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Infrared and X-ray diffraction study of the effect of protonation of DNA on its B-to-A transition

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The influence of H^+ on the secondary structure of DNA and on its B-to-A transition has been studied by employing X-ray diffraction and infrared spectroscopy. Helical parameters for DNA molecules with different degrees of protonation were determined. It was shown that H^+ binding stabilizes the B-form of DNA in fibers over a wide range of water and inorganic salt content. Only 0.03 H^+ bound per nucleotide is sufficient to prevent the B-to-A transition caused by decreasing relative humidity in DNA fibers containing 4% NaCl. The effectiveness of B-form stabilization by H^+ is explained by changes in DNA-solvent molecule interactions, especially in the major groove of double helices.

1. Introduction

Since the discovery in 1953 by Franklin and Gosling [1] of DNA polymorphism, the question still remains as to the manner in which the solvent promotes adoption of a definite conformation by the double helix. In this respect, particular attention has been paid to the identification of the mechanism of stabilization of the B-form and the driving forces of the B-to-A (or A-to-B) transition in DNA [2–5].

During studies in which Dickerson et al. [6,7] localized some of the water molecules in the hydration shell of d(CGCGAATTCGCG) crystals, the authors detected a chain of H-bonded water molecules in the minor groove of the double helix which they designated 'the water spine'. The authors suggested that it was the water spine that provided the driving force inducing the DNA to adopt the B-form due to interactions between the water spine and the adenylate and thymidylate residues. These interactions gave rise to a fixed arrangement of the adjacent base-pairs in the appropriate conformation.

Another hypothesis was put forward in an X-ray diffraction study of the positions of Cs^+ in DNA fibers [8], according to which the existence of the B-form of DNA is due to stabilization of the width of the minor groove by cations (or hydrated cations) located within the groove.

These hypotheses on the mechanisms of stabilization of the B-form qualitatively explain why some double helices with specific base sequences, such as poly(dA) · poly(dT), prefer the B-form over a wide range of relative humidities [9], whereas other polynucleotides, e.g., poly(dG) · poly(dC), favor the A-form [10].

However, the hypotheses do not explain why, in particular, poly(dG) · poly(dC) and poly(dAAG) · poly(dCTT) which have no water spine in their minor grooves still adopt the B-form at high relative humidities (R.H.) and, conversely, why poly(dAG) · poly(dCT) does not adopt the A-form even at 66% R.H. [11]. It is also unclear as

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to the reason why raising the salt content in DNA fibers stabilizes the B-form against low relative humidity [12,13].

The main limitation of the current hypotheses on the role of solvent in stabilization of the B-form is that they take into account only hydration of the minor groove of the double helix, namely, of the A · T base-pairs. Therefore, it would appear reasonable to consider also the role of the solvent in hydration of the major groove, in particular, of G · C pairs. To do so, it is necessary to use agents which act specifically on the G · C pairs from the major groove side of DNA. In our opinion, H^+ may fit the requirements.

It is well known that at low degrees of DNA protonation, H^+ binds with G · C pairs in the double helix [14–16]. There are reasons to believe that H^+ binds with the N_7 atoms of the guanine residues from the major groove side of the DNA molecule [17]. After being protonated, guanine may change to a zwitterionic form via transfer of a proton from the N_1 of guanine to N_3 of cytosine [18]. As a result, a number of specific changes appear in the ultraviolet, Raman and NMR spectra due to cytosine protonation [15,19,20]. Thus, one can use protons as a means for investigating the role of the solvent in the formation of the DNA molecular structure.

In this work, we studied the effect of DNA protonation on the B-to-A transition and attempted to elucidate its mechanism. Films and fibers of DNA at degrees of protonation remote from that of acid denaturation were investigated by X-ray diffraction and infrared spectroscopic techniques.

