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Raman microspectroscopic study on low-pH-induced DNA structural transitions in the presence of magnesium ions

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Low-pH-induced DNA structural changes were investigated in the pH range 6.8–2.10 by Raman microspectroscopy. Measurements were carried out on calf thymus DNA in the presence of low concentrations of Mg^{2+} ions. Vibrational spectra are presented in the wavenumber region 500–1650 cm^{-1}. Large changes in the Raman spectra of calf-thymus DNA were observed on lowering the pH value. These are due to protonation and unstacking of the DNA bases during DNA melting and also to changes in the DNA backbone conformation. The intensities of the Raman bands of guanine (681 cm^{-1}), adenine (728 cm^{-1}), thymine (752 cm^{-1}) and cytosine (785 cm^{-1}), typical of the C2′-*endo-anti* conformation of B-DNA, are discussed. The B-form marker near 835 cm^{-1} and the base vibrations in the higher wavenumber region (1200–1680 cm^{-1}) are analysed. Effects of low pH value upon the protonation mechanism of opening AT and changing the protonation of GC base pairs in DNA are discussed.

INTRODUCTION

The possibility that DNA may contain 'conformational signals' in addition to 'sequence signals' was raised several years ago.[1] The B-form, the canonical right-handed double helix, is the predominant DNA conformation in chromosomes and in the nucleus of the eukaryotic cells. Alternative DNA conformations have also attracted much interest, owing to their relevance to biological function.[2] Among them, protonated DNA structures are of particular importance. Studies are required to elucidate whether the equilibrium between protonated and non-protonated AT and GC base pairs can be influenced by chemical parameters (e.g. pH, counterions).

There is no general consensus in the literature about the precise nature of the changes that occur in DNA as the pH is lowered. It seems that a subtle interplay between pH and counterion concentration may result in appreciable changes in DNA conformation.[3]

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In this work, Raman microspectroscopy was used to study the low-pH-induced DNA structural changes in the pH range 6.8–2.1 in the presence of low concentrations of Mg^{2+} cations. Particular attention paid to the opening mechanism of protonation of AT base pairs, changing the protonation of GC base pairs and unstacking of GC and AT base pairs during DNA melting. Experiments with calf thymus DNA in the presence of sodium ions in the wavenumber range 650–850 cm^{-1} at pH values from 7.4 to 2.8 were reported by Puppels *et al*.[2] For meaningful measurements to be made on living cell components, it is of the utmost importance to use a very sensitive Raman microspectrometer. This makes it possible to reach the small measuring volumes which are necessary to obtain biologically significant results.

Measurements were carried out in the Department of Applied Physics, University of Twente, Enschede, The Netherlands.

EXPERIMENTAL

Preparation of MgDNA complexes at low pH

DNA complexes, in the presence of magnesium ions, were investigated at pHs 6.83, 6.00, 4.40, 3.79, 3.45, 3.10, 2.82, 2.35 and 2.10. Measurements were made at physiological Na^+ ions concentrations.

To obtain the MgDNA complex at neutral pH, calf thymus DNA (Type I, D-1501, Sigma, St. Louis, MO, USA)

at a concentration of 15 mg ml^{-1} was dialysed against Tris buffer (10 mM Tris, 150 mM NaCl, 5 mM $MgCl_2 \cdot 6H_2O$). From the same stock solution, calf thymus DNA was dialysed against glycine buffer (50 mM glycine, 150 mM NaCl, 5 mM $MgCl_2 \cdot 6H_2O$) to obtain MgDNA samples in the pH range 6.00–2.10. For all the samples described here the pH values were measured in the dialysing buffers and in the DNA samples. The pH of glycine buffer solutions was adjusted with HCl.

At pH 2.35 and 2.10, insoluble aggregates and sedimentation of the MgDNA complexes were observed.

