Evidence for Hoogsteen GC Base Pairs in the Proton-Induced Transition from Right-Handed to Left-Handed Poly(dG-dC)·Poly(dG-dC)

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ABSTRACT: The structure of double-helical poly(dG-dC)·poly(dG-dC) is investigated at various pH values with Raman spectroscopy, absorption spectroscopy, and circular dichroism. A comparison is made between the B-form with Watson−Crick base pairing at 1 mM [Na^+] and pH 7.2, the Z-form with Watson−Crick base pairing at 4 M [Na^+] and pH 7.2, and a different structure at 1 mM [Na^+] and pH 4.5 as well as at 150 mM [Na^+] and pH 3.1. The CD spectrum of poly(dG-dC)·poly(dG-dC) under the latter conditions does not show a negative band at 290 nm. The structure is a double-helical structure different from the B-form and the Z-form according to circular dichroism, Raman, and absorption spectroscopic studies. The Raman spectra evidence that the structure contains Hoogsteen base pairing. This can be accommodated in the double helix when the cytosine group is protonated and the sugar−guanine conformer has adopted a $C_{2'}$-endo/syn conformation. It is shown that this antiparallel-stranded Hoogsteen base paired structure can be maintained under varying conditions, balancing the decrease in pH with an increased salt concentration. It is further concluded that the proton-induced transition from a Watson−Crick to a Hoogsteen base pair is aided by a decrease of [Na^+] at pH 4.5 and occurs prior to a conversion from a right-handed helix to a left-handed helix.

The secondary structure of poly(dG-dC)·poly(dG-dC) in aqueous solutions of low ionic strength and at physiological pH is generally recognized as a double-stranded helix with the strands connected by Watson−Crick base pairing, the so-called B-form. In a high-salt environment, it was established, by means of CD and Raman spectra, that the polynucleotide has a left-handed double-helical structure (Pohl & Jovin, 1972; Thamann et al., 1981; Benevides & Thomas, 1983).

Apart from studies on effects of salt concentration, several investigations have been performed on the influence of pH on DNA conformation. Courtois et al. (1968) used optical rotatory dispersion and suggested a conformational change of guanine residues in DNA upon acid titration between pH 3 and 4. It was hypothesized as a possible interpretation of their results that upon protonation on guanine N7, guanine becomes unstacked and rotates out of the helix, reverses from the anti into the syn position, pairs in a Hoogsteen manner, and thus shares the proton with N3 on cytosine. Zimmer and Triebel (1969) concluded from spectrophotometric pH-titration measurements at 20 mM [K^+] that reversible and irreversible conformational changes occur in DNA upon acid treatment. From viscosimetric and spectrophotometric investigations on DNA at different pH values and ionic strengths, it was shown by Kas'yanenko et al. (1986) that DNA is in a double-helical form until at least pH 3.5 at 0.1 M salt concentration. Chen (1984) suggests from spectroscopic studies on the salt titration for poly(dG-dC)·poly(dG-dC) in solutions with pH 7.0 and pH 3.8 that base protonation facilitates the B to Z interconversion.

The possible presence of protonated Hoogsteen base paired DNA sequences in equilibrium with the Watson−Crick base paired form at pHs up to 7 is mentioned by Pulleyblank et al. (1985) for $d(TC)_n$·$d(GA)_n$. They suggest that the guanine residues are either Hoogsteen or reverse Hoogsteen base paired to protonated dC residues. A structure in which dA·dT Watson−Crick base pairs alternate with Hoogsteen dG·dCH^+ pairs in the syn conformation seems to be the most stereochemically acceptable structure. As an explanation for the observed cross-reactivity of the protonated form of $d(TC)_n$·$d(GA)_n$ with a polyclonal antibody raised against the brominated Z form of $d(GC)_n$·$d(GC)_n$, they suggest that the protonated structures of $d(TC)_n$·$d(GA)_n$ and Z-DNA are sufficiently similar, or that they can be distorted by interaction with the antibody binding site into conformations sufficiently similar that they can be recognized by the same antibody.

In an NMR study, Guéron et al. (1990) demonstrated the presence of Hoogsteen GC base pairs at pH 3.2 and lower. Here it is suggested that even at neutral pH Hoogsteen GC base pairs exist as a minority species. Robert-Nicoud et al. (1984) indicated that a dramatic increase occurs in the anti-Z-DNA immunofluorescence intensity of polytene chromosomes upon short exposure to low pH. In addition, they concluded from CD and UV spectral studies that the Z-conformation of poly(dG-dC)·poly(dG-dC) is preferentially induced and stabilized in acid. This suggests that protonation can lead to a Z-like conformation in GC polynucleotides. Kumar and Maiti (1994) describe a model with formation of Hoogsteen base pairs together with a change of the handedness of the helix at low pH as a conclusion from CD and absorption spectroscopic measurements.

From a Raman microspectroscopic study on polytene chromosomes at low pH, Puppels et al. (1994) proposed that

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there is more evidence for the presence of Hoogsteen GC base pairs at low pH than for Z-form DNA, although the presence of Z-DNA is not completely excluded here.

