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Molecular dynamics in calf-thymus DNA, at neutral and low pH, in the presence of Na^+, Ca^{2+} and Mg^{2+} ions: A Raman microspectroscopic study

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Abstract. In this paper the Raman total half bandwidths of calf-thymus DNA vibrations have been measured as a function of pH, monovalent and divalent cations' type and concentration. The dependence of different band parameters on DNA molecular subgroup structure, on pH and on Na^+, Ca^{2+} and Mg^{2+} ions concentrations, respectively, are reported. It is shown that changes in (sub)picosecond dynamics of molecular subgroups in calf-thymus DNA can be monitored with confocal Raman microspectroscopy.

The half bandwidths and the global relaxation times for the vibrations at 728 cm^{-1} (dA), 785 cm^{-1} (dC), 1094 cm^{-1} (PO_2^-), 1377 cm^{-1} (dA, dG, dT, dC), 1488 cm^{-1} (dG, dA) and 1580 cm^{-1} (dG, dA) of calf-thymus DNA are presented. The full-widths at half-height (FWHH) of the bands in calf-thymus DNA are typically in the wavenumber range from 7.4 to 31 cm^{-1}. The bandwidths in the Raman spectra are sensitive to a dynamics active on a time scale from 0.34 to 1.44 ps.

Low pH-induced melting of double helical structure in calf-thymus DNA results for some bands in shorter global relaxation times, as a consequence of the increased interaction of the base moieties with the solvent molecules.

The molecular dynamics characterizing the 785, 1094, 1377 and 1580 cm^{-1} vibrations, is faster in the case of high divalent cations DNA sample (pH 7), as compared to the respective low divalent cations DNA sample (pH 7), for both Ca^{2+} and Mg^{2+} ions. The vibrational energy transfer process of the guanine band at 1488 cm^{-1} is slower for the high salt DNA sample, pH 7 as compared to the corresponding low salt DNA sample, pH 7, for both Ca^{2+} and Mg^{2+}. Molecular dynamics characterizing the vibration at 1488 cm^{-1} is faster for DNA sample at high Na^+ ions (pH 7), as compared to the DNA sample at low Na^+ ions (pH 7).

As far as the CaDNA and MgDNA complexes are concerned (pH 7), the global relaxation times of some base vibrations decrease for the case of magnesium ions, as compared to the case of the same concentration of calcium ions. The different ionic radius of the two types of metal cations (0.72 Å for Mg and 0.99 Å for Ca) were considered in explaining these results.

Molecular relaxation processes of DNA subgroups, upon lowering the pH, in the presence of Na^+, Ca^{2+} and Mg^{2+} ions are presented. Particularly, at low Ca^{2+} concentration, upon lowering the pH, the molecular dynamics of DNA subgroups corresponding to vibrations at 728, 1376, 1488 and 1580 cm^{-1} is much faster, probably due to the denaturation process of the double helical DNA.

Keywords: (Sub)picosecond dynamics, calf-thymus DNA, monovalent and divalent cations, pH, Raman microspectroscopy

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1. Introduction

Infrared and Raman spectroscopies offer the most significant information concerning the behaviour of a condensed system in the picosecond timescale ([1] and references therein). Among the techniques available for the study of molecular dynamics, Raman scattering has the distinct advantage of simultaneously analyzing both reorientational and vibrational relaxation processes ([1,2] and references therein).

Macromolecular motion in fluids is generally too slow to be observed in the Raman time window that is accessible in the frequency domain ($\sim$0.1 ps to $\sim$8 ps). In principle motion of molecular subgroups can be fast enough [3].

Raman spectra of complex molecules do not consist of a set of sharp lines, as time-dependent forces broaden the vibrational bands [4]. Dynamic parameters of atomic and molecular motions determine the vibrational band shapes. A number of processes have been considered to broaden the vibrational bands in the frequency domain ([4] and references therein).

In a chosen nucleic acid, the structure can be influenced by the presence of proteins, ionic salts, pH, metal ions and intercalators. Different vibrational bands in one molecule can behave quite different when the structure of the nucleic acid is changed [5]. Many dynamical processes, such as vibrational energy exchange, vibrational resonance coupling, vibrational dephasing and rotational broadening may occur in a nucleic acid aqueous system in the (sub)picosecond time domain, which may contribute to the bandwidth of the vibrational modes. Different vibrations may be sensitive to different relaxation mechanisms, and this kind of information is available from spontaneous Raman measurements ([4] and references therein).

It was shown that the Raman bandwidths in polynucleotides range from 8 wavenumbers to 35 wavenumbers, and the corresponding time scale of the perturbing forces ranges from fractions of a picosecond to several picoseconds [4].

