Does DNA acid fixation produce left-handed Z structure?

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Abstract The effects of acetic acid ($HCOOCH_3$) on the solution structure of calf-thymus DNA are studied at pH 7.3–2.5 with acid/DNA(P) (phosphate) molar ratios (r) of 1/40, 1/20, 1/10, 1, 2, 10, 20 and 40. Fourier Transform infrared (FTIR) difference spectroscopy is used to establish correlations between spectral changes and base protonation, DNA conformational transition and structural variations of the acid–DNA complexes in aqueous solution. The FTIR difference spectroscopic results showed that protonation of cytosine and subsequent unpairing of the G-C base pairs begins at pH 4–3 and continues up to pH 2.5, where a complete base separation and base unstacking occur. Similarly, protonation of A-T base pairs starts at pH 4–3 and is completed at pH 2.5, where base separation and base unstacking are observed. The protonation of the G-C base pair leads to the formation of Hoogsteen-type H-bonding, before a complete G-C disruption. The biopolymer protonation leads to the formation of several non-B-DNA structures, including left-handed Z conformation.

Key words: DNA; Acid; Denaturation; Conformation; FTIR spectroscopy

1. Introduction

In recent years, growing interest has been generated in DNA conformation other than the commonly known B structure. It has been demonstrated that the kinetics of B to Z transition in poly(dG-dC). poly(dG-dC) is strongly affected by protonation, which leads to the assumption of a possible induction of Z-structure or some left-handed conformation at low pH [1]. So far, the formation of a true Z-DNA structure has not been reported for both synthetic or native DNAs upon acid fixation. The formation of a triple helices H-form and a parallel stranded helical conformation involving oligonucleotides is influenced by base protonation [2,3]. The indication of the existence of Z-DNA in acid-fixed polytene chromosomes [4,5] and the idea of the formation of non-B-DNA structures prompted extensive investigations on the structural flexibilities of the acid-fixed DNA complexes [6–10]. As it is increasingly evident, the low pH has a significant influence in the stability of several novel conformations of DNA duplex [1–10]. These conformations in turn would be of considerable biological interest in their possible role in DNA recombination or in RNA structures. Therefore, it is important to study the effects of acid-fixed DNA structures in more detail and to analyse the non-B-DNA conformations formed during biopolymer protonation. Vibrational spectroscopy is a powerful tool, which has been often used to characterize the nature of acid–DNA interaction [6,9,11–13].

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In this work, FTIR difference spectroscopy is used to study the effects of the acetic acid on the solution structure of the calf-thymus DNA at different H^+/DNA(P) molar ratios (r) of r = 1/40 to 40 and pH 7.3–2.5. Comparisons are also made between acid-fixed DNA and those of the corresponding DNA denaturation by heat [14] and the results are discussed in the text. Furthermore, the effect of the acid fixation on DNA backbone conformation is evaluated and the presence of several non-B-DNA structures is reported here.

2. Experimental

2.1. Materials and methods

Highly polymerized calf-thymus DNA sodium salt (7% Na content) was from Sigma and used as supplied. Acetic acid and other chemicals were reagent grade and used without further purification.

2.2. Preparation of stock solution

Na-DNA is dissolved to 4% w/w (0.1 M DNA(phosphate) in 0.1 M NaCl solution at 5°C for 24 h with occasional stirring to ensure the formation of homogeneous solution. The appropriate amounts of HCH_3COO (1 mM to 2 M) is added dropwise to DNA solution to give a mixture of desired H^+/DNA(P) molar ratios of 1/40 to 40 at a final DNA concentration of 2% w/w or 0.05 M DNA(phosphate). The pH solution is adjusted to 7.3–2.5, using acetic acid or NaOH solution.

IR spectra are recorded on a Bomem DA3–0.02 FTIR spectrometer equipped with a nitrogen-cooled HgCdTe detector and KBr beam splitter. The solution spectra are taken using AgBr windows with resolution of 2–4 cm^{-1} and 100–500 scans. Water subtraction is carried out as in our previous report [15]. A good subtraction is achieved as shown by a flat baseline around 2200 cm^{-1} where water combination mode is located [15]. The difference spectra [(DNA solutioin+acid solution)−(DNA solution)] are produced, using a DNA band at 968 cm^{-1} as internal standard. This band, due to deoxyribose C-C stretching vibrations, exhibits no spectral changes (shifting or intensity variations) on DNA complex formation and it is cancelled upon spectral subtraction.