It is clear that there is no 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. The elucidation of the nature of these changes is the topic of this paper. Therefore, a combined study of absorption spectroscopy, thermal melting profiles, circular dichroism, and Raman spectroscopy was performed. The absorptive techniques enable a direct comparison with the results that have appeared in the literature, while Raman spectroscopy is excellently suited to study local structural properties of base conformers. This paper will present direct evidence that the guanine−sugar residues in poly(dG-dC)·poly(dG-dC) undergo a major change in conformation under mild conditions (1 mM [Na^+] and at pH 4.5). We also present evidence that this conformation is maintained at pH 3.1 and 150 mM [Na^+]. Under both conditions, the base pairing is characterized as Hoogsteen with a predominant presence of $C_{2'}$-endo/syn conformation. Under these conditions, no evidence has been obtained for the presence of the left-handed Z-conformation that can be induced in poly(dG-dC)·poly(dG-dC) at neutral pH and 4 M [Na^+].

MATERIALS AND METHODS

Sample Preparation. Poly(dG-dC)·poly(dG-dC) was purchased from Pharmacia (Uppsala, Sweden; lot numbers AH7910102, 0078273, and 4107910021).

The concentration of poly(dG-dC)·poly(dG-dC) in the absorbance measurements was ∼30 μg/mL (4.5×10^{-5} M) in mononucleotides. UV absorption measurements and melting profiles were carried out on a Shimadzu UV2101PC (Shimadzu Corp., Japan) scanning spectrophotometer in stoppered quartz cuvettes of 1 cm path length in a cell holder connected with a temperature-controlled water bath. The CD measurements were carried out on a JASCO J-715. The CD spectra are obtained at 15 °C. The optical melting profiles were registered from 210 to 320 nm over a temperature range from 15 to 94 °C for pH values of 7.2, 5.2, 4.9, 4.5, and 3.0 at 1 mM NaCl/1 mM Tris or 1 mM NaCl/1 mM glycine. For this purpose, a concentrated stock solution of polynucleotide was prepared in 1 mM Tris/1 mM NaCl, pH 7.2, solution (25 A_{260} units in 1 mL), and aliquots of this solution were dialyzed overnight at 4 °C against buffers with the proper pH and salt concentration. For measurements at a lower pH, a solution of 1 mM glycine/1 mM NaCl was used. The pH of the buffers was adjusted with HCl. A small sample of the dialyzed polynucleotide was diluted with buffer of the same pH and salt concentration to obtain the desired concentration for UV measurement (A_{260} ∼ 0.5). These procedures were used to avoid direct addition of acid to the polynucleotide solution. The melting profile was also measured at pH 3.1 and physiological salt concentration (150 mM NaCl/50 mM glycine).

Raman spectra of the polynucleotide at pH 7.2/low salt and pH 7.2/high salt were measured on a standard scanning spectrometer described previously (Terpstra, 1995), employing a 90° scattering geometry. The excitation wavelength is 514.5 nm from an argon ion laser using a laser power of 0.6 W. The power at the sample was increased approximately 2-fold by retro-reflection of the laser beam from a spherical mirror behind the cuvette. The collection efficiency was improved by signal collection not only in 90° but also in 270° by a properly chosen spherical mirror in this direction. The Raman spectra of the polynucleotide were measured with a spectral resolution of 4.5 cm^{-1}. The spectra were recorded with 25 s per spectral point. The step size was 1 cm^{-1}. Measurements were carried out in $2 \times 2 \times 10$ mm^3 fused silica cells (custom-made by Hellma, total volume 25 μL) at 15 °C.

The Raman spectra of poly(dG-dC)·poly(dG-dC) at pH 4.5 and 3.1 were obtained in back-scattering geometry using a Raman microspectrometer (Sijtsema, 1997). The measurements were carried out in a 0.6×0.6 mm square glass capillary, with 0.12 mm wall thickness. The samples were mounted in a metal holder. The temperature was controlled by a water bath at 15 °C. To prevent water condensation from the surrounding air, the capillary was flushed with nitrogen at the same temperature (15 °C). The Raman microscope was equipped with a 40× Nikon dry objective (NA = 0.65). Laser light was used from an argon ion laser operating at a wavelength of 514.5 nm. The Raman spectrum was focused on a liquid nitrogen cooled CCD camera (Princeton Instruments, 1100×330 pixels). The laser power was 20 mW on the sample, and the measuring time was 14 min. The RAMPAC (de Mul, 1993) software package was used to perform data analysis.

The poly(dG-dC)·poly(dG-dC) pH 7.2 sample was prepared by dissolving 25 A_{260} units in 800 μL of 1 mM Tris, 1 mM NaCl, pH 7.2. This solution was dialyzed overnight at 4 °C against the same buffer of pH 7.2. Next this solution was concentrated to 100 μL under a stream of nitrogen. The final solution had an A_{260} of approximately 250 (∼15 mg/mL).

The Z-form was prepared in a similar way: 25 A_{260} units of poly(dG-dC)·poly(dG-dC) in 800 μL of 0.5 M NaCl in 1 mM Tris/1mM NaCl, pH 7.2. After overnight dialysis against the same buffer, this solution was concentrated to 100 μL, resulting in a solution with an A_{260} of 250 and a salt concentration of 4 M in NaCl.