Isotropic and anisotropic spontaneous Raman spectra were obtained from solutions of poly(rA) and rAMP in buffer [6]. The temperature dependence of these spectra was measured to elucidate the influence of macromolecular dynamics and solvent dynamics on the bandwidths of base vibrations in the single stranded polynucleotide poly(rA) [6].

Particularly, phosphate groups are very important in the structure, dynamics and interactions of mono- and polynucleotides [7,8]. The symmetric stretching mode of this group, ν_s (PO_3^{2-}), gives rise to an IR band whose band shape parameters have been shown to be sensitive to concentration and temperature effects [8]. The IR ν_s (PO_3^{2-}) band shape of cytidine 5′-monophosphate (5′-CMP) in H_2O solution at different concentrations, has been studied and the results have been interpreted in terms of the dynamics of the PO_3^{2-} group. It has been found that the relaxation of this mode in aqueous solution seems to be predominantly vibrational [8]. Besides, the analysis of the ν_s (PO_3^{2-}) FTIR band shape of 5′-CMP in ^2H_2O solutions at different concentrations and temperatures, has been done in terms of molecular dynamics and self-association processes of this mononucleotide. A possible aggregation process of 5′-CMP has been detected from the second derivative and the integrated intensity of the band [9].

A band-shape analysis of the IR ν_s (PO_3^{2-}) band of disodium deoxycytidine 5′-monophosphate, 5′-dCMP, in ^2H_2O and H_2O has been performed in relation to the relaxation processes of this vibrational mode [10]. The second derivative spectra reveal the presence of 5′-dCMP aggregates when concentration reaches $\sim$0.28 mol dm^{-3} [10].

In this paper, the complex system of calf-thymus DNA, in an aqueous buffer solution, is studied by confocal Raman microspectroscopy, in the presence of monovalent and divalent cations, at high and low pH. Monitoring the changes in the full-widths at half-height (FWHH) and, correspondingly, in the

global relaxation time of the molecular subgroups in DNA, upon lowering the pH and changing the type and concentration of the metal ion, is of interest.

For meaningful measurements to be made on living cells components, it is of the utmost importance to use a very sensitive Raman microspectrometer. This makes it possible to reach the small measuring volumes that are necessary to obtain biologically significant results [11,12].

2. Experimental

The experimental details were given in [13] for reduced and low pH DNA complexes, in [11] for CaDNA complexes, and in [12] for NaDNA and MgDNA complexes. Reduced and low pH DNA complexes were obtained in the presence of 150 mM NaCl and 5 mM $MgCl_2 \cdot 6\ H_2O$. CaDNA and MgDNA complexes were studied in the presence of 10 mM NaCl, respectively. The Raman spectra were recorded with a confocal Raman microspectrometer at the Department of Applied Physics, University of Twente, Enschede, The Netherlands and are presented elsewhere [11–13].

The spectra were processed by means of the software package RAMPAC [14]. Each measurement on a DNA sample was followed by a second one (background signal measurement) just next to that of the DNA complex, in order to determine the signal contributions from the buffer, which were then subtracted from the resulting DNA sample spectrum [12].

Peak positions and FWHH of the bands were determined using SpectraCalc software. The FWHHs were evaluated from the half maximum Raman bands.

3. Results and discussions

In the study of the molecular relaxation processes [15], one of the well-known procedures of obtaining the relaxation times and the activation energy was developed by Rakov [16].

The total half bandwidth of the depolarized Raman lines is consisting on the following contributions:

– an intrinsic bandwidth, δ_0, considered temperature independent in that time;
– another contribution $\Delta(T)$ temperature dependent.

The corresponding relationship can be written as:

$$\Delta\nu_{1/2} = \delta_0 + \Delta(T) = \delta_0 + \frac{1}{\pi c \tau_{\mathrm{r}}}. \tag{1}$$

One can determine the potential barrier against reorientation:

$$\tau_{\mathrm{r}} = \tau_0 \exp\left(\frac{U_{\mathrm{or}}}{kT}\right), \tag{2}$$

where τ_0 is the period of the molecule oscillation around the equilibrium position, and U_{or} is the energy barrier or the activation energy.

The Rakov relationship can be written as:

$$\Delta\nu_{1/2} = \delta_0 - \frac{1}{\pi c \tau_0} \exp\left(\frac{-U_{\mathrm{or}}}{kT}\right). \tag{3}$$

From the $(\Delta_{1/2} - \delta_0)$ *vs* $\frac{10^3}{T}$ dependencies one can obtain U_{or} as the slope of this linear dependence.

