a much more direct type of interaction (covalent binding) with one particular oxygen atom of the phosphate group [12].

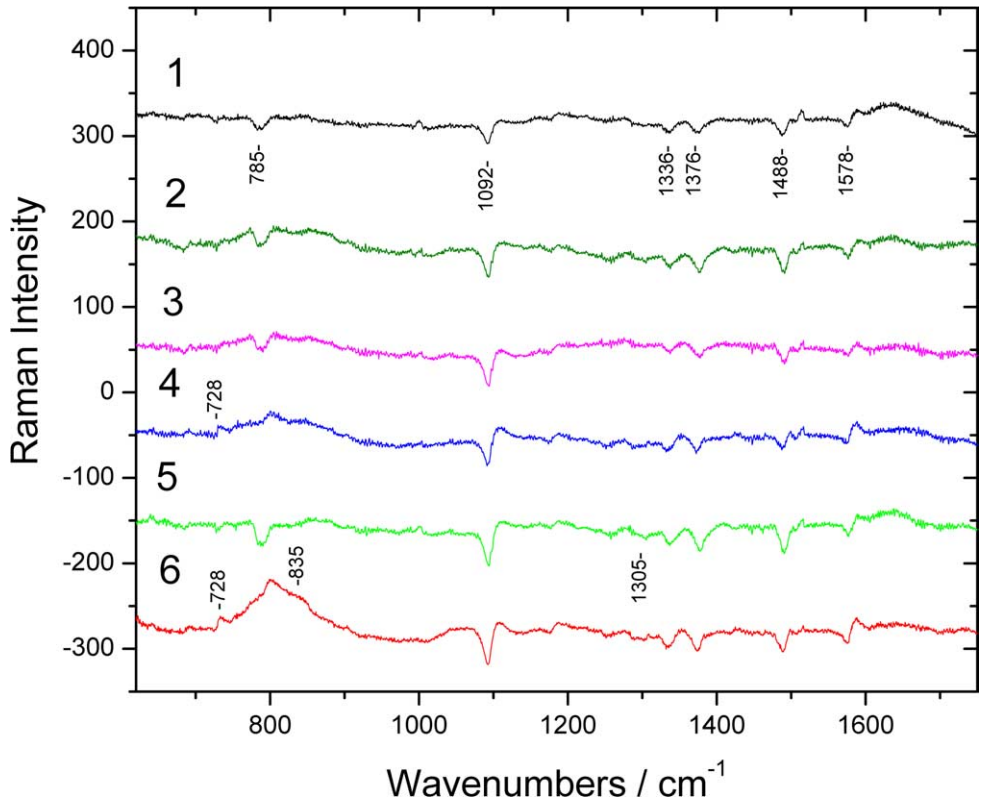


Fig. 2. Raman difference spectra, calculated from the spectra in Fig. 1. The spectrum of aqueous DNA was subtracted from the spectrum in the presence of 5 mM (line 1), 200 mM (line 2), 300 mM (line 3), 400 mM (line 4), 500 mM (line 5) and 600 mM (line 6) $Mn^{2+}$ ions, respectively. All spectra were scaled to have equal intensity in the 1014 $cm^{-1}$ DNA band before subtraction.

Conformational transition towards the C-form of DNA was observed in solution in the presence of $Mn^{2+}$ ions [21]. Our spectra did not support such a drastic change in DNA structure. The symmetric $PO_2^-$ vibration has a rather high wavenumber (1104 $cm^{-1}$) in the Raman spectrum of C-DNA [12,25] and this is not observed by us. Besides, a Raman band at 875 $cm^{-1}$, characterizing the C form of DNA [25] is not present in our data.

Most of the changes in the Raman spectra in 1200–1600 $cm^{-1}$ attributed to purine and pyrimidine ring vibrations, can be assigned to metal ion binding to DNA bases and are indicative of base unstacking [8,14,26]. Unstacking is connected with the loss of the regularly ordered arrangement among the nucleobases and among sugar-phosphate residues of the backbone [1].