The sample of poly(dG-dC)·poly(dG-dC) at pH 4.5 was prepared from 100 A_{260} units dissolved in 300 μL of 1 mM NaCl/1 mM Tris, pH 7.2. Twenty-five microliters of this solution was dialyzed overnight against 1 mM NaCl/1 mM glycine, pH 4.5. From this solution, a small volume was transferred to a capillary.

The sample at pH 3.1 was prepared by dialyzing 50 μL of the above stock solution, diluted with 150 μL of 15 mM NaCl/5 mM glycine, against a 15 mM NaCl/5 mM glycine, pH 3.1, solution. After dialysis, the solution was concentrated 10 times, which results in a solution of the polynucleotide in 150 mM NaCl/50 mM glycine.

RESULTS AND DISCUSSION

In Figure 1, the guanosine-cytidine base pairs in the Watson−Crick and the Hoogsteen conformations are compared.

In Figure 2, the absorbance spectra of poly(dG-dC)·poly(dG-dC) at pH values of 7.2, 5.2, 4.9, 4.5, and 3.0, and at 1 mM Na^+ salt concentration, are shown at a temperature of 15 °C. Clear changes are observed when the pH is lowered from 7.2 to 4.5. The decrease in intensity at 258 nm, the

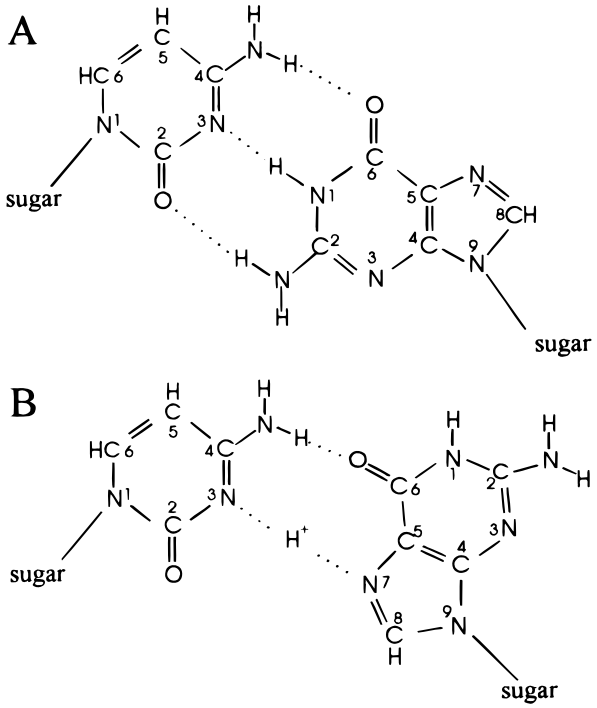

FIGURE 1: Comparison of the structures of the Watson−Crick base pairing with the Hoogsteen base pairing in the guanosine-cytidine base pair. (A) Watson−Crick base pairing; (B) Hoogsteen base pairing.

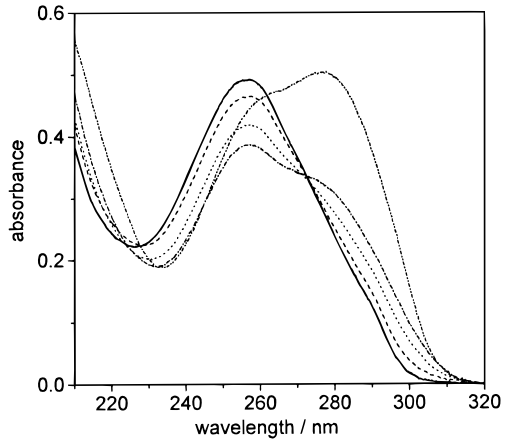

FIGURE 2: Absorbance spectra of poly(dG-dC)·poly(dG-dC) at different pHs: (—) pH 7.2; (- - -) pH 5.2; (···) pH 4.9; (- · -) pH 4.5; (- ·· -) pH 3.0. The salt concentration is 1 mM NaCl /1 mM Tris or 1 mM NaCl /1 mM glycine. The temperature is 15 °C. Gradual changes in the absorption spectrum occur until the pH reaches 4.5. An isosbestic point at 273 nm indicates a biphasic change of the double-stranded B-DNA structure into another double stranded structure. The double-strandedness is absent at pH 3.0 at this salt concentration.

increase in intensity at 285 nm, and the overall red shift of the absorbance from the 260 nm region to the 290 nm region are clear signs of the protonation of cytosine residues and the concomitant structural changes in the double-helical Watson−Crick base paired poly(dG-dC)·poly(dG-dC). An isosbestic point occurs at 273 nm. Also in the short wavelength region between 210 and 230 nm corresponding changes are observed. The absorption spectrum at pH 3.0 reveals additionally a large increase around 285 nm resulting from increased protonation. In this spectrum, there is no correspondence with the isosbestic point at 273 nm.