Spectra

All Raman spectra experiments with MgDNA complexes were carried out on a confocal Raman microspectrometer, built up at the University of Twente, Enschede, The Netherlands, largely identical with the first developed micro Raman setup.[2] The microscope was equipped with a 63× Zeiss Plan Neofluar water immersion objective. Laser radiation of 514.5 nm from an argon ion laser was used for excitation. A laser power of 300 mW and a spectral resolution of 4–5 cm^{-1} were used.

The spectra were processed by means of the software package RAMPAC.[4] Each measurement on a DNA sample was followed by a second one (background signal measurement), immediately after the DNA sample, in order to determine the signal contributions from buffer (water or glycine), which were then substracted from the resulting DNA sample spectrum.

Each spectrum is the result of 12–35 or more measurements, which were then averaged. The averaged spectra thus obtained showed only minor variations, not of any consequence for the interpretations given below. The wavenumber calibration of the Raman spectrum was recorded with the same instrument setting on the basis of an indene Raman spectrum. The spectra were corrected for the wavenumber-dependent detection efficiency of the confocal Raman microspectrometer.[2]

The spectra were scaled to have equal intensity in the 1094 cm^{-1} line of the DNA backbone PO_2^- symmetric stretching vibration. The intensity of this band is not sensitive to DNA protonation down to at least pH 2.35.[2,5] Protonation of the DNA backbone phosphate groups occurs around pH 1, leading to a decrease in the intensity of the PO_2^- symmetric stretching Raman line. However, since no measurements were carried out at pH values lower than 2.1, this will not affect the general validity of the results presented here.[2] Further, under the conditions employed for the present experiments, the interactions between divalent metal cations and phosphodioxy anions should be dominated by electrostatics.[6]

The events occurring in the MgDNA systems on lowering the pH were monitored by a comparative analysis of the spectra obtained for the samples with different stages of DNA protonation.

RESULTS AND DISCUSSION

Raman spectra of non-protonated and protonated DNA samples are presented in Fig. 1.

Nucleoside conformation, backbone geometry and PO_2^- interaction

Raman spectra of calf thymus DNA in the presence of magnesium ions were analysed here in the wavenumber region relating to nucleoside conformation, backbone geometry and PO_2^- interaction (600–1150 cm^{-1}). The following spectra–structure correlations are discussed: (a) the guanine nucleoside marker between 620 and 685 cm^{-1} diagnostic of sugar pucker and glycosyl torsion of dG residues;[2,3,7,8] (b) the adenine residue marker near 728 cm^{-1}; (c) the band near 752 cm^{-1} identifying the C2-*endo-anti* conformers of dT; (d) bands in the region 800–1100 cm^{-1} sensitive to backbone geometry and secondary structure; and (e) marker near 1094 cm^{-1}, characterizing the electrostatic environment of the PO_2^- group.[2,7,8]

In our spectra, the guanine 681 cm^{-1} line[2,3,7,8] decreases in intensity on lowering the pH, but it does not disappear at very low pH. This line is a marker band for the guanine C2′-*endo-anti* conformation, typical of the B-DNA backbone. At pH 2.8 a shoulder is still observed in the Raman spectrum of the DNA molecule, indicating that the B-DNA backbone is not completely altered at this pH value, in the presence of Mg^{2+} ions. This situation is not valid at pH 2.1, where this band completely disappeared. Further, a Raman marker typical for the C2′-*endo-syn* conformer of dG residues appears at 669 cm^{-1}. Hence, on lowering the pH, the population of the dG conformers changes from the C2′-*endo-anti* form to a mixture of different conformers.

In our spectra, the characteristic band of adenine residues near 728 cm^{-1} shows a sudden increase in intensity at pH 2.8, together with a shift to lower wavenumbers (723 cm^{-1}).

