

Zn²⁺–DNA interactions in aqueous systems: A Raman spectroscopic study

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Abstract. The influence of Zn²⁺ ions on the structure of natural calf thymus DNA was studied by Raman spectroscopy. Measurements were done at room temperature and pH 6.2 ± 0.1, in the presence of 10 mM Na⁺, and of Zn²⁺ in a concentration range varying between 0 and 250 mM, respectively. No condensation of DNA was observed.

As judging from the marker bands near 681 cm⁻¹ (dG), 729 cm⁻¹ (dA), 752 cm⁻¹ (dT), and 787 cm⁻¹ (dC, dT) altered nucleoside conformations in these residues are supposed to occur, in different intervals of Zn²⁺ ions concentration. Changes in the conformational marker centered around 835 cm⁻¹, upon Zn²⁺ binding to DNA, were detected. Binding of zinc(II) ions to the charged phosphate groups of DNA, stabilizing the double helical structure, is indicated in the spectra. We have found that binding of metal ions at N3 of cytosine takes place at zinc(II) concentrations between 150–250 mM and interaction of Zn²⁺ ions with adenine is observed in a concentration range from 10 to 250 mM. Binding of zinc(II) ions to N7 of guanine and, possibly, in a lesser extent to adenine was also observed as indicated by the Raman marker bands near 1490 and 1581 cm⁻¹. 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 Zn²⁺ cations. No evidence for DNA melting was identified.

Keywords: Zn²⁺ 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 [12,18], although DNA–metal cation interactions and their influence on DNA structure have been investigated extensively by a variety of techniques [8,17,18,26]. The active biological role of divalent metal ions (Mt²⁺) in the function of genetic apparatus has been attracting permanent interest in the interaction of these ions with nucleic acids [11].

Particularly, several different effects of zinc(II) ions on the DNA structure have been presented [1,3,4, 9–11,13,28,33,34].

Recently it has been observed that Zn²⁺ can induce the human telomeric sequence AG(3)(T(2)AG(3))(3) to fold the G-quadruplex structure from the random coil. Studies were done by UV absorbance difference spectra and circular dichroism (CD) spectroscopy [33]. The thermodynamic parameters of

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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 investigated [15,16]. These parameters showed that the divalent cation destabilizes the antiparallel G-quartet of d(G4T4G4) [16]. 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 [15,16].

Besides, interaction of zinc ions with d(CGCAATTGCG) in a 2.9 Å resolution X-ray structure has been investigated. It has been found that Zn^{2+} ions are important for DNA packing [28].

Fibres of complexes of polypurine–polypyrimidine with divalent cations have been studied by X-ray diffraction [3]. In the presence of Mg^{2+} , poly(dC) and poly(dG) form a very stable triple helix at neutral pH, based on G–G–C triplexes, whereas Zn^{2+} prevents its formation, both at neutral and acidic pH. The poly(dC) · poly(dG) complex with Zn^{2+} is of the B form. With poly[d(A–G)] · poly[d(C–T)] a different triple helical structure is formed, both with Zn^{2+} and Mg^{2+} [3]. Besides, specific interactions with Zn^{2+} ions in low water activity conditions were found to be necessary to stabilize the parallel intramolecular DNA triple helix with G and T bases in the third strand [13].

It has also been shown that Zn(II) complexes can catalyze the cleavage of supercoiled DNA (pUC 19 plasmid DNA) (Form I) to produce nicked DNA (Forms II and III) with high selectivity [34].

Circular dichroism spectra of poly(dG–dC) in the presence of some zinc complexes exhibit the characteristic inversion associated with the formation of a left handed helix. The transition of B–Z DNA is cooperative and slow [9]. The concentration of zinc complex at the mid point of the transition is strongly dependent upon the nature of the ligand bound to zinc. This leads to questions concerning Z DNA–zinc bound ligand interactions in addition to direct zinc coordination and thus a possible role of zinc proteins in DNA conformational changes [9].

Using methods of IR spectroscopy, light scattering, and gel-electrophoresis, DNA structural transitions were studied under the action of Cu^{2+} , Zn^{2+} , Mn^{2+} , Ca^{2+} and Mg^{2+} in aqueous solution [11]. In this work $CuCl_2$, $CaCl_2$, $MnCl_2$, $MgCl_2$ and $ZnCl_2$ have been used. It has been found that Cu^{2+} , Zn^{2+} , Mn^{2+} and Ca^{2+} ions bind both to DNA phosphate groups and bases, while Mg^{2+} ions bind only to phosphate groups of DNA. Upon interaction with divalent metal ions studied (except for Mg^{2+} ions) DNA undergoes structural transition into a compact form. The mechanism of DNA compaction under Mt^{2+} ion action was found not to be dominated by electrostatics [11].