2. Materials and methods

DNA from calf thymus and sturgeon sperm (molecular mass 15 MDa) containing less than 1% protein and RNA impurities was used. All stock solutions were dialysed at 4° C for 24 h against 0.1 M NaCl and 10 mM EDTA, followed by 2 days vs. 10 mM NaCl, and then lyophilized.

DNA films and fibers of different degrees of protonation were obtained via the following three procedures:

(i) HCl solution was added to the lyophilized DNA preparation or to its solution in 10 mM NaCl. The degree of protonation of the DNA in the films and fibers obtained from such acidified solutions was estimated based on the consideration that all H^+ added was bound to DNA. This assumption is valid when the concentration of H^+ added to the DNA solution is much lower than the concentration of DNA (approx. 20 mM), i.e., at pH down to 3.5. The validity of this assumption was checked by referring to the recovery of pH on dissolving the acidified DNA film (or fiber) in the same volume of water from which it had been obtained. For example, when a DNA film of degree of protonation (α) 0.13 was dissolved in the same volume of water from which it had been initially prepared, the resulting DNA solution had a pH value (4.10) close to the initial one (3.95). Such pH shifts did not occur for $\alpha < 0.1$. Thus, the calculations provide at least the upper limit of the α value.

(ii) Films and fibers of unprotonated DNA were placed in a sealed chamber under the vapors of saturated NaCl solution and 0.5 M HCl. This procedure permitted us to record the infrared spectra of DNAs of differing degrees of protonation for one and the same film, to control the water content in the film and to eliminate the dependence of the infrared spectra on film thickness.

(iii) DNA solution was dialysed against deionized water. Under these conditions the Na^+ counterions were partially replaced by H^+, which resulted in DNA protonation. The degree of DNA protonation in a film obtained from such a solution was calculated on the basis of its Na^+ content determined by flame photometry.

DNA films were made by drying drops (150–200 μl) of 3 mM DNA solution on fluoride plates at a constant temperature of 23° C and R.H. 93%. To record the infrared spectrum, a plate with the film on it was mounted in a sealed chamber in which the appropriate relative humidity was maintained by saturated solutions of inorganic salts [20]. Prior to measurements, the samples were stored under an atmosphere of the given relative humidity for not less than 12 h. This time interval was found to be sufficient for hydration

equilibrium in the DNA film to be reached.

For fiber preparation, a piece of DNA gel was dried in a special holder and kept at 93% R.H. and 8–10° C for 2 days. The piece was stretched in the holder and the fiber obtained was oriented by surface tension forces. Prior to diffraction experiments, the DNA fiber was placed in an X-ray diffraction microchamber and stored at a constant relative humidity for not less than 12 h.

Infrared spectra were registered on a UR-10 spectrophotometer. The Na^+ content of samples was determined with an FPL-1 flame photometer. The pH of solutions was evaluated by a pH-340 pH-meter. X-ray diffractograms were made with a BSV-22 tube as radiation source and a chamber with a sample-to-film distance of 5 cm.

3. Results

Most of the data were obtained on DNA samples protonated via procedure (i), i.e., by addition of HCl to DNA solutions or lyophilized samples.

3.1. X-ray-diffraction data on the influence of protonation on the B-to-A transition in DNA

The degree of DNA protonation varied from 0 to 0.5. Films and fibers contained 2–6% excess NaCl. Diffractograms of the fibers were obtained over the range 0–93% R.H.

For all relative humidity values at $\alpha > 0.3$ and for all α values at R.H. $\leqslant 35\%$, there was only a wide diffuse spot at the center of DNA diffractograms. This indicated the absence of any regular arrangement of the macromolecules and their monomer units under the conditions stated.

At high relative humidity values (84–93%) the DNA fibers of protonation degree 0–0.3 gave B-type diffractograms (fig. 1a). Beginning from values as low as $\alpha \simeq 0.05$ the diffractograms became different from that characteristic of unprotonated DNA. The distances between the layer lines increased slightly thus indicating decreased pitch and increased twist of the double helix. Moreover, all the reflections were smeared out because of the disordering of macromolecules (fig. 1b).