The temperature melting profiles at 275 nm are presented in Figure 3 for each of the pH values in Figure 2. A large

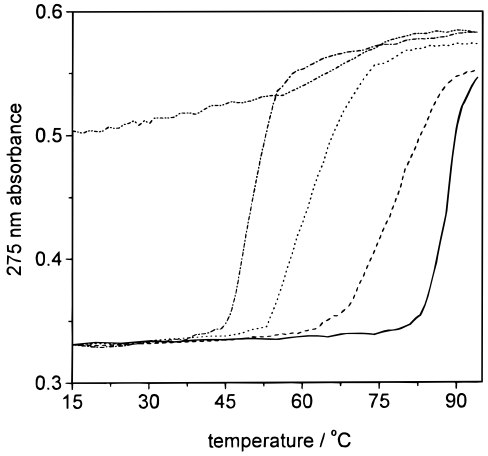

FIGURE 3: Melting profiles of poly(dG-dC)·poly(dG-dC) at different pHs. (—) pH 7.2; (- - -) pH 5.2; (···) pH 4.9; (- · -) pH 4.5; (- ·· -) pH 3.0. The salt concentration is 1 mM NaCl /1 mM Tris or 1 mM NaCl /1mM glycine. A gradual decrease of the melting temperature of the double-stranded poly(dG-dC)·poly(dG-dC) is observed until pH 4.5. No corresponding melting transition is observed at pH 3.0 at this salt concentration.

part of the hypochromicity increase in poly(dG-dC)·poly(dG-dC) during melting at pH 7.2 can be observed as a result of the salt concentration of 1 mM Na^+. At this salt concentration and pH 7.2, the melting temperature is approximately 85 °C. It is further observed that the melting temperature is strongly reduced when the pH is lowered to pH 4.5. At pH 4.5, the melting temperature is 50 °C. The absorbance change in the melting transition of poly(dG-dC)·poly(dG-dC) increases approximately 10% until pH 4.5. The melting-induced absorbance change is very small at pH 3.0. The gradual behavior of poly(dG-dC)·poly(dG-dC) with a decrease of pH to 4.5 suggests that the polynucleotide is in a double-stranded conformation in this pH range in agreement with previous observations (Kas'yaneneko, 1986). It is further concluded that at pH 3.0 and 1 mM [Na^+] poly(dG-dC)·poly(dG-dC) is not in a double-stranded conformation anymore.

In Figure 4A and Figure 4B are presented, respectively, the absorbance and CD spectra of the B-form (pH 7.2, 1 mM [Na^+]), with the Z-form (pH 7.2, 4 M [Na^+]) and the "pH 4.5-form" at 1 mM [Na^+] and the corresponding circular dichroism spectra. It is well-known that the B-form is different from the high-salt Z-form (Pohl & Jovin, 1972) and from the "pH 4.5-form" (Courtois et al., 1968; Kumar et al., 1994). From Figure 4A,B, it is clear that indeed the absorption and CD spectra of the "Z-form" are completely different from those of the "pH 4.5-form". Like in Z-form DNA, there is a red shift in the absorption spectrum of the "pH 4.5-form". The red shift at decreased pH is partially due to the protonation of cytosine residues, which leads to an increase of the absorption around 280 nm. It is also clear from Figure 4A that at pH 4.5 additional oscillator strength occurs beyond 300 nm which is not present in the Z-form of poly(dG-dC)·poly(dG-dC). The CD spectrum of poly(dG-dC)·poly(dG-dC) at pH 4.5 is very weak in comparison with those from both the B-form as well as the Z-form. A weak optical activity can be observed beyond 300 nm in the protonated form. No negative CD occurs at 290 nm. Previously (Chen, 1984; Kumar, 1994), a negative CD at that wavelength was interpreted as the formation of a left-handed helical structure.

Table 1: Raman Spectra of Poly(dG-dC)·Poly(dG-dC) at Different pHs and Salt Concentration

band position (cm^{-1}) for band assignments[a]					
B	**H**	**Z**	intensity	characteristic for	origin
597	–	596			cytosine
–	–	625	$C_{3'}$-endo/syn		guanine
–	675	–	$C_{2'}$-endo/syn		guanine
681	–	685	B ≫ Z	$C_{2'}$-endo/anti	guanine
783	786	782	B = HS = Z		cytosine/backbone
–	–	793	shoulder		backbone
–	–	810		PO_2-antisymm stretch	backbone
830	824	–		PO_2-antisymm stretch	backbone
1093	1094	1093	B = HS > Z	PO_2-symm stretch	backbone
1177	1181	1180			guanine
1218	–	1211			cytosine
1240	–	1245			cytosine
1259	1259	1264	Z narrow/HS very broad		cytosine
1293	1288	1290	in HS only shoulder		guanine
1317	–	1316	B ≪ Z		guanine
–	1328	–			guanine
1334	–	–			guanine
1362	1359	1356	Z > B > HS		guanine
1420	1424	1426	B,Z > HS		guanine
1489	1488	1483	B,Z > HS	H-bonding at guanine N_7	guanine
1530	1538	1536			cytosine
1577	1578	1579			guanine

[a] B, Watson−Crick base paired B-conformation at 1 mM NaCl/1 mM Tris, pH 7.2; H, Hoogsteen base paired conformation at 1 mM NaCl/1 mM glycine, pH 4.5; Z, Watson−Crick base paired Z-conformation at 4 M NaCl/1 mM Tris, pH 7.2.