Different metal complexes of the ribonucleotides 5'-CMP and 5'-GMP have been studied [4]. The chief motive behind this research is the interest provoked by the presence of metal ions as necessary stabilizers of the negative charges of phosphate groups in nucleic acids. The effect that the presence of different metal ions produces on the band principally assigned to the ν_s PO_3^{2-} mode has been studied using FT-IR and FT-Raman spectroscopy [4]. It has been found that the fourth period transition metal ions [Cr(III), Co(II), Cu(II) and Zn(II)] interact directly and indirectly with the phosphate group. Direct phosphate-metal interactions increase as we move to the right of the period. Thus, it was verified that in Zn(II) complexes the proportion of the two types of bonds is approximately the same [4].

Synthesis and crystal structure of two unusual dimeric Zn(II)–cytosine complexes were reported [1]. M–DNA is a type of metalated DNA that forms at high pH and in the presence of Zn, Ni and Co, with the metals placed in between each base pair, as in guanine–Zn–cytosine base pair. A comprehensive ab initio study of eight G–Zn–C models in the gas phase has been done to help discern the structure and electronic properties of Zn–DNA [10].

In our present study, the complex system of calf thymus DNA, in an aqueous buffer solution, is studied by Raman spectroscopy, at different concentrations of Zn^{2+} ions, respectively. Zn^{2+} concentration varied between 0 and 250 mM. DNA structural changes, induced by Zn^{2+} ions are of interest.

2. Experimental section

2.1. Chemicals

Lyophilized fibrous DNA (Type I) from calf thymus (1501 Sigma, Lot Nr. 105K7025) have been used. Zinc acetate dihydrate and Sodium chloride were from Merk, Germany, Bis-(2-hydroxyethyl)-imino-tris-(hydroxymethyl)-methan, in the following Bis-Tris, was from AppliChem, Germany.

2.2. Preparation of ZnDNA complexes

Lyophilized fibrous DNA (Type I) from calf thymus was dissolved in 10 mM Tris, pH 7.0, 150 mM NaCl (20 mg/ml) and sonicated under cooling in 1 min steps. An ultrasonic homogenizer VIBRACELL 75022, Bioblock Scientific, Illkirch, France have been used. Aliquots of 200 μ l from the DNA stock solution were dialyzed at 4°C against 10 mM Bis-Tris buffers, 10 mM NaCl, pH = 6.2 ± 0.1 , containing 0, 10, 50, 100, 150, 200 and 250 mM Zinc acetate dihydrate $(CH_3COO)_2Zn \cdot 2H_2O$, respectively, using dialysis tubes with a 1000 Da molecular mass cut-off (Roth, Karlsruhe, Germany). All samples were centrifuged (1.4×1000 rpm, 2 min) before the Raman measurements, using an Eppendorf 5702 R centrifuge. DNA concentrations were estimated spectrophotometrically with a ND-1000 UV-Vis photometer (NanoDrop products, Wilmington, USA).

2.3. Raman spectroscopy

Sample solutions of approximately 80 μ l were sealed in homemade cylindrical quartz cuvettes. Standard Raman measurements have been done at room temperature, using a KAISER Raman HoloSpec f/1.8i spectrometer. Raman spectra were excited with a 785 nm laser line. HOLOGRAMS software for KAISER spectrometer was used for data acquisition. The laser power at the sample space was about 60 mW. Software package GRAMS (Thermo Galactic, USA) was used to perform Raman spectra analysis. DNA sample solution spectra were corrected by subtraction of the buffer spectrum.

Raman spectra of dissolved DNA have been scaled to give equal intensity in the 1014 cm^{-1} DNA band, assigned to the sugar moiety [8,17], 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 [8,18].

Difference bands will be considered as significant when the intensity of the difference band is at least 2 times higher than the signal-to-noise ratio.

3. Results and discussion

Raman spectra of calf thymus DNA were measured at pH 6.2 ± 0.1 , at a constant sodium salt concentration of 10 mM NaCl and zinc acetate dihydrate $[(CH_3COO)_2Zn \cdot 2H_2O]$ concentrations between 0 and 250 mM.

