Raman Microspectroscopic Study of Effects of Na(I) and Mg(II) Ions on Low pH Induced DNA Structural Changes

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ABSTRACT: In this work a confocal Raman microspectrometer is used to investigate the influence of Na^+ and Mg^{2+} ions on the DNA structural changes induced by low pH. Measurements are carried out on calf thymus DNA at neutral pH (7) and pH 3 in the presence of low and high concentrations of Na^+ and Mg^{2+} ions, respectively. It is found that low concentrations of Na^+ ions do not protect DNA against binding of H^+. High concentrations of monovalent ions can prevent protonation of the DNA double helix. Our Raman spectra show that low concentrations of Mg^{2+} ions partly protect DNA against protonation of cytosine (line at 1262 cm^{-1}) but do not protect adenine and guanine N(7) against binding of H^+ (characteristic lines at 1304 and 1488 cm^{-1}, respectively). High concentrations of Mg^{2+} can prevent protonation of cytosine and protonation of adenine (disruption of AT pairs). By analyzing the line at 1488 cm^{-1}, which obtains most of its intensity from a guanine vibration, high magnesium salt protect the N(7) of guanine against protonation. A high salt concentration can prevent protonation of guanine, cytosine, and adenine in DNA. Higher salt concentrations cause less DNA protonation than lower salt concentrations. Magnesium ions are found to be more effective in protecting DNA against binding of H^+ as compared with calcium ions presented in a previous study. Divalent metal cations (Mg^{2+}, Ca^{2+}) are more effective in protecting DNA against protonation than monovalent ions (Na^+). © 2003 Wiley Periodicals, Inc. Biopolymers (Biospectroscopy) 72: 225–229, 2003

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INTRODUCTION

The predominant DNA conformation in chromosomes and in the nucleus of eucaryotic cells is the B-form, which is the canonical right-handed double helix. Until the end of the 1970s, it was generally accepted that this biopolymer is very regular and independent of the nucleotide sequence.[1] Today it is a well-established fact that the DNA double helix exhibits local polymorphism at the levels of the single base pair and the dinucleotide step. DNA structure polymorphy is sequence dependent and influenced by environmental conditions (pH, ions, superhelical stress, length of particular tracts).[1] Alternative DNA structures and conformations, such as cruciforms,[2] triplex DNA, parallel-stranded DNA,[3] extended stretches of unpaired bases, and potential Z-DNA forming stretches of DNA, have attracted much interest.[4] Biophysical, chemical, and enzymatic assays

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proved that these non-B-DNA structures, which are unorthodox structures formed under conditions of torsional stress, may play important organizing and/or regulatory roles *in vivo*.[5]

Canonical B-DNA has no strong protonation sites, and because of this it does not pick up protons with the exception of totally nonphysiological conditions of an extremely low pH. However, protonation is a significant phenomenon for DNA. Unusual DNA structures, in which Hoogsteen is a possible base-paired arrangement of protonated dG:dC$^+$, are supposed to be implied in a new mode of recognition.

However, there is no general consensus on the precise nature of the changes that occur in DNA as the pH is lowered. It seems that a subtle interplay between chemical parameters (pH and ion concentration) may result in appreciable changes in DNA conformation.[6,7]

In this work a confocal Raman microspectrometer was used to investigate the influence of Na^+ and Mg^{2+} cations on the DNA structural changes induced by low pH.

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.[6]

MATERIALS AND METHODS

Preparation of NaDNA Complexes at Neutral and Low pH

Calf thymus DNA (type I, D-1501, Sigma, St. Louis, MO) at a concentration of 15 mg/mL was dialyzed against glycine buffers with the required quantities of monovalent cations (50 m*M* glycine, 10 m*M* NaCl and 50 m*M* glycine, 2.01*M* NaCl) to obtain NaDNA complexes at low pH. Reference samples at neutral pH were obtained in phosphate and Tris-HCl buffers for the same biopolymer and monovalent cations concentrations (10 m*M* NaCl, 2.01*M* NaCl), respectively. The pH values at neutral and low pH were measured in the dialyzing buffers and the DNA samples, respectively.