The temperature "independent" part, due to the vibrational relaxation, δ_{v}, presents small temperature dependence, reversal as the one due to the reorientational relaxation. The vibrational contribution becomes important for large molecules, in aqueous solutions. Using Raman measurements with polarized light, it is possible to do the selection of these two contributions. One can assume into a first approximation, the existence of a global relaxation time, τ, obtained from the total Raman half bandwidth. This parameter can be related with the intrinsic parameters of the analyzed system through the relationship:

$$\tau_{\text{v,1R,2R}} = \frac{1}{\pi c \Delta\nu_{1/2}^{\text{v,1R,2R}}}, \tag{4}$$

where the half bandwidth includes the vibrational ($\Delta\nu_{1/2}^{\text{v}}$) and rotational ($\Delta\nu_{1/2}^{\text{1R,2R}}$) contributions and c is the velocity of light. $\Delta\nu_{1/2}^{\text{1R,2R}}$ is obtained from IR and Raman bands, respectively.

The dominant contribution of one or another molecular relaxation process can be controlled through:

(a) the selection of the molecular system; in the case of large molecules, solved in polar media (e.g. water), one can assume that the vibrational relaxation dominates (the most efficient); one can neglect the reorientational contribution, being a very slow molecular motion;

(b) the selection of the parameters, e.g. temperature dependence, one can separate these contributions; with the temperature increase, the half bandwidth increases, see Eq. (1); if one observe a weak temperature dependence or even a decrease of the half bandwidth with the temperature increase, one can speak about the dominant contribution of the vibrational relaxation;

(c) a corresponding selection of the solvents, e.g. in strong polar media, the vibrational contribution is dominant; in the case of inert, non-polar solvent media, on the contrary, the rotational relaxation must be taken into account.

By using these approximations, molecular dynamics studies for mononucleotides [9] or deoxymononucleotides [8] in aqueous solutions were done.

The development of fast and accurate curve fitting programs allows the analysis of the vibrational spectra of complicated biological molecules containing often more than 40 vibrational bands ([4] and references therein). In this paper we will concentrate on the vibrational bandwidths. Only the relatively isolated nucleic acids vibrations are considered.

A study into the Raman vibrational bandwidths of molecular subgroups in calf-thymus DNA, upon lowering the pH and in the presence of Na^+, Ca^{2+} and Mg^{2+} ions, respectively, is of interest (see Tables 1–8). It is shown that changes in (sub)picosecond dynamics of molecular subgroups in calf-thymus DNA can be monitored with confocal Raman microspectroscopy.

For the case of aqueous solutions of DNA molecules we can suppose that mainly, the dominant relaxation mechanism is the vibrational one. The values of the global relaxation time suggest also the existence of a vibrational relaxation time, because the reorientational movement is much more slower for the DNA macromolecule in aqueous solution. Particularly, the absence of reorientational broadening in polynucleotides indicates that the bases in polynucleotides reorient through an angle of 41° in times slower than 21 ps ([4] and references therein).

3.1. DNA complexes at reduced and low pH values

The Raman band parameters obtained for the adenine vibration at 728 cm^{-1} [11,17–19], the cytosine ring breathing mode at 785 cm^{-1} [17,20–22], the DNA backbone PO_2^- symmetric stretching vibration at

Table 1
pH dependent half bandwidths (cm^{-1}) of different vibrations in calf-thymus DNA

pH	Vibrational modes, ν (cm^{-1})					
	728 (A)	785 (C)	1094 (PO_2^-)	1377 (A, G, T, C)	1488 (G, A)	1580 (G, A)
	FWHH, $\Delta\nu_{1/2}$ (cm^{-1})					
2.10	11.6	21.9	27.1	–	14.2	–
2.35	11.6	24.5	21.9	–	14.4	–
2.82	12.9	21.9	20.6	–	14.8	–
3.10	10.9	25.3	22.2	18.4	16.4	15.8
3.45	10.2	25.3	20.2	31.0	15.7	28.4
3.79	11.0	22.1	21.9	23.2	15.7	21.9
4.40	11.0	25.4	21.4	20.6	15.6	18.1
6.00	10.7	25.1	18.8	20.6	14.7	16.8
6.83	11.2	24.9	21.0	21.0	15.2	18.4

Table 2
pH dependent global relaxation times, for molecular subgroups in calf-thymus DNA

pH	Vibrational modes, ν (cm^{-1})					
	728 (A)	785 (C)	1094 (PO_2^-)	1377 (A, G, T, C)	1488 (G, A)	1580 (G, A)
	Relaxation time, τ (sec $\times 10^{-13}$)					
2.10	9.14	4.84	3.92	–	7.48	–
2.35	9.13	4.33	4.85	–	7.37	–
2.82	8.24	4.85	5.15	–	7.17	–
3.10	9.74	4.19	4.78	5.77	6.47	6.72
3.45	10.39	4.20	5.15	3.42	6.76	3.74
3.79	9.64	4.80	4.85	4.58	6.76	4.85
4.40	9.64	4.18	4.96	5.15	6.81	5.87
6.00	9.94	4.24	5.65	5.15	7.22	6.32
6.83	9.50	4.27	5.06	5.06	6.98	5.77