Figure 1 shows the Raman spectra of sonicated calf-thymus DNA obtained at 0, 10, 50, 100, 150, 200 and 250 mM zinc acetate dehydrate $[(CH_3COO)_2Zn \cdot 2H_2O]$, in the region $550\text{--}1800\text{ cm}^{-1}$. Raman

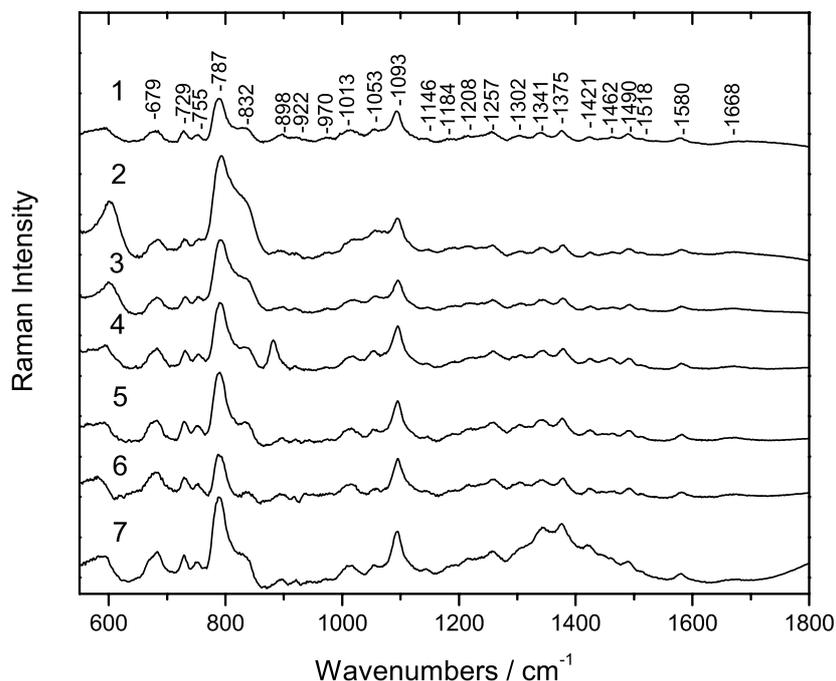


Fig. 1. Raman spectra of sonicated calf thymus DNA in the presence of 10 mM Na^+ , at different Zn^{2+} concentrations: 0 mM (line 1), 10 mM (line 2), 50 mM (line 3), 100 mM (line 4), 150 mM (line 5), 200 mM, (line 6) and 250 mM (line 7). The spectra presented in the region 550–1800 cm^{-1} are background corrected. Peak positions of prominent Raman bands are labeled. The spectra were scaled to have equal intensity in the 1014 cm^{-1} DNA band assigned to the sugar moiety. For all measurements, the laser power at the sample space was about 60 mW. Raman spectra were excited with a 785 nm laser line.

spectra of DNA samples have been scaled to give the same intensity for the 1014 cm^{-1} DNA band, assigned to the sugar moiety [8,17].

Wavenumber positions of the major peaks are given in Fig. 1 and are in accordance with those given previously in the literature [6,18,31,32].

Figure 2 shows Raman difference spectra 1–6 that were obtained by subtraction of the spectrum of dissolved DNA from the corresponding DNA spectra obtained for samples containing Zn^{2+} ions. The negative (troughs) and positive (peaks) bands of the difference spectra indicate zinc(II) dependent changes in the DNA structure, in the presence of 10 mM Na^+ ions.

A detailed comparative analysis of the Raman spectra of calf-thymus DNA, in the presence of different Zn^{2+} ions concentrations is given in Table 1, based on spectra presented in Fig. 1. Proposed Raman band assignments are also included. In some cases, assignments are described in terms of a specific DNA atom or functional group, which makes the related Raman band very useful for recognition of specific DNA-ligand interactions [25].

In the following the Raman spectra will be analyzed in the wavenumber region 600–1150 cm^{-1} that contains information about nucleoside conformation, backbone geometry and PO_2^- interaction [7,8,21, 23,30–32].