3. Results and discussion

3.1. Acid–DNA complexes

At low acid concentration r = 1/40 (pH 6.10), no major IR spectral changes are observed for calf-thymus DNA on proton complexation. A small increase in intensity is observed for several DNA in-plane vibrations at 1717 cm^{-1} (G,T), 1663 cm^{-1} (T,G,A,C), 1609 cm^{-1} (mainly A) and 1492 cm^{-1} (C,G) [15–19] in the difference spectra of acid–DNA complexes (Fig. 1, r = 1/40). The calculated intensity ratios of these vibrations as a function of proton concentration are shown in Fig. 2. Similarly, the band at 1717 cm^{-1} (G,T) shifts towards a lower frequency at 1715 cm^{-1}, while the PO_2 antisymmetric stretching mode at 1222 cm^{-1} is observed at 1217 cm^{-1} on acid interaction (Fig. 1). The observed spectral changes are due to an indirect proton interaction (via H_2O) with the backbone PO_2 group, since base protonation occurs at lower pH (pH <5) for cytosine N-3 (pK_a 4.24), adenine N-1 (pK_a 3.20) and guanine N-7 (pK_a 2.3) [20].

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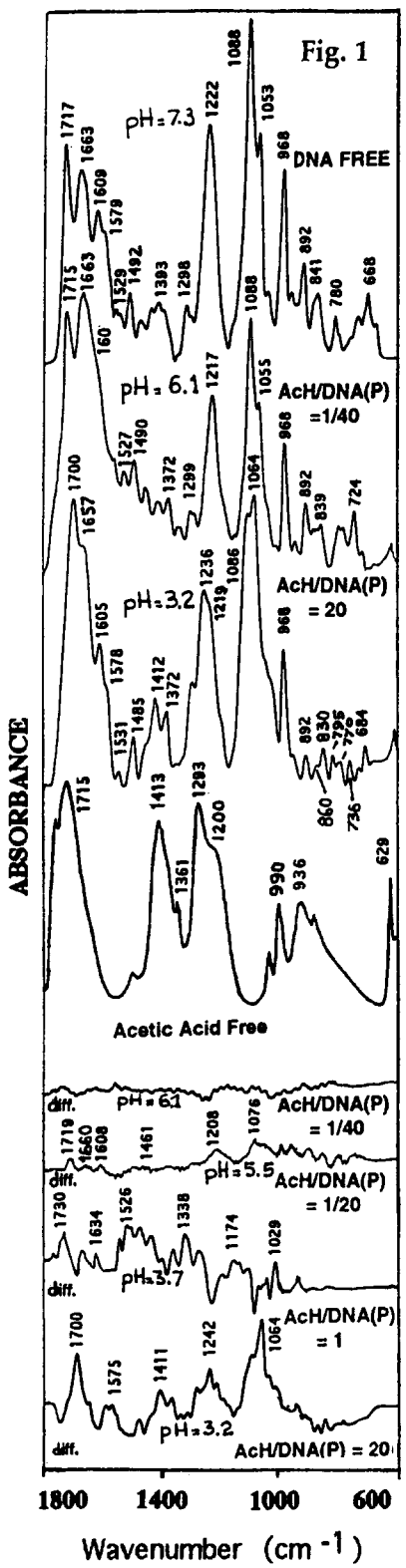

Fig. 1. FTIR spectra and difference spectra [(DNA solution+acetic acid solution)-(DNA solution)] of calf-thymus DNA and its proton complexes at different pHs and H^+/DNA(P) molar ratios in the region of 1800-600 cm^{-1}.