At R.H. $< 81\%$ an A-type diffraction pattern was observed for unprotonated DNA fibers (fig. 1c). During DNA protonation it changed drastically. A diffuse diagonal cross appeared on the A-form diffraction pattern even at a very low degree of DNA protonation ($\alpha = 0.002$ in the samples containing 4% NaCl, i.e., 1.5 Na^+ per nucleotide) (Fig. 1d). The intensity of the cross increased with α, whereas those of the A-pattern spots decreased. The DNA diffractograms in the range α 0.002–0.02 can be interpreted as being a superimposition of patterns characteristic of the crystalline A-form and the paracrystalline B-form. At $\alpha > 0.03$ only the 10_1 type of double helix was seen in the diffractograms (fig. 1e).

Fig. 2 shows plots which we termed 'X-ray diffraction titration curves'. Here, one can discern the range of values where changes in the diffraction pattern of DNA take place. The S-shaped curves represent the dependence of the I_{130}/I_{110} ratio on the degree of DNA protonation, where I_{130} and I_{110} denote the integral intensities of reflections 1 3 0 and 1 1 0, respectively, The former is observed only in the A-type DNA diffractograms, the latter being more characteristic of those of the B-type. Raising the degree of DNA protonation resulted in an increase in I_{110} and a decrease in I_{130}. Hence, the B-type diffraction pattern became more intense and distinct on the A-DNA diffractograms (fig. 1d). The I_{130}/I_{110} ratio which was equal to 2.1 for the pure A-form gradually fell to values of less than 1 at $\alpha \simeq 0.01$ and approx. 0 at $\alpha = 0.03$ (since the reflection 1 3 0 could not be distinguished from the background).

Helical parameters of DNA molecules for a number of values are listed in table 1. The final row in table 1 gives the helical parameters of the Li DNA C-form [23] for purposes of comparison.

The X-ray diffraction data obtained show that DNA protonation (up to $\alpha = 0.03$ for DNA fibers containing 2–6% NaCl) prevents the B-A transition in the double helix induced by a decrease in relative humidity. It should be noted that the observed stabilization of the B-form in the range $\alpha = 0.03$–0.05 is partly reversible. Prolonged (> 1 week) exposure of such DNA fibers to an atmosphere of 20% R.H. induced transformation from