The spectral interval 600–800 cm^{-1} contains Raman bands that originate from vibrations involving concerted ring stretching motions (ring breathing) of purine or pyrimidine residues, often in combination with stretching of the glycosidic bond and possibly also stretching of bonds within the linked deoxyribose ring [5]. These bands thus combine the relatively high Raman intensity typical of base residue

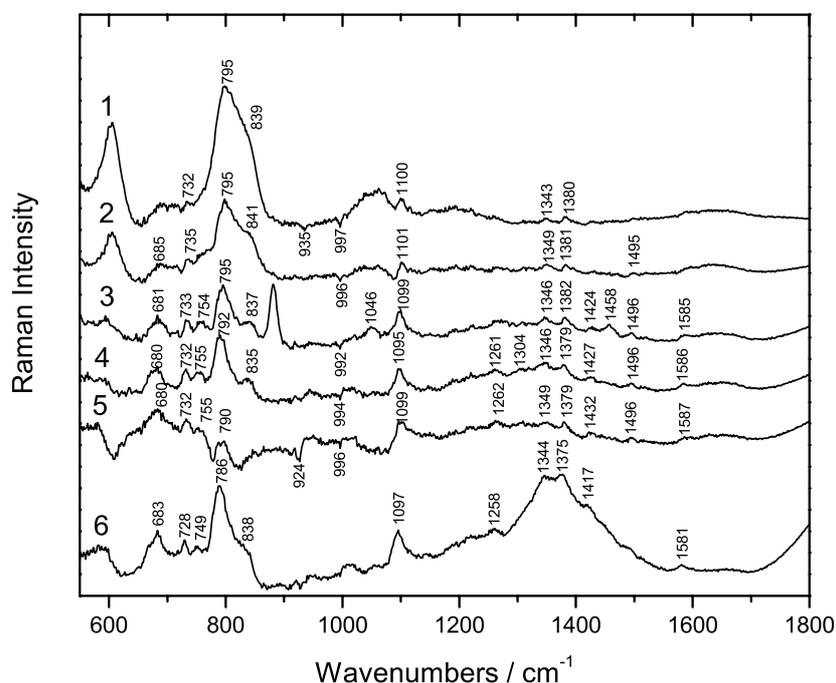


Fig. 2. Raman difference spectra obtained by subtracting the spectrum of dissolved DNA from the corresponding DNA spectrum obtained in the presence of zinc acetate dihydrate, at the following Zn^{2+} ions concentrations, respectively: 10 mM (line 1), 50 mM (line 2), 100 mM (line 3), 150 mM (line 4), 200 mM (line 5) and 250 mM (line 6). All spectra were scaled to have equal intensity in the 1014 cm^{-1} DNA band before subtraction.

vibrations with the sensitivity in wavenumber value expected from changes in glycosyl torsion angle and/or deoxyribose ring pucker ($C2'$ -endo vs $C3'$ -endo) ([5] and references therein).

The $C2'$ -endo-anti nucleoside conformers [7,18,24,30] are identified by the conformation markers at 681 cm^{-1} (dG) [24,27,30], 729 cm^{-1} (dA) [20,24], 752 cm^{-1} (dT) [20,24] and 787 cm^{-1} (dC) [24,27,30]. These bands do respond to the unstacking of bases [19,22].

The strong band at $790 \pm 5\text{ cm}^{-1}$, due to a complex vibration of $5'-C-O-P-O-C3'$ network, is always overlapped by a strong band of either thymine (790 cm^{-1}), or cytosine (780 cm^{-1}), or both, depending upon the DNA base composition [5].

The marker band of B -form DNA backbone and $C2'$ -endo sugar conformations [24,27] is centered around 835 cm^{-1} [20,24,26,27]. This medium intensity band is due to a complex vibrational mode involving the deoxyribose-linked phosphodiester network ($5'-C-O-P-O-C3'$) of B DNA [5].

The band near 1094 cm^{-1} is sensitive to the electrostatic environment of the PO_2^- group [18,24,30].

Raman bands in the wavenumber region $1150\text{--}1720\text{ cm}^{-1}$ are influenced by the electronic structures of the bases and base pairing [8,23,31,32].

The DNA Raman signature is extensively perturbed in the presence of divalent transition metals ([19] and references therein).

Spectral changes are to be observed in Fig. 2 (difference spectra 3–6) at 681 cm^{-1} , the guanine nucleoside marker, suggesting that in the presence of 100–250 mM Zn^{2+} ions specific interactions of the divalent metal ions with the dG residues take place. The intensity of this band has a nonlinear increase in intensity with respect to zinc(II) ions concentration.