As proton concentration increases r >1/20 (pH <5), a major intensity increase is observed for the bands at 1717, 1663, 1609, 1492 and 1222 cm^{-1}, with positive derivative features at 1719, 1660, 1608, 1461 cm^{-1} (DNA bases) and 1208, 1076 cm^{-1} (PO_2 band) in the difference spectra of acid-fixed DNA complexes (Figs. 1,2, r = 1/20). It is important to note that the major intensity variations observed for the DNA in-plane vibrations are accompanied by the shift of the mainly G-C bands at 1717 to 1713 cm^{-1}, 1529 to 1527 cm^{-1} and 1492 to 1490 cm^{-1}, where the A-T bands at 1663, 1609 and 1579 cm^{-1} exhibit no major spectral shiftings at this stage. This indicates the begining of the base protonation at the G-C pairs, which is also consistent with other spectroscopic evidences that showed major structural alterations for the G-C rich region than that of the A-T bases, when DNA protonation begins [21–23]. The protonation of the G-C base pairs continues as proton concentration increases with major shift of the mainly G-C bands at 1717 (G,T), 1492 cm^{-1} (C,G) towards lower frequencies at 1700 and 1485 cm^{-1}, respectively, while the 1529 cm^{-1} (mainly C) shifts towards a higher frequency at 1531 cm^{-1} (Fig. 1, r = 20 and pH 3.2). The complete base separation of the G-C base pair occurs at higher proton content (r = 40 and pH 2.5 not shown), where further downshift of the G-C vibrations at 1717 to 1695 cm^{-1} and 1492 to 1480 cm^{-1}, with the upshift of the band at 1529 to 1533 cm^{-1} were observed (spectra is not shown). The base unstacking also occurs at high proton concentration (r = 20), with major intensity increase of the G-C bands at 1717, 1492 and 1429 cm^{-1} (Fig. 2). It is important to note that similar increase in the intensity was observed for several G-C in-plane vibrations, in the Raman spectra of calf-thymus DNA during G-C disruption and base unstacking upon thermal denaturation (14).

One of the important features of DNA acid fixation is the formation of protonated Hoogsteen-type G-C base pairs. Recent Raman spectroscopic studies on chromosomes and calf-thymus DNA provide evidence for the reversible formation of protonated G-C base pairs, upon acid fixation [6]. It has also been suggested that protonated Hoogsteen-type G-C base pairs may exist as a minor species in chromosomal DNA under physiological conditions (at pH 7, about 0.01% of the G-C base pairs may be in the protonated Hoogsteen form because the midpoint of transition of Watson-Crick to protonated G-C base pairs is around pH 3). On the basis of intensity variations of the Raman marker lines at 681 cm^{-1} (guanine) and 1488 cm^{-1} (guanine), evidence for the conversion of the guanine conformation from C2′-endo/anti (in B structure) to that of the C3′-endo/syn (left-handed) has been provided [6]. Our present IR spectroscopic results of the acid-fixed DNA complexes also provide direct evidence for guanine C2′-endo/anti conversion to C3′-endo/syn conformation. As protonation progresses, a weak IR band at 790–795 cm^{-1} (C3′-endo/syn) in the DNA spectrum gains intensity, while the B-DNA marker band at 841 cm^{-1} (phosphodiester mode in C2′-endo/anti) losses its intensity and shifts towards a lower frequency (830 cm^{-1} C3′-endo/anti) (Fig. 1, r = 20). The emergence of the band near 795 cm^{-1} is attributed to the conversion of guanine C2′-endo/anti to C3′-endo/syn conformation, since in the left-handed Z-DNA structure a band with medium intensity occurs at about 785 cm^{-1} (C3′-endo/syn), while the band near 840 cm^{-1} (C2′-endo/anti) shifts towards a lower frequency at 830 cm^{-1} [24,25]. Similarly, the shifting of the marked IR band at 1717 cm^{-1} (G,T) to 1700 cm^{-1} is also evidence for the formation of the left-handed Z structure. It should be noted, that in a true Z-DNA structure, the marker IR bands at 1717 cm^{-1} (G,T) shifts towards lower