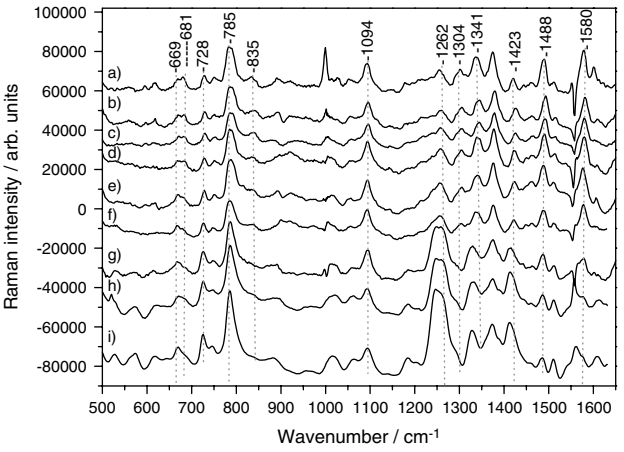

Figure 1. Raman spectra of calf thymus DNA, obtained in the presence of 150 mM NaCl and 5 mM $MgCl_2 \cdot 6H_2O$, at the following pH values: (a) 6.8; (b) 6.0; (c) 4.4; (d) 3.79; (e) 3.4; (f) 3.1; (g) 2.8; (h) 2.35; (i) 2.1.

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Further, in our Raman spectra of MgDNA complexes, the thymine band at 752 cm^{-1}, which identifies the C2′-*endo-anti* residues of dT, shows an increase in intensity at pH 2.8. The intensities of the adenine residue band near 728 cm^{-1} and of the thymine band at 752 cm^{-1}, are proof of unstacking of the AT base pairs during DNA melting at a very low pH value (2.8).

The pH dependences of the 681, 728 and 752 cm^{-1} Raman band intensities of dissolved calf thymus DNA are presented in Figs 2–4. In this study, the cytosine ring breathing mode at 785 cm^{-1} did not show a definite behaviour. At pH 2.8 this band exhibits a sudden increase in intensity, indicating unstacking of the GC base pairs at this pH value.

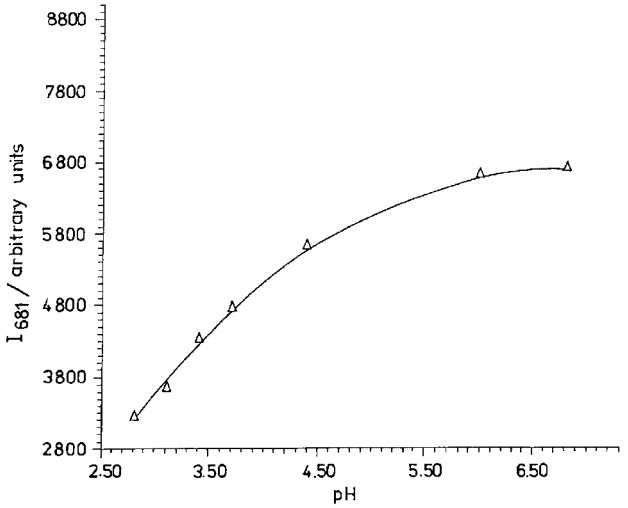

Figure 2. pH dependence of the guanine 681 cm^{-1} Raman band intensity of dissolved calf thymus DNA.

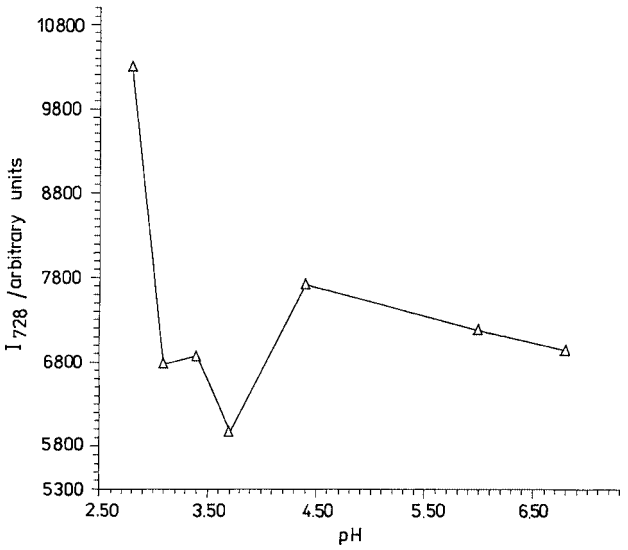

Figure 3. pH dependence of the adenine 728 cm^{-1} Raman band intensity of dissolved calf thymus DNA.