Table 1
Raman wavenumbers (cm⁻¹) and tentative assignments of calf thymus DNA in the presence of different Zn²⁺ ions concentrations

0 mM Zn ²⁺	10 mM Zn ²⁺	50 mM Zn ²⁺	100 mM Zn ²⁺	150 mM Zn ²⁺	200 mM Zn ²⁺	250 mM Zn ²⁺	Tentative assignment ^a [5] and references therein
679	682	682	683	682	681	683	dG
729	731	733	732	731	727	729	dA
755	755	752	757	752	752	751	dT
787	794	792	792	792	788	787	dC, dT, 5'-C-O-P-O-C3' network
832	838 sh ^b	839	838	834	838	839	ν OPO
898	892	897		896	893	895	d
922	922	917	920	918	917	921	d
				946	936	946	d
970	971	976	973	974		972	d
1013	1016	1019	1019	1015	1013	1013	dT, dG, dC, d
1053	1055	1059	1053	1055	1056	1055	ν CO
1093	1094	1096	1095	1095	1094	1094	ν PO ₂ ⁻
1146	1145	1146	1145	1149	1145	1143	dT
1184	1189	1185	1191	1188	1181	1182	dG, dT, dC
1208	1216	1218	1215	1219	1214	1214	dC, dT
1257	1258	1258	1258	1257	1260	1258	dA, dC
1302	1306	1306	1306	1304	1304	1303 sh	dA
1341	1340	1345	1345	1341	1341	1345	dA
1375	1377	1376	1375	1377	1377	1377	dT, dA, dC
1421	1423	1424	1423	1421	1423	1421	d(2'-CH ₂ δ), dA
1462	1463		1458	1463	1463	1464	d(5'-CH ₂ δ)
1490	1492	1490	1488	1491	1490	1488	dG (N7), dA
1518	1514	1513	1512	1510	1511	1511	dA
1580	1581	1584	1581	1581	1580	1581	dG, dA
		1667	1672	1667	1674	1669	dT
1690	1686						dG

^a Abbreviations: ν and δ indicate stretching and deformation vibrations, respectively, of the atoms listed; bk indicates a vibration of the DNA backbone; d indicates a vibration localized in the deoxyribose moiety; dA – deoxyadenosine; dG – deoxyguanosine; dC – deoxycytidine; dT – thymidine; vibrations of the deoxynucleosides include modes either localized in the purine or pyrimidine base or delocalized (base plus furanose moieties) [5].

^b sh – shoulder.

The adenine residues marker around 729 cm^{-1} is present in all the difference spectra (Fig. 2, lines 1–6), indicating dA residues interactions with Zn^{2+} ions.

The peak near 752 cm^{-1} which is indicative for the C2'-*endo/anti* conformers of dT [17,20], was found in the difference spectra 3–6 (Fig. 2), suggesting that specific interactions of the Zn^{2+} ions with dT residues take place in the concentration range 100–250 mM.

The band near 787 cm^{-1} [17,24,27,30], characterizing the C2'-*endo-anti* conformation marker of dC and dT, exhibits a drastic increase in intensity in several of our spectra, suggesting altered nucleoside conformations in these residues (Fig. 2, spectra 1–6). The largest intensity increase was observed for this band at 10 mM Zn^{2+} (difference spectrum 1). A shift of the Raman band from 795 cm^{-1} (10 mM Zn^{2+}) to 786 cm^{-1} (250 mM Zn^{2+}) is to be found in the spectra. A decrease in the peak intensity at this wavenumber has been detected upon increasing the zinc(II) ions concentration from 10 to 200 mM Zn^{2+} . This behaviour suggests different intensities of zinc ions interactions with dC and dT residues, with respect to divalent metal ions concentration.

Changes in the conformational marker centered around 835 cm^{-1} [20,24,27,30] have been found in the difference spectra (Fig. 2).

Raman bands at 895 ± 1 , 922 ± 1 , 1053 ± 2 and $1462 \pm 1\text{ cm}^{-1}$ (Fig. 1, Table 1), which are of weak-to-moderate intensity, are confidently assigned to the deoxyribose moiety ([5] and references therein).

The sugar residues of DNA are also expected to be major contributors to the many weak Raman bands observed in the interval $946\text{--}1053\text{ cm}^{-1}$ (Table 1). It is clear that at least two of the bands in this interval (998 and 1016 cm^{-1}) are base-composition dependent [5].

A small trough was detected near 996 cm^{-1} in the difference spectra 1–5 (Fig. 2), indicating weak changes in the deoxyribose moiety upon metal binding to calf-thymus DNA.