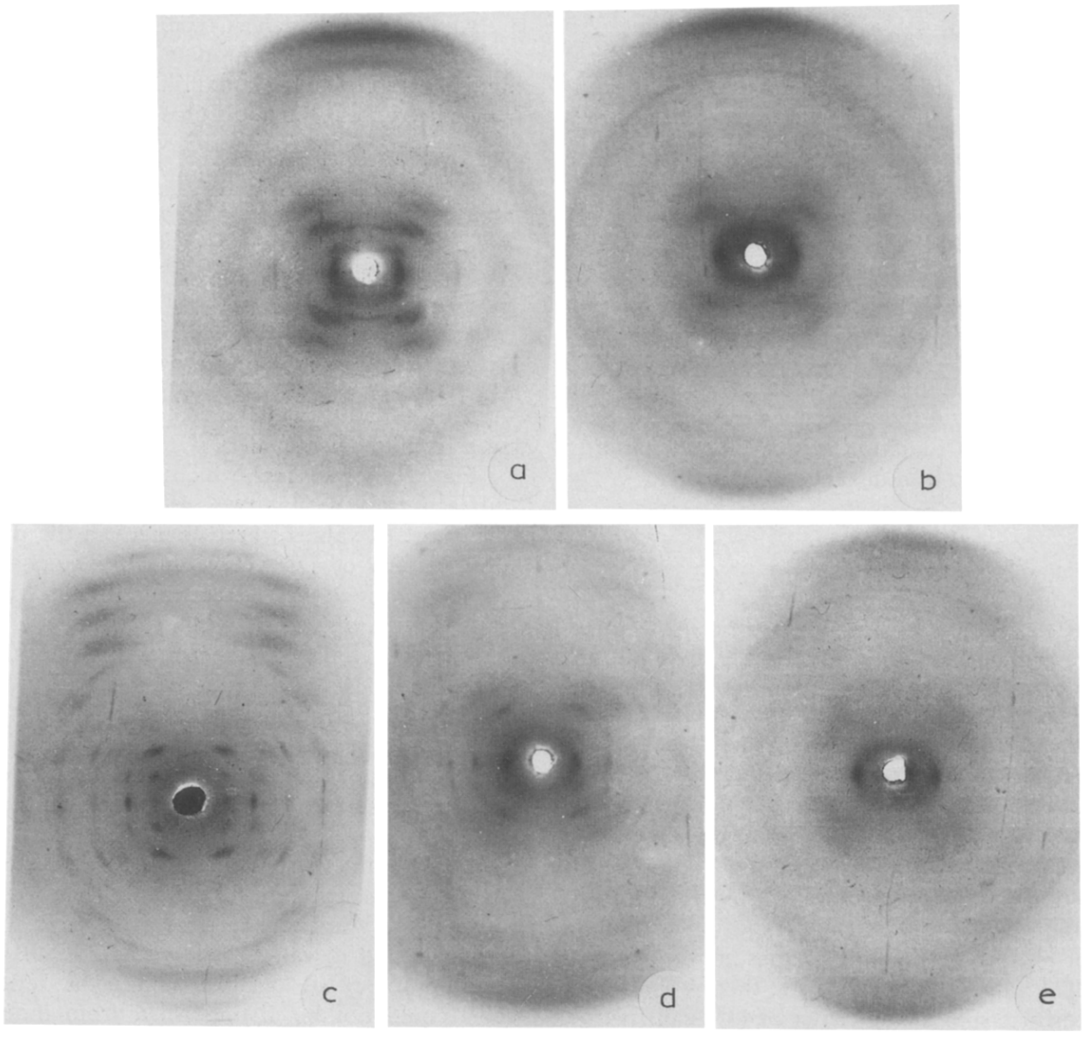

Fig. 1. X-ray diffractograms of DNA fibers of different degrees of protonation (α). Relative humidities: 93%, $\alpha = 0$ (a), $\alpha = 0.05$ (b); 75%, $\alpha = 0$ (c), $\alpha = 0.004$ (d), $\alpha = 0.05$ (e). All fibers contained 4% NaCl. $T = 20\,^{\circ}C$.

the pure B-form to a mixed A + B form. No restoration of the A-form was observed at $\alpha > 0.05$. At these degrees of protonation the double helix becomes more twisted and compressed along the helical axis. As a result, its helical parameters approach those of C-DNA, which was most pronounced at $\alpha = 0.15$.

The conclusion that DNA protonation prevents the B-to-A transition raises the question of whether the binding of H^+ can induce the transition of DNA from the A- to B-form. To resolve this problem, DNA fibers were protonated at a constant 75% R.H. via procedure (ii), i.e., in HCl vapor. Protonated DNA showed drastic structural changes similar to those obtained via procedure (i) (fig. 1c–e). The contribution of the A-type diffraction pattern gradually disappeared and after 36 h exposure of the DNA fiber to the vapor of 0.5 N HCl only a diffuse B-type diffraction pattern remained. This implies that H^+ is able not only to

Table 1

The parameters of Na^+ DNA helices at different degrees of protonation

Relative humidity, (%)	Degree of protonation (α)	p (Å)	P (Å)	τ (°)	Number of base pairs per turn
	0.0	3.38	33.8	36.0	10
93	0.03	3.38	33.6	36.0	10
	0.15	3.32	32.5	36.8	9.8
	0.0	2.56	28.2	32.7	11
75	0.03	3.32	32.1	37.2	9.7
	0.15	3.31	31.0	38.4	9.4
C-form of Li DNA ($\alpha = 0$)		3.30	30.8	38.6	9.3

stabilize the B-form but also to transform DNA from the A- to B-form.