Preparation of MgDNA Complexes at Neutral and Low pH

Calf thymus DNA (type I, D-1501, Sigma) at a concentration of 15 mg/mL was dialyzed against Tris buffers (10 m*M* Tris, 10 m*M* NaCl) containing 5 m*M* $MgCl_2 \times 6H_2O$ and 500 m*M* $MgCl_2 \times 6H_2O$, respectively, to obtain the MgDNA complexes at neutral pH. MgDNA complexes at low pH (~pH 3) were prepared by dialyzing calf thymus DNA from the same stock solution against glycine buffers (50 m*M* glycine, 10 m*M* NaCl) containing 5 m*M* $MgCl_2 \times 6H_2O$ and 500 m*M* $MgCl_2 \times 6H_2O$, respectively. The pH 3 and 7 values were measured in the dialyzing buffers and DNA samples, respectively.

Experimental

All Raman experiments with DNA complexes were carried out on a confocal Raman microspectrometer developed in the Biophysical Technology Group in the Department of Applied Physics at the University of Twente. This instrument was basically identical to the first micro-Raman setup that was developed.[4,6] The microscope was equipped with a 63× Zeiss Plan Neofluar water immersion objective. Laser light of 514.5 nm from an argon ion laser was used for excitation.

The spectra were processed by means of the software package RAMPAC.[8] 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 substracted from the resulting DNA sample spectrum.

Each spectrum is the result of 12 or more measurements, which were averaged. These averaged spectra showed only minor variations, which were not of any consequence for the interpretations given below. The wavenumber calibration of the Raman spectra was recorded with the same instrument setting on the basis of an indene spectrum. The spectra were corrected for the wavenumber-dependent detection efficiency of the confocal Raman microspectrometer.[4]

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.[4,9] Protonation of the DNA backbone phosphate groups occurs at around pH 1, leading to a decrease in the intensity of the PO_2^--symmetric stretching Raman line. However, because no measurements were carried out at pH values lower than 3, this does not affect the general validity of the results presented here.[4]

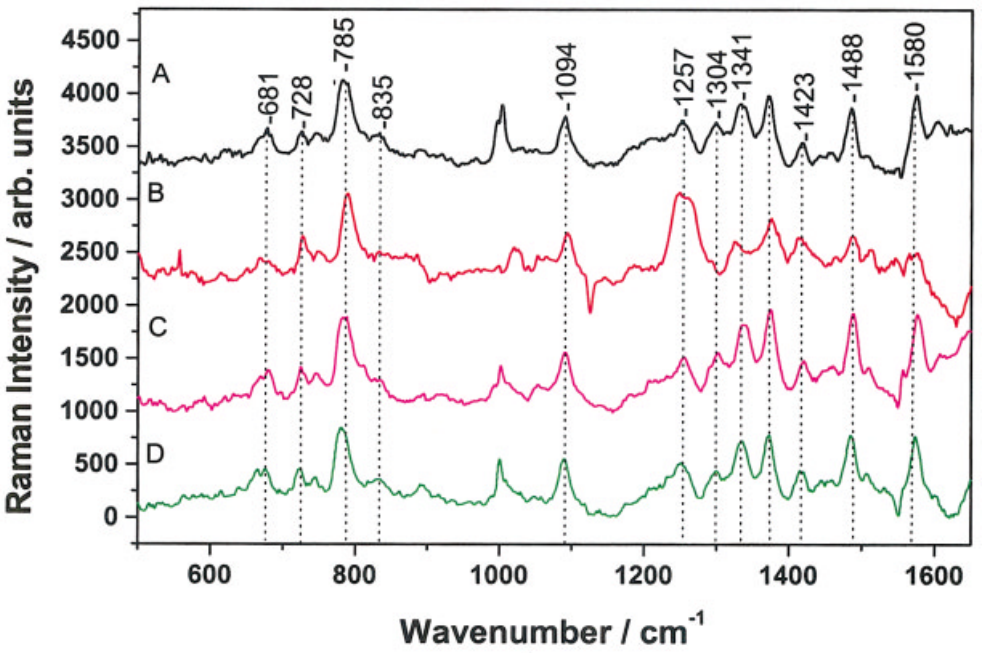

Figure 1. Raman spectra of calf thymus DNA in the presence of low and high concentrations of Na^+ ions. The spectra are presented comparatively at neutral pH (7) and pH 3: 10 m*M* Na^+, pH 7 (spectrum A); 10 m*M* Na^+, pH 3 (spectrum B); 2.01*M* Na^+, pH 7 (spectrum C); and 2.01*M* Na^+, pH 3 (spectrum D). The macromolecular concentration is 15 mg/mL. The data have the background signal substracted. The spectra are scaled to have equal intensity in the 1094 cm^{-1} line.