Binding of Zn^{2+} ions to DNA phosphate groups (1094 cm^{-1}) is indicated in our spectra. The presence of 10–250 mM Zn^{2+} ions is accompanied by a nonlinear increase in the intensity of the PO_2^- symmetric vibration (Fig. 2, difference spectra 1–6). Electrostatic interactions of the negatively charged phosphate groups with the Zn^{2+} ions, stabilizing the double-helical DNA structure, are probably connected with these changes [18]. 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 [14,18].

Bands in the $1200\text{--}1600\text{ cm}^{-1}$ 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 [8,17,30]. Loss of stacking represents loss of the regularly ordered arrangement among the nucleobases and among sugar-phosphate residues of the backbone [2,19].

A peak near 1258 cm^{-1} , band assigned to dC [17,18], appeared in the difference spectra 4–6 (Fig. 2), indicating that metal ion binding at N3 of cytosine takes place at zinc(II) ions concentration between 150 and 250 mM. This band is shifted to higher wavenumbers upon the divalent metal binding to dC (Fig. 2, difference spectra 4–5). No evidence for DNA melting at GC basepairs regions was identified.

A peak is detected near 1343 cm^{-1} (dA) in the difference spectra 1–6, indicating binding of Zn^{2+} ions to dA residues. Interaction of divalent metal ions with adenine is also indicated by the peak at 1304 cm^{-1} , present in the difference spectra 4–6 (Fig. 2) and seems to be the strongest for 250 mM Zn^{2+} .

Changes in the band near 1380 cm^{-1} , difficult to interpret because it characterizes both the purine (dA) and the pyrimidine (dT, dC) residues [5,12,17,30] were also identified.

A peak detected around 1424 cm^{-1} (Fig. 2, difference spectra 3–6) indicates changes in the $d(2'\text{-CH}_2\delta)$ and dA vibrations.

The peak observed at 1458 cm^{-1} , in the difference spectrum 3 (corresponding to 100 mM Zn^{2+}) is a proof of the interaction of divalent metal ions with $d(5'-CH_2\delta)$.

Metal binding to N7 of guanine and to a lesser extent to N7 of adenine [2,8] started at 50 mM Zn^{2+} ions, as judging by the appearance of the difference bands of the bases near 1490 and 1581 cm^{-1} (Fig. 2). The band at 1581 cm^{-1} is attributed to the purines residues (dG, dA), but is mostly due to the guanine vibration ([29] and references therein). Both bands are shifted upon zinc(II) binding to DNA.

In our Raman spectra of the ZnDNA 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 Zn^{2+} cations [8,18].

No condensation of DNA was visually observed at any of the Zn^{2+} concentrations.

4. Conclusions

The results obtained for Zn^{2+} -DNA aqueous 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.

Difference peaks observed near 681 cm^{-1} , the guanine nucleoside marker, suggest that in the presence of $100\text{--}250\text{ mM Zn}^{2+}$ ions specific interactions of the divalent metal ions with dG residues take place. Interactions of Zn^{2+} ions with dA residues starts at 10 mM divalent metal ions concentration (as judging from the band near 729 cm^{-1}), while interaction of Zn^{2+} ions with dT residues was observed in the range of $100\text{--}250\text{ mM Zn}^{2+}$ (752 cm^{-1}). As judged from the marker band of dC and dT near 787 cm^{-1} , altered nucleoside conformations in these residues are supposed to occur, in the Zn^{2+} concentration range between $10\text{--}250\text{ mM}$. All these bands are related to the unstacking of bases.

Changes in the conformational marker centered around 835 cm^{-1} [20,24,27,30], upon Zn^{2+} binding to DNA, were detected.

Binding of Zn^{2+} ions to DNA phosphate groups (1094 cm^{-1}) is indicated in the spectra.

We have found that divalent metal ion binding at N3 of cytosine takes place at zinc(II) ions concentrations between 150 and 250 mM , as analyzing the Raman peak at 1258 cm^{-1} , assigned to dC [17,18]. Besides, a peak was detected near 1343 cm^{-1} (dA) in the difference spectra, indicating binding of Zn^{2+} ions to the adenine in the concentration range $10\text{--}250\text{ mM Zn(II)}$ ions.

Metal binding to N7 of guanine and to a lesser extent to N7 of adenine [2,8] started at 50 mM Zn^{2+} ions, as judging by the appearance of the difference bands of the bases near 1490 and 1581 cm^{-1} .

In our Raman spectra of the ZnDNA 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 Zn^{2+} cations.

No evidence for DNA melting or DNA condensation was identified.

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