DNA protonation in the fiber by HCl addition via procedures (i) and (ii) leads to an increase in the free NaCl content because of partial replacement of Na^+ by H^+. Therefore, for the fiber the water content also increases slightly [24]. As a result, H^+ might indirectly influence the DNA secondary structure by increasing the water and/or salt content. To exclude the possibility of such an indirect effect, the DNA was protonated via procedure (iii), i.e., by dialysis vs. deionized water. In this case, the DNA fibers do not contain excess inorganic salt at any degree of protonation. Moreover, the water content decreased with DNA protonation (at a fixed relative humidity value) [24].

At $\alpha = 0.1$, viz., with 0.9 Na^+ and 0.1 H^+ per nucleotide, the DNA protonated via procedure (iii) changed from the B- to A-form with decreasing relative humidity from 93 to 75%. Increasing the degree of protonation resulted in a diffraction pattern of the A + B type at R.H. 75% (fig. 2, curve 2). At $\alpha = 0.2$–0.4 the double helix remained in the B-form at both high (93%) and low (75%) R.H. Thus, for DNA protonated via procedure (iii) protonation also inhibits the B-to-A transition but this effect takes place at higher α values as compared to procedures (i) and (ii), i.e., in the presence of excess salt in the sample. Therefore, the X-ray diffraction titration curve for the former DNA (fig. 2, curve 2) is shifted to higher α values as compared to that for the latter DNA (fig. 2, curve 1).

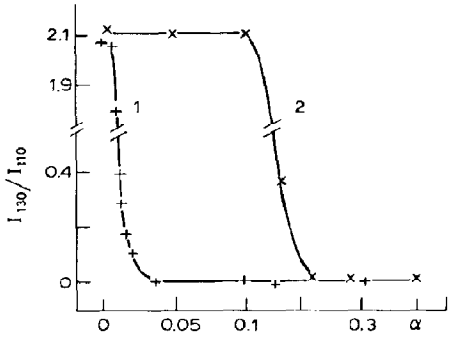

Fig. 2. Dependence of the ratio of integral intensities of reflections 130 and 110 on the degree of DNA protonation. (Curve 1) Fibers containing 4% NaCl, (curve 2) fibers of DNA protonated by dialysis without salt; relative humidity, 75%; T, 20 °C.

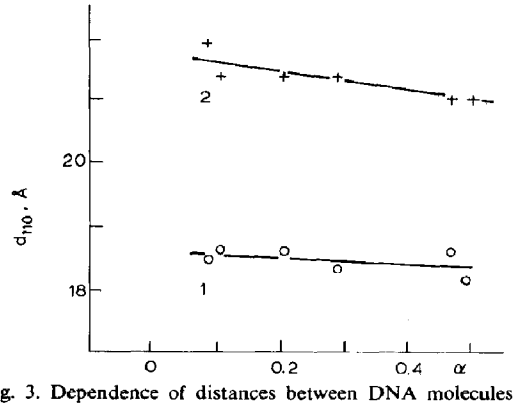

Fig. 3. Dependence of distances between DNA molecules in salt-free fibers on degree of protonation of DNA. Relative humidities, 75 (curve 1) and 93% (curve 2); T, 20 °C.