RESULTS AND DISCUSSION

The Raman microspectroscopic measurements were carried out on calf thymus DNA at neutral pH (7) and pH 3 in the presence of low and high concentrations of Na^+ and Mg^{2+} cations, respectively. 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 681 cm^{-1} line).[4,6] The influence of Na^+ and Mg^{2+} cations on the DNA structural changes induced by low pH has been of interest. The effects of monovalent and divalent cations on the protonation mechanism of opening AT and changing the protonation of GC base pairs in DNA are discussed.[6]

Figure 1 presents a comparison of the Raman spectra of DNA complexes at two concentrations of Na^+ ions and at two pH values (pH 7 and 3).

Figure 2 presents the Raman spectra of DNA complexes obtained at two concentrations of magnesium ions and at two pH values (pH 7 and 3).

The Raman spectra of calf thymus DNA in the presence of low and high concentrations of monovalent and divalent ions are analyzed here in the wavenumber region describing the nucleoside conformation, backbone geometry, and PO_2^- interaction (600–1150 cm^{-1}).[9–15] The following spectra–structure correlations are of interest: the guanine nucleoside marker between 620 and 685 cm^{-1} that is diagnostic of sugar pucker and glycosyl torsion of dG residues[4,5,7,10,11]; the adenine residue marker near 728 cm^{-1}; the band near 752 cm^{-1} identifying the C2-endo/anti conformers of dT; the bands in the 800–1100 cm^{-1} region that are sensitive to backbone geometry and secondary structure; and the marker near 1094 cm^{-1}, characterizing the electrostatic environment of the PO_2^- group.[4,10,11]

Large changes in the Raman spectra of calf thymus DNA were observed upon lowering the pH in samples containing low concentrations of Na^+ ions. The Raman bands of the ring breathing vibrations of the guanine (681 cm^{-1}),[4,7,10] adenine (729 cm^{-1}),[4,6] thymine (749 cm^{-1}),[4,6] and cytosine (784 cm^{-1}),[4,7,10] which are markers for the C2′-endo-anti conformation of the nucleotides that is typical of B-DNA,[4,10] do respond to unstacking of the bases.[16] The guanine line is hypo-