1094 cm^{-1} [17,21,23], the purine (dA, dG) and pyrimidine (dT, dC) residues band at 1377 cm^{-1} [19–21], the guanine (N-7) and adenine rings vibration at 1488 cm^{-1} [17,19] and the purines (dG, dA) 1580 cm^{-1} vibration ([24] and references therein) of calf-thymus DNA, at different pH values are summarized in Table 1 and Table 2, respectively. We have marked in the tables the cases for which the Raman half bandwidths could not be read.

The full-widths at half-height (FWHH) of the bands in calf-thymus DNA have been measured at 9 different pH values and are typically in the wavenumber range from 10.2 to 31 cm^{-1} (see Table 1).

Besides, the global relaxation times were evaluated on the basis of Eq. (4). From the vibrations at 728, 785, 1094, 1377, 1488 and 1580 cm^{-1} it can be observed that the global relaxation times, for molecular subgroups in dissolved calf-thymus DNA are slower than 0.34 and faster than 1.04 ps (see Table 2). The bandwidths in the Raman spectra are sensitive to dynamics active on a time scale from 0.1 to 10 ps [4].

Figures 1–5 present the global relaxation times of molecular subgroups vibrations in calf-thymus DNA, as a function of pH, for the modes at 728 cm^{-1} (dA), 1094 cm^{-1} (PO_2^-), 1377 cm^{-1} (dA, dG, dT, dC), 1488 cm^{-1} (dG, dA) and 1580 cm^{-1} (dG, dA), respectively. Low pH-induced DNA structural changes are responsible for their behaviour.

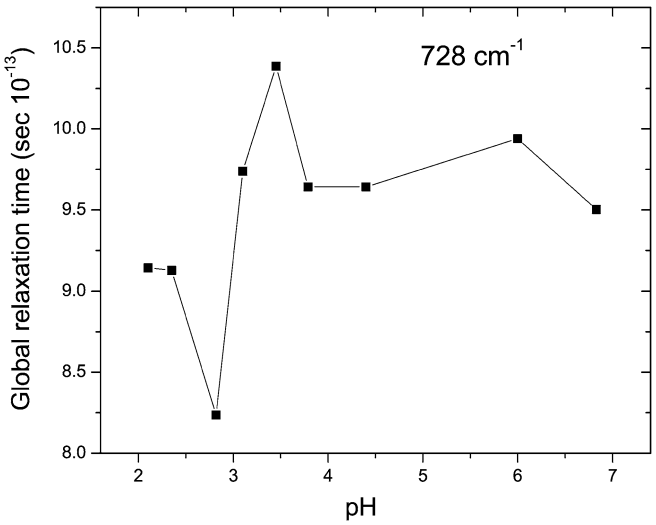

Fig. 1. Global relaxation time of the adenine vibration at 728 cm^{-1} in calf-thymus DNA, as a function of pH.

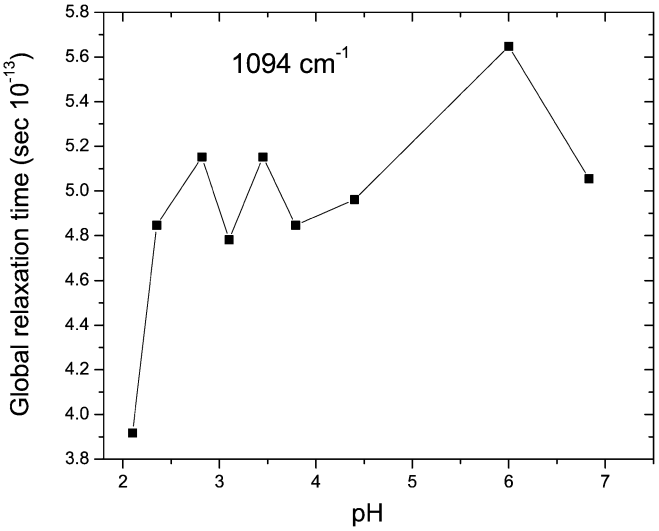

Fig. 2. Global relaxation time of the DNA backbone PO_2^- symmetric stretching vibration at 1094 cm^{-1} in calf-thymus DNA, as a function of pH.