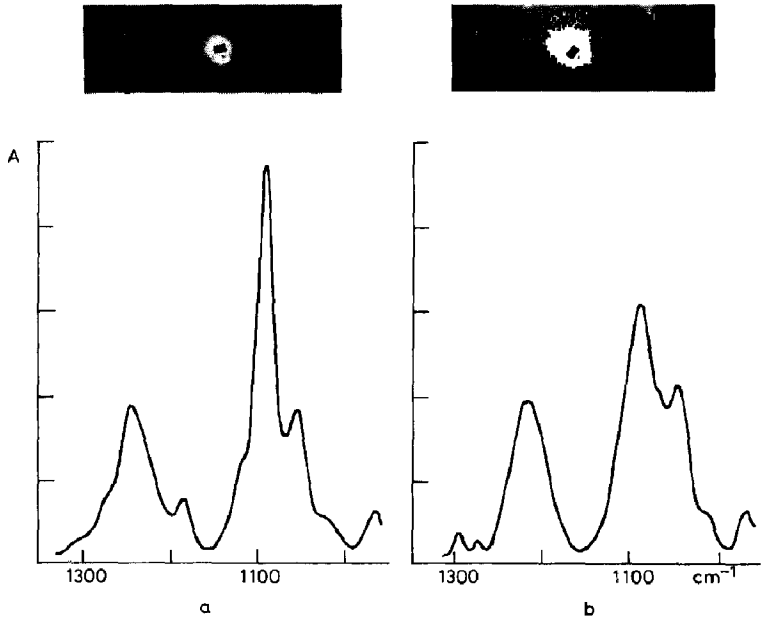

Fig. 4. Infrared spectra and debyegrams of unoriented DNA films at 75% R.H., A-form (a) and at 93% R.H., B-form (b). Arrow indicates the ring from the lavsan window.

The helical parameters of protonated B-DNA at high R.H. (93%) differed slightly from those of the unprotonated type: the helix pitch was 32.4 Å instead of 33.9 Å and the twist angle was 37° instead of 36°. The d_{110} value which characterizes the distance between neighbouring DNA helical axes also changed only slightly at $\alpha = 0$–0.05 (fig. 3). This suggests that the thickness of the hydration shell of DNA did not change significantly on protonation. On the other hand, a decrease in the relative humidity from 93 to 75% at any constant α values within the range 0–0.5 decreased d_{110} by about 3 Å. These data indicate that the A-to-B DNA transition may or may not be accompanied by an increase in the hydration shell thickness for change in relative humidity from 75 to 93% at $\alpha = 0$ or for change in α from 0 to 0.5 at 75% R.H., respectively. In other words, on protonation at low relative humidity, the B-DNA conformation is stabilized directly by H^+ binding rather than by an increase in total hydration.

Table 2

The parameters of infrared bands of DNA A- and B-forms

Relative humidity (%)	Form	$\nu_{as}(PO_2^-)$ (cm^{-1})	D_{1086}/D_{1224}	D_{1183}/D_{1193}
93	B	1224±2	1.6±0.3	0.54±0.15
75	A	1237±3	2.4±0.2	1.2 ±0.1

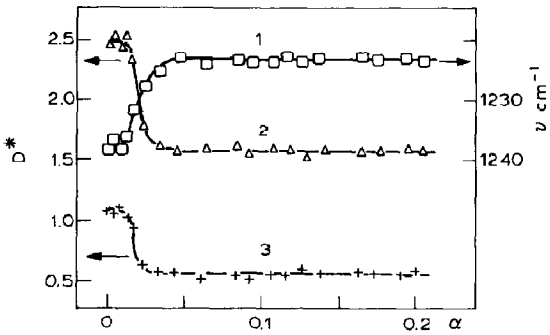

Fig. 5. The effect of DNA protonation on the parameters of some infrared bands in DNA films containing 4% NaCl. 75% R.H.; $T = 25°$ C. (Curve 1) Frequency of antisymmetric vibrations of PO_2^- groups; (2) absorbance ratio D_{1086}/D_{1224}; (3) absorbance ratio D_{1183}/D_{1193}.

Thus, irrespective of both the DNA protonation procedure and the inorganic salt concentration in the fiber, the binding of H^+ to DNA stabilizes the B conformation and prevents the B-to-A transition caused by the decrease in relative humidity. At a constant low value of the relative humidity, where unprotonated DNA adopts the A-form, H^+ directly induces the A-to-B transition.