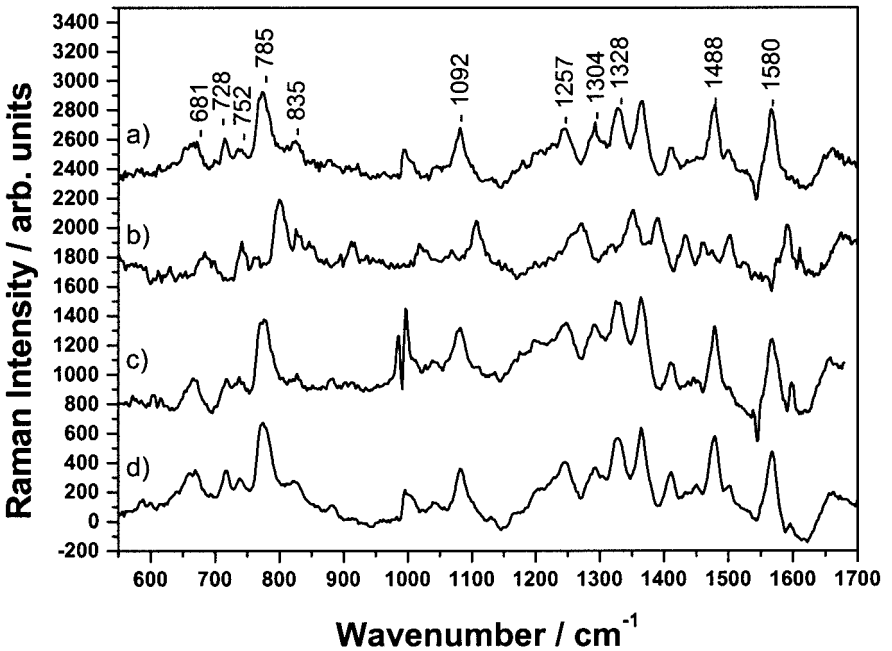

Figure 2. Raman spectra of calf thymus DNA in the presence of low and high concentrations of Mg^{2+} ions. The spectra are presented comparatively at neutral pH (7) and pH 3: 5 m*M* Mg^{2+}, pH 7 (spectrum a); 5 m*M* Mg^{2+}, pH 3 (spectrum b); 500 m*M* Mg^{2+}, pH 7 (spectrum c); and 500 m*M* Mg^{2+}, pH 3 (spectrum d). The macromolecular concentration is 15 mg/mL. The data have the background signal substracted. The spectra are scaled to have equal intensity in the 1094 cm^{-1} line.

chromic; its intensity decreases upon unstacking during DNA melting.[4] The other bands present an increase in their Raman intensities upon unstacking. The band centered at around 830 cm^{-1},[4,6,7,10] which is a marker of B-form DNA backbone and C2′-endo sugar conformations,[4,7] decreases in intensity upon lowering the pH. Cytosine protonation in DNA is thought to occur via a mechanism involving a rotation of guanine around the glycosidic bond from an anti to a syn position, leading to an intermediate protonated Hoogsteen GC base pair.[4,17,18]

The main spectra–structure correlations in the wavenumber region describing base electronic structures and base pairing (1150–1680 cm^{-1})[11–15] for DNA complexes at neutral and low pH are described in the following.

Bands in the 1200–1600 cm^{-1} region, which are assigned to purine and pyrimidine ring vibrations, are sensitive indicators of ring electronic structures[10] and are expected to exhibit perturbations upon proton binding to DNA. Among the most informative of these are the band of guanine at 1488 cm^{-1}, which shifts upon binding of electrophilic agents to the N(7) acceptor, and bands at 1240 and 1257 cm^{-1}. The band at 1488 cm^{-1} decreases in intensity upon binding of H^+ to guanine N(7).[4,6,7,19] The band near 1240 cm^{-1} is due predominantly to dT, but it has a minor contribution from dC. The band near 1257 cm^{-1} has been assigned to dC.

The broad band centered near 1668 cm^{-1} is assigned to coupled C═O stretching and N—H deformation modes of dT, dG, and dC.[10] It is sensitive to denaturation, reflecting altered hydrogen bonding states of the exocyclic groups (C═O, NH, NH_2).

Thus, among the changes occurring in the DNA structure at pH 3 in the presence of low concentrations of Na^+ are cytosine protonation, adenine protonation, adenine and thymine unstacking, AT unpairing, DNA backbone conformational changes, and so forth. Our experimental data show that at low concentrations of Na^+ ions the DNA molecule is not protected against protonation, particularly against protonation of cytosine (1257 shifted to 1262 cm^{-1}), adenine (1304 cm^{-1}), and guanine at N(7) (1488 cm^{-1}) . High concentrations of monovalent metal ions prevent protonation of the DNA double helix.

Our Raman spectra show that low concentrations of Mg^{2+} cations partially protect DNA against protonation of cytosine (characteristic line at 1262 cm^{-1}) and do not protect adenine (characteristic line at 1304 cm^{-1}) and N(7) of guanine (line at 1488 cm^{-1}) against binding of H^+. This is consistent with extensive melting of AT-rich regions and limited melting of GC-rich regions. High concentrations of Mg^{2+} can prevent protonation of cytosine and adenine (disruption of AT pairs) in DNA. High magnesium salt was also found to protect the N(7) of guanine against binding of H^+.

CONCLUSIONS

We obtained Raman spectra of excellent quality for calf thymus DNA at neutral and low pH in the presence of low and high concentrations of Na^+ and Mg^{2+} ions, respectively. A high salt concentration can prevent protonation of guanine, cytosine, and adenine in DNA. Higher salt concentrations cause less DNA protonation than lower salt concentrations. Magnesium ions are found to be more effective in protecting DNA against binding of H^+ as compared with calcium ions presented in a previous study.[6] Divalent metal cations (Mg^{2+}, Ca^{2+}) are more effective in protecting DNA against protonation than monovalent ions (Na^+).

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