Low pH-induced melting of double helical structure in calf-thymus DNA results for some vibrations in smaller global relaxation times, and larger bandwidths, respectively, as a consequence of the increased interaction of the base moieties with the solvent molecules. This behaviour is most evident for the bands at 1377, 1488, 1580 cm^{-1} and partially confirmed for the adenine band at 728 cm^{-1}. For this last band, the global relaxation time increases at 1.04 ps for the pH value 3.45, before starting to decrease. The vibrational energy transfer processes are the most rapid for the adenine 728 cm^{-1} vibration around the pH 2.82 (global relaxation time 0.82 ps), for the vibration near 1377 cm^{-1} at pH 3.45 (global relaxation time 0.34 ps), for the guanine band around 1488 cm^{-1} at pH 3.1 (global relaxation time 0.65 ps) and for the band around 1580 cm^{-1} at pH 3.45 (global relaxation time 0.37 ps). Upon lowering the pH

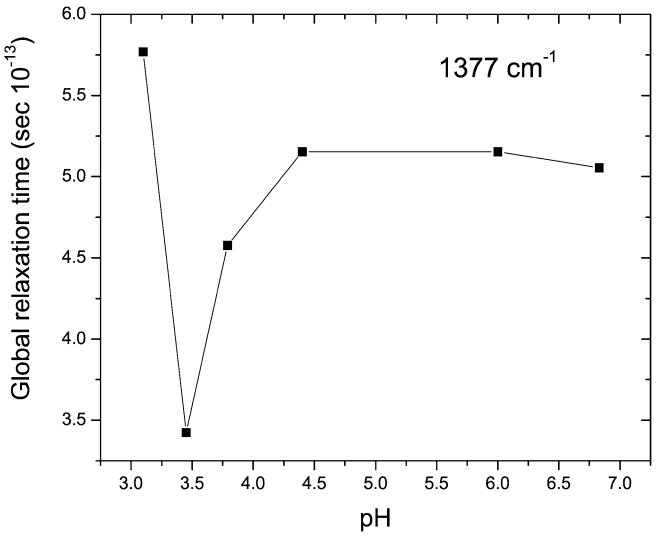

Fig. 3. Global relaxation time characteristic to the purine (dA, dG) and pyrimidine (dT, dC) residues band at 1377 cm^{-1} in calf-thymus DNA, as a function of pH.

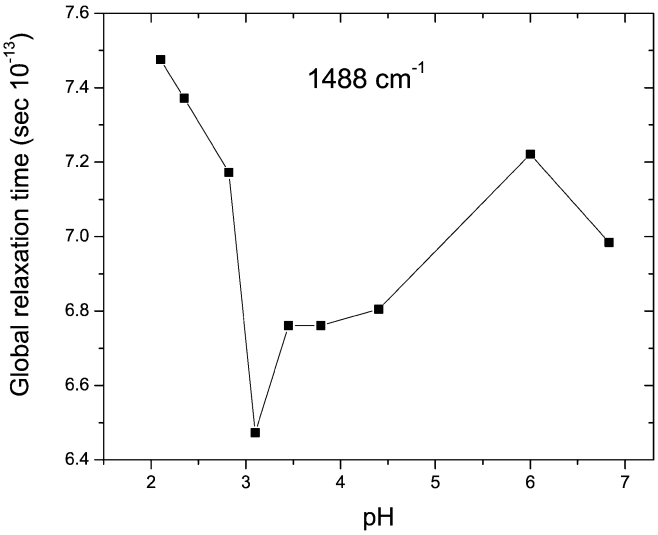

Fig. 4. Global relaxation time of the guanine (N-7) and adenine rings vibration at 1488 cm^{-1} in calf-thymus DNA, as a function of pH.

under these values, the global relaxation times increase at the highest values for the bands at 1377, 1488 and 1580 cm^{-1} and this slower dynamics is probably due to the aggregation and sedimentation of calf-thymus DNA complexes, in association with acidic melting of the double helix [13]. An increase of the global relaxation time is also observed upon DNA protonation at very low pH for the adenine vibration at 728 cm^{-1}, but the respective values are not so high as compared to those of the bases bands.