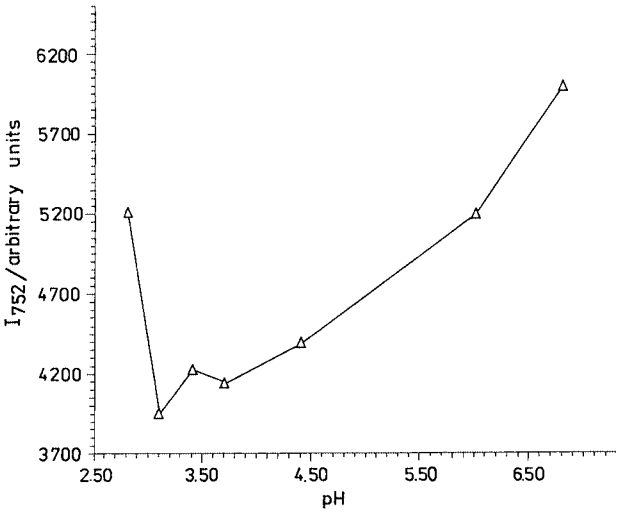

Figure 4. pH dependence of the thymine 752 cm^{-1} Raman band intensity of dissolved calf thymus DNA.

Useful spectral information can be obtained from the large band, centred around 835 cm^{-1}, assigned to a marker of the B helix and C2′-*endo* sugar conformations.[2,3,7,8] This band undergoes a decrease in intensity on lowering the pH and disappears almost completely at pH 2.8.

The band near 1094 cm^{-1} of the DNA backbone PO_2^- symmetric stretching vibration was used as a scaling band.

Base electronic structures and base pairing

In the following, spectra are analysed in the wavenumber region relating to base electronic structures and base pairing (1150–1680 cm^{-1}).[2,7–9] The effects of lowering the pH value on the protonation mechanism of opening AT and changing the protonation of GC base pairs in DNA are discussed.

The spectroscopic result show that on lowering the pH value to 3.1 in the presence of low concentrations of Mg^{2+} ions, DNA is effectively protected against protonation of cytosine (band at 1262 cm^{-1}) and is not protected against binding of H^+ to adenine (band at 1304 cm^{-1}) and to guanine N-7 (marker near 1488 cm^{-1}).[2,7–9] It therefore appears that in this pH interval, low concentrations of Mg^{2+} ions act as a mechanism of selectivity for protonation of cytosine and adenine in DNA. Particularly large changes in DNA structure are observed in the pH range 3.1–2.1. At pHs 2.35 and 2.1, insoluble aggregates and sedimentation of DNA were observed.

In the following, we analyse the base electronic structures and the base pairing changes of calf thymus DNA complexes on lowering the pH from 6.8 to 2.1.

In particular, Raman bands characterizing thymine (1241 cm^{-1}), cytosine (1257 cm^{-1}), protonated cytosine (1262 cm^{-1}), adenine (1304 cm^{-1}), protonated adenine residues (1323 cm^{-1}), dA residues (1341 cm^{-1}), dG and dA residues (1488 cm^{-1}), dG and dA residues (1580 cm^{-1}) and dT residues (1665 cm^{-1}) (C═O) are presented. The presence

of insoluble molecular aggregates and of sedimentation in DNA complexes is discussed.[2,7–9]

The band near 1241 cm^{-1} is due in principal to thymidine (dT), with a minor contribution from dC residues. The intensity of this band presents a slight increase on lowering the pH from 6.8 to 3.1 and exhibits a significant increase at pH 2.8.