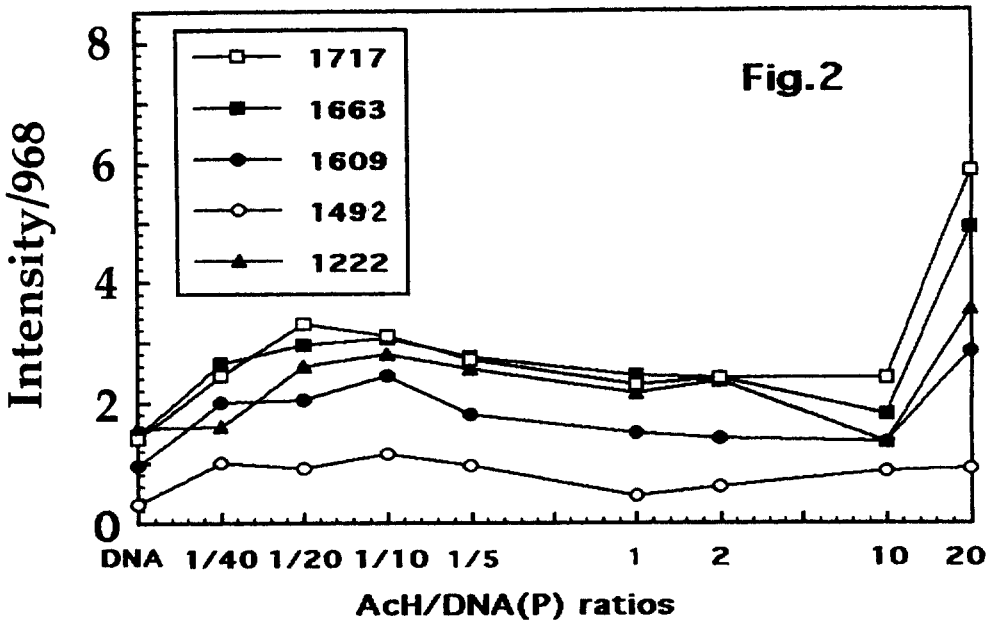

Fig. 2. Calculated intensity ratios of several DNA in-plane vibrations (cm^{-1}) due to the A-T and G-C base pairs and the backbone PO_2 group as a function of acid concentration (AcH/DNA (P) molar ratios) for r = 1/40 (pH 6.10), r = 1/20 (pH 5.50), r = 1/10 (pH 5.00), r = 1/5 (pH 4.40), r = 1 (pH 3.80), r = 2 (pH 3.60), r = 10 (pH 3.40), r = 20 (pH 3.20) and r = 40 (pH 2.50).

frequency at 1700-1690 cm^{-1} the PO_2 antisymmetric stretching band at 1222 appears at 1215 cm^{-1} and the sugar-phosphate near 840 cm^{-1} moves towards 830 cm^{-1} and a new band appeas near 790 cm^{-1} [24]. Other IR evidences to support the formation of the Hoogsteen-type G-C structure come also from major intensity variations and shiftings of the two mainly C-G bands at 1529 (C,G) and 1492 cm^{-1} (C,G). As protonation continues, a gradual shift of the band at 1492 cm^{-1} towards a lower frequency at 1490–1485 cm^{-1} is observed, while the other band at 1529 cm^{-1} shifts towards a lower frequency at 1527 cm^{-1} in the begining and then to a higher frequency at 1531 cm^{-1} (Fig. 1, r = 20). Further shift of these two bands to 1480 and 1533 cm^{-1} are also observed as protonation leads to a complete disruption of the G-C base pairs (r = 40, spectra is not shown). It is also worth mentioning that cation-DNA interaction with the guanine N-7 site leads to a major increase in intensity and shifting of the band at 1492 cm^{-1} towards a lower frequency [15]. Similarly, the band at 1493 cm^{-1} (N-7-C-8 mode) in the IR spectrum of the hydrated Na_2-GMP (guanosine-5′-monophosphate) salt is shifted towards a lower frequency at 1479 cm^{-1} in the N-7-protonated H_2-GMP acid [26]. Thus, it is reasonable to assume that the major shift of the band at 1492 cm^{-1} towards a lower frequency (Dn = 7 cm^{-1}) in the acid fixed DNA complexes is due to the protonation of the guanine N-7 site and the formation of the Hoogsteen-type G-C structure during DNA acid fixation. Similar behaviors are observed in the Raman spectra of DNA of polytene chromosomes upon acid fixation, where the mainly guanine line at 1488 cm^{-1} exhibited major modifications upon Hoogsteen-type G-C hydrogen bond formation [6]. Other spectroscopic evidences also suggest the formation of the Hoogsteen-type of G-C base pairs during acid–DNA complexation [27,28].

The protonation of adenine bases occurs at r >1/10 (pH <5), where the mainly A-T bands at 1663 and 1609 cm^{-1} shift towards lower frequencies at 1657 and 1605 cm^{-1}, respectively (Fig. 1). The protonation of the A-T bases is also accompanied by intensity increase of the bands at 1663 and 1609 cm^{-1} (Fig. 2). As acid concentration increases, the downshift of the A-T bands continues to 1653 cm^{-1} and 1600 cm^{-1} respectively, where a complete separation of the A-T bases occurs (r = 40, pH 2.5). The base unstacking of the A-T region also followed by major intensity increase of the A-T bands at 1663 and 1609 cm^{-1} (Fig. 2, r >20). Similar intensity increase is observed for several A-T in-plane vibrations in the Raman spectra of calf-thymus DNA upon thermal denaturation [14].