Apart from the data above, the global relaxation time of the band near 1094 cm^{-1} of the DNA backbone PO_2^- symmetric stretching vibration [13], has a tendency to decrease upon decreasing the pH. The fastest dynamics was observed for this band at pH 2.1 (global relaxation time 0.39 ps) and this is in con-

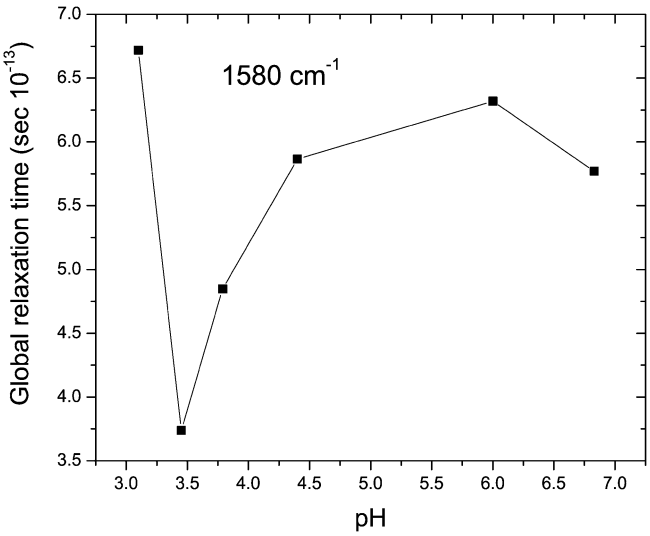

Fig. 5. Global relaxation time of the purines (dG, dA) 1580 cm^{-1} vibration in calf-thymus DNA, as a function of pH.

tradiction with the behaviour of the relaxation processes characterizing the DNA bases bands at 1377, 1488 and 1580 cm^{-1}. In this study the Raman band parameters of the cytosine ring breathing mode at 785 cm^{-1} did not show a definite behaviour [13].

Previously, it was established [4] that the bandwidths of the 1336, 1480 and 1575 cm^{-1} adenine vibrations increase toward the value for the mononucleotides upon thermal melting of poly(rA). This temperature behaviour of the bandwidths of poly(rA) were assigned to an increasing exposure of the bases to the solvent upon melting of the secondary structure. Moreover all the studied adenine, thymine, and uracil vibrations have a smaller bandwidth in stacked structures than in unstacked structures and mononucleotides [4].

3.2. NaDNA, CaDNA and MgDNA complexes

The half bandwidths of calf-thymus DNA vibrations have also been measured at neutral (pH 7) and low (pH 3), for NaDNA, CaDNA and MgDNA complexes (Tables 3, 5, 7). For each cation type, two metal ions concentrations were considered. The corresponding global relaxation times evaluated on the basis of Eq. (4) are presented in the Tables 4, 6, 8. We have marked in the tables the vibrations for which we were not able to measure the Raman half bandwidths in calf-thymus DNA.

This choice of low pH was based on the observation that the midpoint of transition of Watson–Crick GC base pairs to protonated GC base pairs lies at around pH 3 (analyzing the guanine 681 cm^{-1} line from the Raman spectrum of calf-thymus DNA) [12,17].

The studied bandwidths are in the wavenumber range 7.4–28.6 cm^{-1}. The limit values of this interval were obtained for CaDNA vibrations. The molecular relaxation process has a global relaxation time smaller than 1.44 ps and larger than 0.37 ps.

The molecular relaxation processes are faster for the bands at 785, 1094 and 1488 cm^{-1}, in the case of DNA sample at 2.01 M Na^{+} (pH 7) as compared to the DNA sample at only 10 mM Na^{+} (pH 7), respectively. At 500 mM Ca^{2+}, pH 7 the characteristic global relaxation times of DNA molecular subgroups decrease for the bands at 728, 785, 1094, 1376 and 1580 cm^{-1}, as compared to those corresponding to the DNA sample at 5 mM Ca^{2+}, pH 7. Besides, the vibrational energy transfer is faster for the 785,

Table 3
Half bandwidths (cm^{-1}) of different vibrations in calf-thymus DNA, in the presence of Na^+ ions

Metal ion, pH	Vibrational modes, ν (cm^{-1})					
	728 (A)	785 (C)	1094 (PO_2^-)	1377 (A, G, T, C)	1488 (G, A)	1580 (G, A)
	FWHH, $\Delta\nu_{1/2}$ (cm^{-1})					
2.01 M Na^+, pH 7	–	27.3	22.1	23.7	19.5	18.2
10 mM Na^+, pH 7	–	26.0	16.5	24.7	16.3	18.3
2.01 M Na^+, pH 3	–	9.8	–	–	–	–
10 mM Na^+, pH 3	–	20.1	19.9	28.3	16.8	–

Table 4
Global relaxation times for molecular subgroups in calf-thymus DNA, in the presence of Na^+ ions

Metal ion, pH	Vibrational modes, ν (cm^{-1})					
	728 (A)	785 (C)	1094 (PO_2^-)	1377 (A, G, T, C)	1488 (G, A)	1580 (G, A)
	Relaxation time, τ (sec $\times 10^{-13}$)					
2.01 M Na^+, pH 7	–	3.89	4.80	4.48	5.44	5.83
10 mM Na^+, pH 7	–	4.08	6.43	4.30	6.51	5.80
2.01 M Na^+, pH 3	–	10.89	–	–	–	–
10 mM Na^+, pH 3	–	5.27	5.33	3.75	6.32	–