To confirm this conclusion, we performed infrared spectroscopic measurements of DNA films at different relative humidity and α values. This technique enables one to monitor both the A-to-B DNA transition and the water content of the film.

3.2. Infrared spectroscopic data on the B-to-A transition in DNA films with different degrees of protonation

Differences in conformation and molecular packing between DNA double helices of the A- and B-types are clearly manifested in the range of sugar-phosphate backbone frequencies (950–1300 cm^{-1}) in the infrared spectrum of DNA (fig. 4). To characterize these differences, the following parameters may be proposed: the antisymmetric vibrational frequency of the PO_2^- groups, the intensity ratio of symmetric and antisymmetric vibrations of PO_2^- groups, and the presence or absence of the 1183 cm^{-1} band. We used the D_{1183}/D_{1193} ratio as a criterion for judging the appearance of the 1183 cm^{-1} band.

The parameters listed in table 2 were used to characterize the changes in DNA conformation induced by protonation and relative humidity changes. According to their values, DNA molecules in the film are in the B-form in the range 93–84% R.H. and $\alpha = 0$–0.3.

At 75% R.H., a very low degree of DNA protonation was sufficient for the macromolecule to be transformed from the A- to B-form (fig. 5). One can observe that with increasing α from 0.02 to 0.04, $\nu_{as}(PO_2^-)$ decreased from 1237 to 1224 cm^{-1}, the D_{1086}/D_{1224} ratio changed from 2.5 to 1.5, and the 1183 cm^{-1} band disappeared. These results are in good agreement with the X-ray diffraction data. Thus, the infrared spectroscopic data also indicate the stabilization of B-DNA by H^+.

Infrared spectra for DNA films protonated in HCl vapor (procedure (ii)) show that the water content of the film increased only very slightly with DNA protonation (data not presented), i.e., that the A-to-B transition is induced directly by H^+ binding to the macromolecule.

4. Discussion

We have obtained X-ray diffraction and infrared spectroscopic evidence to show that DNA protonation results in the stabilization of the B-form in the range of relative humidity down to 66%. It may be said that protonation and hydration of DNA, while stabilizing the B-form, act in the same direction. Consequently, the lower the relative humidity, the greater is the number of protons needed to prevent the B-to-A transition (over a given range of α and relative humidity values). On the other hand, decreasing relative humidity partly restores the A-form of DNA at a constant, very low degree of protonation ($\alpha <$ 0.03). A similar type of compensation may also take place between the degree of DNA protonation and inorganic salt content. This is seen from the shift of the X-ray diffraction titration curve (fig. 2). However, H^+ is much more effective than Na^+ in inducing the A-to-B transition. For example, to stabilize B-DNA in the fiber containing 4% NaCl at 75% R.H. it is necessary to add 0.5 Na^+ [11] but only 0.03 H^+ (fig. 5) per nucleotide. Therefore, the number of protons needed to induce the A-to-B transition under these conditions is about 20-times less than that of Na^+.

What is the nature of such a stabilizing effect of protons and their high efficiency? We suppose that this effect is mediated by changes in the DNA hydration shell, thus stabilizing the B-type conformation of protonated DNA at a low water content. It is significant that the binding sites for H^+ reside on G · G nucleosides which prefer the A-form [5] and cannot participate in the formation of the water spine in the DNA minor groove [4,6].

It should also be noted that protonation of a G · C base-pair increases the number of its proton-donor centers exposed to the solvent and

decreases the number of proton-acceptor centers, changing their ratio from 2 : 4 to 3 : 3. Such equalization may facilitate the formation of water bridges between adjacent base-pairs in the DNA major groove.

The present results demonstrate that the B-form of DNA can be stabilized by agents capable of modifying the charge distribution and hydration in the major groove of the double helix. This indicates an important role of solvent molecules of the DNA major groove in formation of the B-type double helix and in the B-to-A conformational transition.

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