The band near 1257 cm^{-1} has been assigned to dC residues. In our spectra, this band exhibits a slight increase in intensity on lowering the pH from 6.8 to 3.1 and a sudden increase in intensity at pH 2.8. This effect takes place concomitant with a shift of the band toward higher wavenumbers and of the appearance of the protonated cytosine line near 1262 cm^{-1}. This line helps in monitoring two phenomena taking place in our molecular system: (a) the cytosine protonation and (b) the unstacking of the GC base pairs at low pH, during DNA melting. Cytosine protonation leads to a slight increase and melting of DNA leads to a major increase in intensity of this band. This suggests that at pH 2.8 a sudden opening of GC (guanine–cytosine) base pairs takes place in the DNA molecule.

Changes in the 1257 cm^{-1} Raman band intensity of dissolved calf thymus DNA with pH are presented in Fig. 5. The Raman band at 1304 cm^{-1}, corresponding to dA and dC residues in the system, exhibits a decrease in intensity as the pH is lowered. We can distinguish two regions: a moderate decrease in Raman intensity from pH 6.8 to 3.1 and a major decrease in intensity from pH 3.1 to 2.8. At pH 2.8 this band completely disappeared. This is proof that almost all dA residues in the system are protonated and unstacked. At the same time, at this pH value, a well defined band is found at 1323 cm^{-1}, corresponding to protonated adenine.

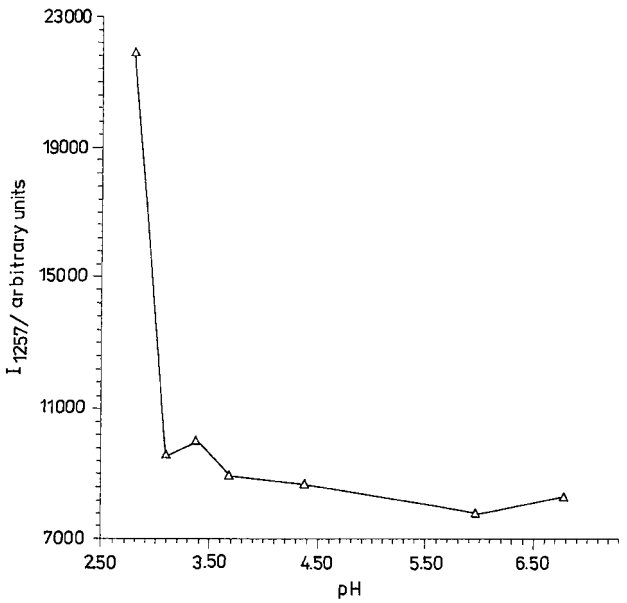

Figure 5. pH dependence of the cytosine 1257 cm^{-1} Raman band intensity of dissolved calf thymus DNA.

It is shown that the AT pairs are protonated just before the denaturation and that their behaviour seems to be different from that of protonated GC pairs. AT pairs seem to be unable to assume a new pairing when protonated. Only one H-bond remains between A^+ and T, i.e. between the 6-NH_2 of adenine and the 4-C═O of thymine. This pairing must be more unstable than that of the protonated GC pairs, in which two H-bonds remain.[10] It is shown that just before the acidic denaturation, the most unstable regions in DNA are the AT-richest regions.

Our spectroscopic results correspond, in the pH range 6.8–3.1, to substantial denaturation of DNA secondary structure in the AT-rich regions and to limited melting of this molecule in the GC-rich regions. In the pH range 3.1–2.8 all base pairs in the system are suddenly opened. This is also consistent with the fact that Mg^{2+} cations bind preferentially to GC rather than to AT regions.[6]

In our spectra, on changing the pH value from 6.8 to 2.8, a decrease in the intensity of 1341 cm^{-1} band is to be observed, corresponding to the dA residues. Simultaneously with this effect, a shift of the band towards lower wavenumbers takes place. Further, a well defined band appears around 1377 cm^{-1}, difficult to interpret because it characterizes both the purine (dA, dG) and the pyrimidine (dT, dC) residues. On lowering the pH value from 6.8 to 2.8, this band shows a decrease in intensity.