The main question, which can be raised here is to what extent the A-T backbone conformation has been altered during acid fixation. The A-T backbone losses its B structure as protonation progresses. Evidence for this comes from the loss of intensity and shifting of the IR marker band at 841 cm^{-1} (sugar-phsophate mode in C2′-endo/anti) to 830 cm^{-1} (C3′-endo/anti) in acid-fixed DNA complexes (Fig. 1). The major intensity variations and shifting of the A-T bands at 1663 and 1609 cm^{-1} towards lower frequencies at 1657 and 1605 cm^{-1} are also evidences for A-T structural changes upon base protonation. It is important to note that although the G-C protonation occurs prior to that of the A-T bases, the complete disruption of the G-C base pairs continues after A-T bases are completely separated. Evidence for this comes from major spectral changes of the G-C vibrations during acid fixation up to pH 2.5 (r = 40), whereas the overall spectral alterations of the A-T bands occur above pH 2.5.

3.2. DNA conformation

Recently, the IR marker bands for B-, A-, C- and Z-DNA structures are reported and the conditions for the formation of each conformation are determined [25]. The IR spectra of the acid-fixed DNA complexes show the presence of several non-B-DNA structures in coexistance, before complete helix denaturation. The presence of the two components of the PO_2

antisymmetric stretch at 1219 and 1236 cm^{-1} with the phosphodiester band at 830 cm^{-1} (C3′-endo/anti) and the carbonyl band at 1700 cm^{-1} (T,G) are attributed to the C-DNA structure, while the presence of a weak band at 860 cm^{-1} with a component band of the backbone PO_2 group at 1236 cm^{-1} is due to A-DNA conformation (Fig. 1, r = 20). On the other hand, the presence of a marker band at 1700 cm^{-1} (carbonyl stretchings of G and T) with the sugar-phosphate bands at 830 cm^{-1} (C3′-endo/anti) and 795 cm^{-1} (C3′-endo/syn) are coming from left-handed Z-DNA structure (Fig. 1). Although the B-DNA structure is strongly perturbed by acid fixation, the major structural alterations come at high proton concentration (pH <5), where the B structure is chiefly altered to those of the A, C and Z conformations. The gradual reduction of the intensity observed for several G-C and A-T vibrational frequencies at pH 5–3.4 (r = 1/10 to 10) are attributed to the alteration of the B-DNA structure and the formation of the non-B-DNA conformations, which is consistent with the presence of the IR marker bands observed in the spectra of acid-fixed DNA complexes (Figs. 1 and 2).

4. Concluding remarks

The IR results presented here clearly show that acid fixation induces conformational changes in native DNA and promotes the formation of local left-handed Z structure. However, it is worth mentioning that earlier studies led to the suggestion that N-3 of cytosine is the initial site of protonation, due to the fact that cytosine has the highest pKa [29,30], while recent spectroscopic investigations propose the formation of Hoogsteen-type G-C base pairs, which results from protonation of guanine N-7 site and the subsequent anti to syn conformational transition and share the proton with N-3 of cytosine base [21,27]. Recent NMR study of oligonucleotide suggests the protonation of the cytosine N-3 resulting in an anti parallel orientation in syn conformation and forming two bonds with guanine base [31]. Other NMR spectroscopic studies on the oligonucleotides indicate that the protonation of cytosine at first is followed by adenine and consequently adopts a parallel stranded double helix with symmetrical non-Watson-Crick homobase pairs [32]. The formation of triple helical H-form by homopurine-homopyrimidine tracts, involves the protonated oligo (dC^+) strand folding back to the major groove of helix and forming Hoogsteen-type dG-dC^+ base pairs [2,33]. Recent Raman spectroscopic results also show the formation of Hoogsteen-type G-C base pair for the acid-fixed polytene chromosomes [6]. The formation of Z structure, however, requires the alteration of anti to syn conformation for guanine bases, which also has been observed in the crystal structure of protonated duplexes with G-C^+ base pairs [33]. Furthermore, the pH dependence of the kinetics of B to Z transition [1] and a major increase of anti Z-DNA antibody binding to *Chironomous thummi* larvae chromosomal DNA when DNA is exposed to low pH [34], support our observations. Our IR results presented here for the first time show direct evidence (IR marker bands) for the formation of the Hoogsteen-type G-C base pairs and the coexistance of several non-B-DNA structures, such as A, C and Z conformations, during acid fixation.

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