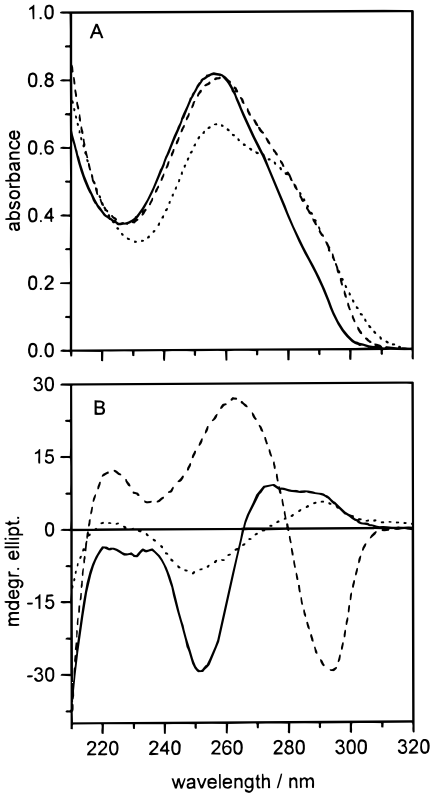

FIGURE 4: (A) Comparison of the absorbance spectra of poly(dG-dC)·poly(dG-dC) in the B-form (pH 7.2, 1 mM [Na^+]), in the Z-form (pH 7.2, 4 M [Na^+]), and in the Hoogsteen base paired form at pH 4.5 and 1 mM [Na^+] (dotted line). (B) Ibid, the circular dichroism spectra.

The nature of the conformation of poly(dG-dC)·poly(dG-dC) at pH 4.5 at 1 mM Na^+ concentration was further investigated by Raman spectroscopy. In Figure 5, the Raman spectra of poly(dG-dC)·poly(dG-dC) are shown at 1 mM [Na^+] and pH 7.2 (B-form, Figure 5B), at 4 M [Na^+] and pH 7.2 (Z-form, Figure 5C), and at 1 mM [Na^+] and pH 4.5 (Figure 5A). In Table 1 the Raman band assignments are

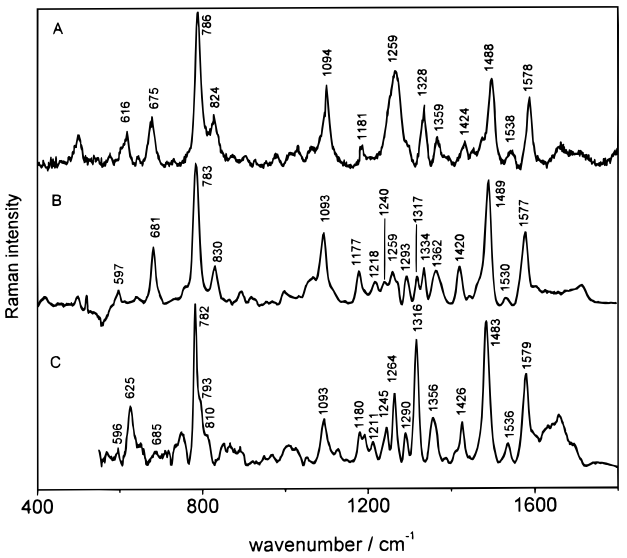

FIGURE 5: Comparison of the Raman spectra of poly(dG-dC)·poly(dG-dC) in the B-form (pH 7.2, 1 mM [Na^+]) (spectrum B; middle), in the Z-form form (pH 7.2, 4 M [Na^+]) (spectrum C; bottom) and in the Hoogsteen base paired form (pH 4.5, 1 mM [Na^+]) (spectrum A; top).

presented. The 1 mM [Na^+], pH 7.2 spectrum shows the typical features of poly(dG-dC)·poly(dG-dC) in the B-conformation. The guanine vibrational motions in the region between 1300 and 1500 cm^{-1} couple with the motions of the sugar group. The existence of this coupling leads to large differences in the Raman spectra of the B- and the Z-form of poly(dG-dC)·poly(dG-dC). The guanine bands at 1317 and 1334 cm^{-1} in 1 mM [Na^+] are replaced by a single, intense band at 1316 cm^{-1} in 4 M [Na^+], neutral pH. Positive shifts occur for the bands at 1177, 1420, 1530, and 1577 cm^{-1} (B-form) to respectively 1180, 1426, 1536, and 1579 cm^{-1} (Z-form). Other bands shift to smaller frequencies. The bands at 1293, 1362, and 1489 cm^{-1} (B-form) shift to respectively 1290, 1356, and 1483 cm^{-1} (Z-form).

Changes in the cytosine bands between 1200 and 1280 cm^{-1} are also apparent. All these changes are in agreement with earlier Raman spectroscopic investigations of the B- and Z-form DNAs (Thamann et al., 1981; Benevides & Thomas, 1983).