A Raman difference spectrum of high-molecular-weight DNA in the presence of 100 mM $Mn^{2+}$ was previously published [8]. The largest hyperchromicities were observed at 1247 and 1263 $cm^{-1}$, however at these wavenumbers hyperchromicity was not observed in the difference spectra (Fig. 2). This is probably due to the fact that we have used sonicated calf thymus DNA but not high-molecular-weight DNA since vibrational spectra of DNA-metal complexes might be influenced by the molecular length of DNA molecules [8,9]. The band near 1240 $cm^{-1}$ is assigned to dT (with a minor contribution from dC), and the band near 1257 $cm^{-1}$ is assigned to dC [14]. The lack of the peak at 1241 $cm^{-1}$ indicates that unstacking of thymine doesn't take place in our experimental conditions. The behaviour of the band at

1257 $cm^{-1}$ shows that metal binding at N3 of cytosine in the $Mn^{2+}$ concentration range studied here is not observed.

Raman bands of the bases at 1305, 1336, 1376, 1488 and 1578 $cm^{-1}$ are consistent with metal binding to N7 of guanine and to adenine [8]. As observed in other studies ([1] and references therein), $Mn^{2+}$ coordination with N7 of adenine is only about 10% of that with N7 of guanine.

*1305 $cm^{-1}$*: No significant binding of $Mn^{2+}$ to adenine is indicated in the experimental ion concentration range, except of a minimal trough at 1305 $cm^{-1}$ that appeared at 500 mM $Mn^{2+}$ (Fig. 2, spectrum 5).

*1336 $cm^{-1}$*: Band at 1336 $cm^{-1}$, attributed to adenine and guanine, decreases in intensity in all Raman spectra presented in Fig. 1.

*1376 $cm^{-1}$*: Another trough appears at 1376 $cm^{-1}$ in the difference spectra, at all $Mn^{2+}$ ion concentrations. Purine (dA, dG) and dT residues contribute to this band [14,26]. Therefore discrimination between the individual contributions of these bases is difficult.

*1423 $cm^{-1}$*: The band at 1423 $cm^{-1}$ (Fig. 1) that attributes to $CH_2$ vibrations and reflects properties of the DNA backbone [14,26] does not undergo significant changes, therefore any features are missing at this wavenumber in our difference spectra.

*1488 $cm^{-1}$*: The loss in intensity of the guanine band at 1488 $cm^{-1}$ indicates binding of divalent metal ion to the N7 acceptor of guanine [16,22,24]. Such an intensity loss results in a trough in the difference spectra at 1488 $cm^{-1}$, as visible in the difference spectra at any studied $Mn^{2+}$ concentration (Fig. 2). This feature provides evidence for the binding of $Mn^{2+}$ to N7 of guanine.

*1578 $cm^{-1}$*: A trough appears at 1578 $cm^{-1}$, attributed to purine residues (dA, dG), but mostly due to guanine vibrations ([25] and references therein).

*1668 $cm^{-1}$*: The band at 1668 $cm^{-1}$ is expected to reflect changes in hydrogen bonding states of exocyclic donor and acceptor groups of the bases and changes induced in the structure of water by divalent cations [8]. We did not observe an intensity change of this band in the Raman spectra of the $Mn^{2+}$–DNA complexes and consequently no difference band at this wavenumber (Fig. 2), suggesting no obvious change in hydrogen bonding or water structure.

Raman intensities of the main DNA vibrational markers do not exibit a linear behaviour versus $Mn^{2+}$ concentration at room temperature and this was also observed for some wavenumbers by other authors, using the IR absorption technique [1].

## 4. Conclusions

The experimental results shown above exemplify in detail the structural changes of calf thymus DNA induced by $Mn^{2+}$ ions in the concentration range 0–600 mM.

Spectroscopic data show that $Mn^{2+}$ ions bind to the charged phosphate groups of DNA, stabilizing the double helical structure [25]. The presence of the B-form marker at 835 $cm^{-1}$ indicates that DNA conformation is not affected in the concentration range 0 and 0.5 M. A critical concentration has been found by us at 100 mM $Mn^{2+}$, at which DNA undergoes a structural transition into a compact form. Altered nucleoside conformations in dC residues are supposed to occur as judging from the marker band of dC near 785 $cm^{-1}$. DNA denaturation was not observed in our Raman spectra.

Raman bands of the bases at 1305, 1336, 1376, 1488 and 1578 $cm^{-1}$ are consistent with metal binding to N7 of guanine [13,18]. To much less extent, binding of $Mn^{2+}$ to adenine is suggested. Unstacking of these bases is indicated in the spectra upon binding of $Mn^{2+}$ to DNA [8].

$Mn^{2+}$ binding to N3 of cytosine is not observed in the whole range of metal ion concentrations used in this study.

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