The band centred near 1423 cm^{-1} is typical of non-protonated adenine. At pH 2.8 a clear shift of this band toward lower wavenumbers (1415 cm^{-1}) occurs, suggesting adenine protonation and unstacking, at this acidic pH value.

A Raman band of particular interest is around 1488 cm^{-1}, attributed in principle to the guanine (N-7) and adenine rings. On lowering the pH value from 6.8 to 2.8 this band exhibits a decrease in intensity, presenting major changes in the pH range 3.4–2.8. The pH dependence of the 1488 cm^{-1} Raman band intensity of dissolved calf thymus DNA is presented in Fig. 6.

The Raman band near 1580 cm^{-1}, attributed to purines (G, A), exhibits a decrease in intensity on lowering the pH value from 6.8 to 2.8.

The broad band near 1668 cm^{-1} (not shown), assigned to coupled C═O stretching and NH deformation modes of dT, dG and dC, is sensitive to denaturation, reflecting altered hydrogen bonding states of the exocyclic donor and acceptor groups.[7–9] In our spectra, this band shows an increase in intensity on lowering the pH value.

At pH 2.35 and 2.10, spectroscopic and visual evidence proved the existence of insoluble 'aggregates' and sedimentation for calf thymus DNA complexes, in association with acidic melting of the DNA helix.

CONCLUSIONS

We obtained Raman spectra of excellent quality for DNA samples with different degrees of protonation, by using

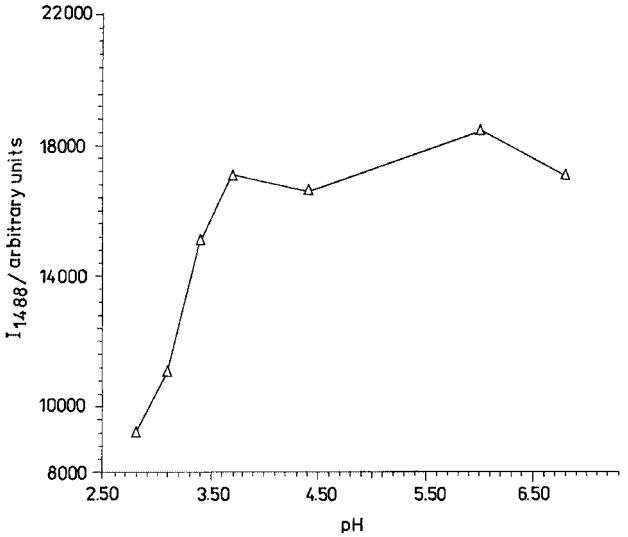

Figure 6. pH dependence of the guanine 1488 cm^{-1} Raman band intensity of dissolved calf thymus DNA.

Raman microspectroscopy, a unique experimental technique for studying biomolecular structures *in situ*.

Our Raman spectra document in detail the free DNA structural changes elicited by low-pH treatment in the presence of Mg^{2+} cations. Mg^{2+} ions were found to inhibit DNA protonation and remains an important factor, influencing the equilibrium between Watson–Crick DNA and alternative DNA structures with potential biological function. Structural evidence for a selective action on the protonation mechanism of opening AT as compared with the protonation of GC base pairs was found.

Other changes occurring in DNA structure at different pH values are adenine protonation, adenine, thymine, cytosine and guanine unstacking, AT and GC unpairing and DNA backbone conformational changes.

In order to obtain more information about the possible significance of the protonated base pairs in cellular processes, studies are required to elucidate whether the equilibrium between protonated and non-protonated GC base pairs[2,8] can be influenced by other chemical parameters.

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