The Raman spectrum of poly(dG-dC)·poly(dG-dC) at pH 4.5 and 1 mM [Na^+] is completely different from the B-form spectrum as well as from the Z-form spectrum. Changes have occurred in bands in the cytidine and guanosine residues as well as in bands originating in the backbone of the helix. We will next discuss these changes. The pK_a of cytidine is reported (O'Connor, 1981; Pulleyblank, 1985) to be around 4.4. The cytidine residues in poly(dG-dC)·poly(dG-dC) are therefore partially protonated at a pH of 4.5. The guanosine residues have a pK_a of 2.2 (O'Connor, 1981), and the phosphate groups have a pK_a of 1.2 (O'Connor, 1981). It is safe to assume that cytidine protonation by far exceeds the protonation of any of the other groups in poly(dG-dC)·poly(dG-dC). It was analyzed (O'Connor, 1976, 1981) that the following bands in cytidine at neutral pH are diagnostic bands for protonation: 596, 1214, 1241, 1290, 1454, and 1530 cm^{-1}. In agreement with a partial protonation of cytidine residues in poly(dG-dC)·poly(dG-dC) (Figure 5A) are the decrease in the weak band at 596 cm^{-1}, the weakening of the band at 1240 cm^{-1}, the increase in intensity of the band at 1259 cm^{-1}, and the shift in the band at 1530 cm^{-1} to 1538 cm^{-1}. From these changes and the fact that the pH is close to the pK_a of the cytidine residues, it is estimated that the fraction of cytidine residues that are actually protonated is 0.5 ± 0.1.

The Raman bands originating in the guanine residues in the partially protonated poly(dG-dC)·poly(dG-dC) show numerous differences from the bands in the classical B-form and Z-form. A single, intense band occurs at 1328 cm^{-1}, while a doublet at 1317 and 1334 cm^{-1} exists in B-form and an intense band at 1316 cm^{-1} exists in Z-form. A band at 1288 cm^{-1} occurs as a shoulder on the intense cytosine contribution at 1259 cm^{-1}, while this band is positioned at 1293 cm^{-1} in B-form and at 1290 cm^{-1} in Z-form. A weak and broad band at 1424 cm^{-1} replaces a strong band at 1420 cm^{-1} in B-form and a strong, narrow band at 1426 cm^{-1} in Z-form. These changes are all exemplary of the strong coupling of the guanine vibrational modes with the sugar vibrational modes and indicate the changes in base−sugar coupling upon protonation. The partial protonation of poly(dG-dC)·poly(dG-dC) leads to a shift of the marker band for the guanosine conformer to 675 cm^{-1}. This band occurs at 681 cm^{-1} in B-form ($C_{2'}$-endo/anti conformation) and at 625 cm^{-1} in Z-form ($C_{3'}$-endo/syn conformation). A band at 671 cm^{-1} has been assigned (Benevides et al., 1984) to a $C_{2'}$-endo/syn conformation of the guanosine residues in an unprotonated GC base pair. We conclude that this bands shifts to 675 cm^{-1} upon protonation of the base pair. The bandwidth of the 675 cm^{-1} band is comparable with the 681 cm^{-1} band (B-form) and the 625 cm^{-1} band (Z-form) and suggests that all the guanine residues are in a narrow range of conformational states. In the Raman spectrum of the protonated poly(dG-dC)·poly(dG-dC), no band occurs at 681 cm^{-1}. It is thus estimated that less than 5% of the guanosine conformers are in the $C_{2'}$-endo/anti conformation.

The intensity of the guanine band at 1489 cm^{-1} has decreased upon protonation. This vibration is known to be very sensitive to ligation or complexation at the guanine N_7 position (Guéron et al., 1990; Courtois et al., 1968; Zimmer et al., 1969). Since no direct protonation of this nitrogen atom takes place at pH 4.5, this change in intensity can only be ascribed to large structural changes of the helix that will place the N_7 position in guanine opposite the protonated N_3 position of cytosine. This structural pattern is typical for a Hoogsteen GC base pair. This type of base pairing can be accommodated by the antiparallel double-stranded helix (Pulleyblank et al., 1985) through a change in the guanosine conformer from $C_{2'}$-endo/anti (B-form) to $C_{2'}$-endo/syn present in protonated residues. Since only a fraction of the cytosine residues is actually protonated, this leads to a weak base pairing interaction. This fact is probably partially responsible for the decreased melting temperature of the protonated helix, apart from the fact that the number of hydrogen bonds for this arrangement must already decrease from three to two.

Bands that originate in the backbone of the helix have also changed. The antisymmetric PO_2-stretching mode couples with motions in the furanose sugar ring (Prescott et al., 1984). This band is observed at 824 cm^{-1} in the protonated helix. In the B-form poly(dG-dC)·poly(dG-dC), this band is observed at 830 cm^{-1}. This position is typical for the furanose ring in a $C_{2'}$-endo conformation (Prescott et al., 1984). In the Z-form spectrum, this band is observed at 810 cm^{-1}. The band due to the symmetric stretching vibration of the PO_2^- vibration at 1094 cm^{-1} in the protonated helix is comparable in intensity with the same band in the B-form but not with that in the Z-form, where it appears weaker. The strong band at 783 cm^{-1} in the B-form spectrum is composed of two accidentaly degenerate vibrations, one originating in the backbone and the other in the pyrimidine ring. In the protonated helix, this band shifts to 786 cm^{-1} from 783 cm^{-1} in the B-form and from 782 cm^{-1} in the Z-form. The backbone band at 793 cm^{-1} in the spectrum of the Z-form can be observed as a shoulder because the bandwidth of the cytosine band at 782 cm^{-1} is reduced. Other vibrational motions of the backbone that are more concentrated in the sugar−phosphate group can be observed between 850 and 1080 cm^{-1}. The Raman spectrum of the protonated poly(dG-dC)·poly(dG-dC) is also in this spectral region different from the Raman spectra of both the B-form as well as the high-salt-induced Z-form. It follows that Raman spectroscopy gives a clear fingerprint of the Hoogsteen base pairing and the resulting DNA structure.