Table 5
Half bandwidths (cm^{-1}) of different vibrations in calf-thymus DNA, in the presence of Ca^{2+} ions

Metal ion, pH	Vibrational modes, ν (cm^{-1})					
	728 (A)	785 (C)	1094 (PO_2^-)	1376 (A, G, T, C)	1488 (G, A)	1580 (G, A)
	FWHH, $\Delta\nu_{1/2}$ (cm^{-1})					
500 mM Ca^{2+}, pH 7	11.3	27.3	24.9	20.8	17.9	18.0
5 mM Ca^{2+}, pH 7	7.4	26.9	20.4	19.8	18.7	17.1
500 mM Ca^{2+}, pH 3	10.8	25.5	22.6	20.8	18.3	18.2
5 mM Ca^{2+}, pH 3	10.00	23.4	19.6	28.6	19.6	18.2

1094, 1377 and 1580 cm^{-1} vibrations, in the case of DNA sample in the presence of 500 mM Mg^{2+}, pH 7 as compared to that of the respective vibrations of DNA molecule in the presence of 5 mM Mg^{2+}, pH 7. This behaviour is not similar with that of the band parameters characterizing the guanine vibration at 1488 cm^{-1}, in the case of the same CaDNA and MgDNA complexes, respectively.

Comparing the results obtained for the DNA sample at 500 mM Ca^{2+}, pH 7 with those corresponding to DNA sample at 500 mM Mg^{2+}, pH 7 the global relaxation times of the base vibrations at 1377, 1488, 1580 cm^{-1} decrease for the case of magnesium ions, as compared to the case of calcium ions. These results might be explained by the different ionic radius of the two types of metal cations (0.72 Å for Mg and 0.99 Å for Ca). The same observation is valid for the vibrations at 1376 and 1488 cm^{-1}, in the cases of DNA samples at 5 mM Ca^{2+}, pH 7 and at 5 mM Mg^{2+}, pH 7, respectively. On the contrary, the global relaxation time of the DNA backbone PO_2^- symmetric stretching vibration at 1094 cm^{-1} increases for MgDNA complexes at pH 7 as compared to CaDNA complexes at pH 7, at both low and high divalent cations concentrations, respectively.

Table 6
Global relaxation times for molecular subgroups in calf-thymus DNA, in the presence of Ca^{2+} ions

Metal ion, pH	Vibrational modes, ν (cm^{-1})					
	728 (A)	785 (C)	1094 (PO_2^-)	1376 (A, G, T, C)	1488 (G, A)	1580 (G, A)
	Relaxation time, τ (sec $\times 10^{-13}$)					
500 mM Ca^{2+}, pH 7	9.43	3.89	4.26	5.10	5.93	5.90
5 mM Ca^{2+}, pH 7	14.35	3.95	5.20	5.36	5.68	6.21
500 mM Ca^{2+}, pH 3	9.87	4.16	4.70	5.10	5.80	5.83
5 mM Ca^{2+}, pH 3	10.66	4.54	5.42	3.71	5.42	5.06

Table 7
Half bandwidths (cm^{-1}) of different vibrations in calf-thymus DNA, in the presence of Mg^{2+} ions

Metal ion, pH	Vibrational modes, ν (cm^{-1})					
	728 (A)	785 (C)	1094 (PO_2^-)	1377 (A, G, T, C)	1488 (G, A)	1580 (G, A)
	FWHH, $\Delta\nu_{1/2}$ (cm^{-1})					
500 mM Mg^{2+}, pH 7	–	27.0	19.5	26.0	20.8	23.4
5 mM Mg^{2+}, pH 7	–	24.3	16.9	20.8	22.2	16.9
500 mM Mg^{2+}, pH 3	–	26.0	19.9	25.8	25.0	18.3
5 mM Mg^{2+}, pH 3	–	23.4	–	–	–	–

Table 8
Global relaxation times for molecular subgroups in calf-thymus DNA, in the presence of Mg^{2+} ions

Metal ion, pH	Vibrational modes, ν (cm^{-1})					
	728 (A)	785 (C)	1094 (PO_2^-)	1377 (A, G, T, C)	1488 (G, A)	1580 (G, A)
	Relaxation time, τ (sec $\times 10^{-13}$)					
500 mM Mg^{2+}, pH 7	–	3.93	5.44	4.08	5.10	4.54
5 mM Mg^{2+}, pH 7	–	4.36	6.28	5.10	4.78	6.28
500 mM Mg^{2+}, pH 3	–	4.08	5.33	4.12	4.25	5.80
5 mM Mg^{2+}, pH 3	–	4.54	–	–	–	–