The explanation presented above is not in disagreement with previous observations of the proton-assisted transition of the right-to-left-handed conformation (Chen, 1984; Kumar, 1990). The left-handedness of the protonated poly(dG-dC)·poly(dG-dC) was concluded from a negative band at 293 nm in the CD spectrum. A negative CD spectrum at 293 nm was obtained under widely varying conditions, i.e., pH 3.8 and 2.6 M [Na^+] (Chen, 1984), pH 3.4 and 0.01 M [Na^+] (Kumar, 1990), and pH 3.03 and 0.01 M [Na^+] (Kumar, 1990). Earlier Courtois et al. (1968) had obtained CD spectra at pH 3.1 and 0.15 M [Na^+] that did not show a negative band at 293 nm. This is seemingly in contradiction with the result of Chen (1984), who showed that at pH 3.8 the left-handed conformation could be induced when the [Na^+] was increased. In order to check the structure of protonated poly(dG-dC)·poly(dG-dC) at pH 3.1, we performed absorption, CD, and Raman measurements at pH 3.1 and 0.15 M

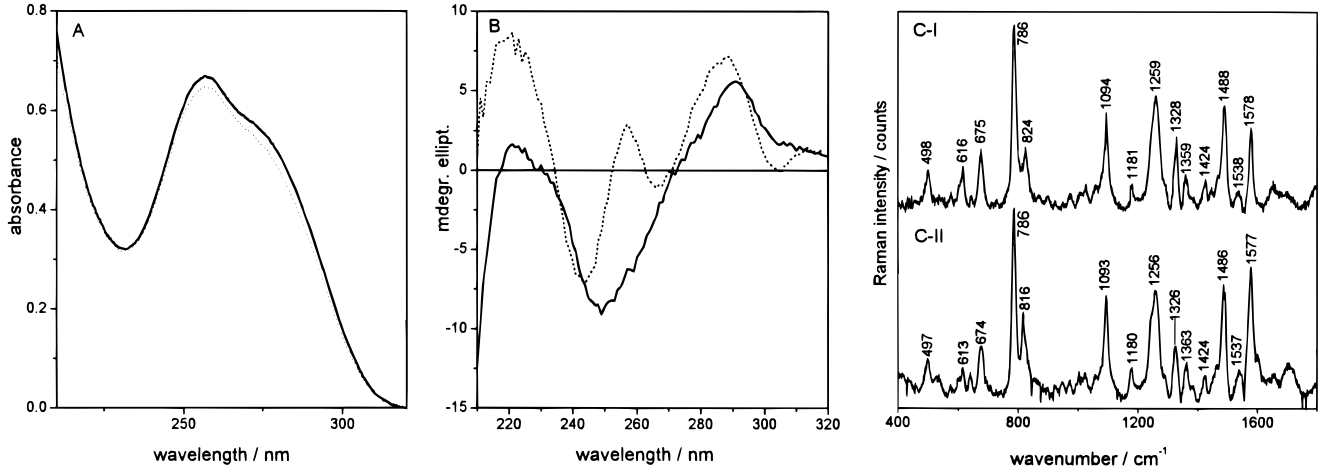

FIGURE 6: Comparison of the spectra of poly(dG-dC)·poly(dG-dC) obtained at pH 4.5 and 1 mM [Na^+] and obtained at pH 3.1 and 150 mM [Na^+]. Under both conditions, poly(dG-dC)·poly(dG-dC) has a double-stranded, Hoogsteen base paired structure. (A) Absorption spectra: solid line, pH 4.5 and 1 mM [Na^+]; dotted line, pH 3.1 and 150 mM [Na^+]. (B) Circular dichroism; ibid. (C) Raman spectra: C-I, pH 4.5 and 1 mM [Na^+]; C-II, pH 3.1 and 150 mM [Na^+].

[Na^+] and compared them with the results obtained in Figures 2, 4, and 5. For clarity, curves from these figures have been repeated in Figure 6. The absorption spectrum (Figure 6A) shows a close correspondence in shape. The CD spectrum (Figure 6B) shows a very weak CD spectrum (compared with the CD spectra of the B-form and the Z-form; Figure 4) in both cases. The spectrum at pH 3.1 is in close correspondence with that published by Courtois et al. (1968). The corresponding Raman spectrum is presented in Figure 6C (lower graph). It is observed to be in an almost one-to-one correspondence with the spectrum of the Hoogsteen base paired poly(dG-dC)·poly(dG-dC) (Figure 6C, upper graph). The Raman spectrum shows from the intensity of the band at 1259 cm^{-1} that the degree of protonation of poly(dG-dC)·poly(dG-dC) is not much different from that at pH 4.5. From the present results, it follows directly that the Hoogsteen base paired structure of weakly protonated poly(dG-dC)·poly(dG-dC) may exist between pH 3.1 and pH 4.5 if the amount of protons available in the solution is balanced by the proper amount of Na^+ ions. In a proton exchange study, Guéron et al. (1990) interpreted certain proton resonances observable at pH 2.6 and 0.1 M [Na^+] as resulting from Hoogsteen base paired and protonated GC^+ residues. It was mentioned that the first indication of these proton resonances could be observed at pH 3.2 (and 0.1 M [Na^+]). Although this latter condition is compatible with a double-stranded Hoogsteen base paired structure, the conditions at pH 2.6 and 0.1 M [Na^+] may correspond with a different and left-handed structure of poly(dG-dC)·poly(dG-dC). In Figure 7 information has been compiled that gives credence to this argument.

In Figure 7, the results are collected from the literature (Courtois, 1968; Zimmer, 1969; Chen, 1984; Kas'nayenko, 1986; Guéron, 1990; Kumar, 1994) together with the results presented in this paper (closed squares). The open triangles represent measurements where a change of handedness was observed from the CD spectrum. Open circles indicate that a structural change in DNA was observed without evidence for a change in handedness of the DNA. The Raman, absorption, and CD spectra in Figures 4−6 support the conclusion that the structural change in DNA is accompanied by a change in base pairing from Watson−Crick to Hoogsteen. A clear dependence is observed for the formation of Hoogsteen base pairs on the interplay between pH and ion

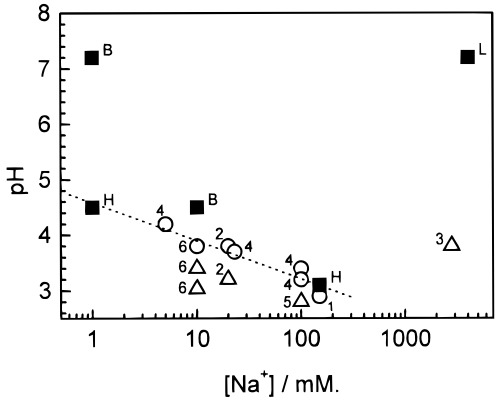

FIGURE 7: Structural properties of poly(dG-dC)·poly(dG-dC) in its dependence on pH and [Na^+] concentration. The labels "B", "H", and "L" signify respectively B-form poly(dG-dC)·poly(dG-dC), Hoogsteen base paired poly(dG-dC)·poly(dG-dC), and left-handed poly(dG-dC)·poly(dG-dC). The closed squares are measurements presented in this paper. The open circles are measurements indicating a large change in DNA structure, but no observable change in handedness from CD data. The open triangles indicate data where such a change in handedness was observed. The open circles and triangles are connected to the following papers by a number: 1 (Courtois, 1968); 2 (Zimmer, 1969); 3 (Chen, 1984); 4 (Kas'nayenko, 1986); 5 (Guéron, 1990); 6 (Kumar, 1994). The occurrence of the Hoogsteen base paired structure is observed in a narrow range of pH values and ion concentrations. The dashed line is merely a guide to the eye.

concentration. The presence of the Hoogsteen base paired structure exists as a narrow band between the B-form poly(dG-dC)·poly(dG-dC) and a form that is characterized as a left-handed form by the negative band at 293 nm in the CD spectrum. The graph shows that poly(dG-dC)·poly(dG-dC) in the Hoogsteen base paired structure adopts a B-form again under a slight increase in salt concentration. This is in agreement with Chen (1984). This observation is not in agreement with the proposal of Guéron et al. (1990) that even at neutral pH the Hoogsteen base pairing may exist. Including the stabilization by ions of the B-form versus the Hoogsteen base paired structure prevents the formation of Hoogsteen base pairs in poly(dG-dC)·poly(dG-dC) at neutral pH and physiologically relevant ion concentrations. It can be estimated from Figure 7 that only at approximately 10 mM [Na^+] Hoogsteen base pairs may exist at neutral pH.

CONCLUSION

A right-handed to left-handed helical transition in poly(dG-dC)·poly(dG-dC) can be induced by increasing salt concentrations and by increasing proton concentrations. The salt-induced transition maintains a Watson−Crick type of base pairing. It is shown in this paper that as the proton concentration is increased a transition in duplex poly(dG-dC)·poly(dG-dC) occurs that consists of Hoogsteen base paired GC residues. The DNA duplex is characterized by a $C_{2'}$-endo/syn conformation of the guanosine residues, a hydrogen bridge connecting to the N_7 group of guanosine, a highly cooperative, single transition in the melting curve, and a weak CD spectrum that is positive above 280 nm. It follows that the proton-induced right-handed to left-handed transition does not preserve the type of base pairing. This in contrast with the salt-induced right-handed to left-handed transition at neutral pH. Furthermore, it is shown that the occurrence of Hoogsteen base pairing also takes place at pH 3.1 if the increase in proton concentration is balanced by an increase in $[Na^+]$.

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