Because of the inhibition of DNA protonation at pH 3, by high concentration of Ca^{2+} ions, the global relaxation times of the base vibrations at 1376, 1488 and 1580 cm^{-1} are not modified too much as compared to those corresponding to the bands of DNA sample at high calcium, pH 7. For the same high Ca^{2+} concentration, the global relaxation times corresponding to DNA vibrations at 728, 785 and 1094 cm^{-1} increase upon DNA protonation. Besides, at low Ca^{2+} concentration, upon lowering the pH, the molecular dynamics of DNA subgroups corresponding to the bands at 728, 1376, 1488 and 1580 cm^{-1} is much faster, probably due to the denaturation process of the double helical DNA. Also in the same case the global relaxation times characterizing the bands at 785 and 1094 cm^{-1} increase upon DNA protonation.

4. Conclusions

Spontaneous Raman scattering can be used to study the fast dynamics of molecules [3]. This paper presents a confocal Raman microspectroscopic study into the vibrational half bandwidths of molecular

subgroups in calf-thymus DNA, upon lowering the pH, and in the presence of Na^+, Ca^{2+} and Mg^{2+} ions, respectively. Besides, the corresponding global relaxation times have been derived. The Raman band parameters were obtained for the modes at 728 cm^{-1} (dA), 785 cm^{-1} (dC), 1094 cm^{-1} (PO_2^-), 1377 cm^{-1} (dA, dG, dT, dC), 1488 cm^{-1} (dG, dA) and 1580 cm^{-1} (dG, dA) of calf-thymus DNA.

The study of vibrational half bandwidths of calf-thymus DNA revealed a sensitivity of these bandwidths to the pH value and to the type and concentration of monovalent and divalent cations, respectively. Moreover this proved to be dependent on the vibration under study.

The Raman half bandwidths of calf-thymus DNA vibrations reveal a dynamic picture on a (sub)picosecond time scale. The full-widths at half-height (FWHH) of the bands in calf-thymus DNA are typically in the wavenumber range from 7.4 to 31 cm^{-1}. It can be observed that the global relaxation times studied in this work, for molecular subgroups in dissolved calf-thymus DNA, are slower than 0.34 and faster than 1.44 ps.

As far as the DNA complexes at reduced and low pH values are concerned the best vibrational energy transfer processes were obtained for the adenine 728 cm^{-1} band around the pH 2.82 (global relaxation time 0.82 ps), for the vibration near 1377 cm^{-1} at pH 3.45 (global relaxation time 0.34 ps), for the guanine band around 1488 cm^{-1} at pH 3.1 (global relaxation time 0.65 ps) and for the band around 1580 cm^{-1} at pH 3.45 (global relaxation time 0.37 ps).

Low pH-induced melting of double helical structure in calf-thymus DNA results for some bands in smaller global relaxation times, and larger bandwidths, respectively, as a consequence of the increasing interaction of the base moieties with the solvent molecules.

For the NaDNA, CaDNA and MgDNA complexes, the studied bandwidths are in the wavenumber range 7.4–28.6 cm^{-1}. The limit values of this interval were obtained for CaDNA vibrations. The molecular dynamics has a global relaxation time between 0.37–1.44 ps.

Vibrational energy transfer process is faster for the 785, 1094, 1377 and 1580 cm^{-1} vibrations, in the case of high divalent cations DNA sample (pH 7), as compared to the respective low divalent cations DNA sample (pH 7), for both Ca^{2+} and Mg^{2+} ions. The molecular dynamics, characterizing the guanine band at 1488 cm^{-1} for the high salt DNA sample, pH 7 as compared to the corresponding low salt DNA sample, pH 7, is similar for the Ca^{2+} and Mg^{2+} ions and different for the Na^+ ions.

As far as the CaDNA and MgDNA complexes are concerned (pH 7), the global relaxation times of some base vibrations decrease for the case of magnesium ions, as compared to the case of the same concentration of calcium ions. The different ionic radius of the two types of metal cations (0.72 Å for Mg and 0.99 Å for Ca) were considered in explaining these results.

The molecular relaxation processes of DNA subgroups, upon lowering the pH, in the presence of Na^+, Ca^{2+} and Mg^{2+} ions are presented. Particularly, at low Ca^{2+} concentration, upon lowering the pH, the molecular dynamics of DNA subgroups corresponding to vibrations at 728, 1376, 1488 and 1580 cm^{-1} is much faster, probably due to the denaturation process of the double helical DNA.

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