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Changes of hydration during conformational transitions of DNA

M. Harmouchi, G. Albiser, and S. Premilat

Laboratoire de Biophysique Moléculaire, UA CNRS 494, Faculté des Sciences, Université de Nancy I, BP 239, F-54506 Vandoeuvre les Nancy, France

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Abstract. Fiber X-ray diffraction and measurement of fiber dimensions yields information about the hydration of DNA in fibers. The results obtained give us the fraction of nucleotides in the B form for the A–B transition or the rate of progression for the B–C transition as functions of the number of water molecules per nucleotide. The present experimental results confirm the importance of cooperativity in the A–B transition and the progressive change of the DNA double helix conformation during the C–B transition. At least twenty additional water molecules per nucleotide are necessary to stabilize the B form for DNA molecules in fibers following the A to B transition whereas only ten are sufficient when the B conformation is obtained starting from the C form.

Key words: DNA – Conformational transitions – Hydration – Fiber X-ray

Introduction

It is well known that water is of major importance for the stabilization of secondary and tertiary structures of biological macromolecules (Tanford 1968; Kuntz et al. 1974; Eldelhoch and Osborne 1976). In the case of DNA, hydration of the molecules is an important factor (Drew et al. 1981; Conner et al. 1984; Lee et al. 1987) which determines the form of the more or less stable double helix conformations. For DNA in fibers, we obtain the B-form at a high r.h. (relative humidity) (Langridge et al. 1960) and when the r.h. is lowered, the A form (Fuller et al. 1965) or the C one (Marvin et al. 1961) is obtained, depending on the type and content of salt. This is also the way one can observe the D (Davies and Baldwin 1963) or the Z form (Leslie et al. 1980), depending on the primary structure of the polynucleotide chains.

Many different experimental methods such as equilibrium sedimentation (Hearst and Vinograd 1961; Cohen and Eisenberg 1968; Tunis and Hearst 1968 a, b; Wolf and Hanlon 1975), gravimetry (Falk et al. 1962), isopiestic measurements (Hearst 1965), spectroscopy (Falk et al. 1963, 1970; Hanlon et al. 1975; Semenov et al. 1988); X-ray diffraction (Saenger et al. 1986; Westhof 1987) have clearly shown that DNA molecules are generally highly hydrated. The average number of water molecules bound to or in the near vicinity of nucleotides determines the DNA helical structure (Dahlborg et al. 1980; Lindsay et al. 1988; Forsyth et al. 1989; Tao et al. 1989; Grimm and Rupprecht 1989). These water molecules contribute to the primary and secondary hydration shells.

In a previous study (Premilat et al. 1990) it was shown how one can follow conformational transitions of DNA by coupling X-ray diffraction with measurements of fiber dimensions. We recently found in the literature that variations of DNA fiber length, characteristic of form transitions of DNA, can also be observed in water-ethanol mixtures (Rupprecht and Piskur 1983). In the present approach, precise evaluation of the water content and its variation with the r.h. during conformational changes associated with the A–B and B–C transitions of natural DNA are presented. It is shown that the minimum number of water molecules necessary to stabilize different DNA structures can be deduced from measurements of fiber dimensions and analysis of X-ray patterns. The present approach shows that the B form can be realized with very different numbers of water molecules, depending on the salt used for the preparation of DNA fibers.

Material and methods

In the present study, preparations of calf thymus DNA, purchased from Pharmacia, were used without further purification. The lyophilized DNA was dissolved in distilled water at pH 7 and the salt concentration was defined by dialysis. The DNA gel from which fibers were drawn was obtained from centrifugation of the solutions at 40 000 rpm. Two different salt concentrations were

Offprint requests to: S. Premilat

used for NaCl solutions of DNA; one with 0.05 Na^+ per nucleotide and the other one with 0.5 Na^+ per nucleotide. The same concentrations of salt were used for Li DNA preparations.

The behaviour of the different DNA fibers submitted to variations of the r.h. was analysed by X-ray diffraction measurements and direct observation with an optical microscope. Details concerning the experimental methods used are given elsewhere (Premilat et al. 1990). One should only note that a small tension was applied to the fiber. This is because measurement of the fiber length can be complicated by slight fluctuations of the fiber which may appear when the r.h. is varied. As shown previously (Albiser et al. 1988), such a tension has no effect on the conformational transition of the DNA.

In order to determine the number of water molecules associated with a nucleotide in a given DNA conformation, one has to know the total number of nucleotides in the fiber. The number, N, of nucleotides situated along the fiber axis is given by the ratio of the fiber length to the rise per base pair in the DNA helix. This ratio remains constant with changes of the r.h. (Premilat et al. 1990). We also need to determine the number, n, of nucleotide pairs in a section of the DNA fiber. This number is equal to the ratio of the section, S, of the fiber to the section, s, of a DNA double helix. The fiber section was obtained from a direct measurement of its diameter and the section of the DNA helix can be deduced from the lattice parameters determined from X-ray patterns obtained at very low relative humidities. Hence, because in the hexagonal lattice there are three DNA molecules in a unit cell and only two in the monoclinic lattice (Langridge et al. 1960; Marvin et al. 1961; Fuller et al. 1965), we obtain, with the unit cell parameters a and b in a plane perpendicular to the helix axis:

$$s = a^2\sqrt{3}/6 \quad \text{(hexagonal)}$$

and

$$s = ab/2 \qquad \text{(monoclinic; } \alpha=\gamma=90°;\ \beta=97°\text{)}.$$

The number of moles of nucleotides in the fiber is thus given by $2N \cdot n/\mathcal{N}$ ($\mathcal{N}$ is Avogadro's number).

The number of water molecules associated with the DNA in the fiber varies during conformational changes and was determined from measurements of the variations of the fiber volume following modifications of the relative humidity. We assume that the volumes of DNA and water can be simply added to obtain the total volume, V_f, of the fiber at a given r.h. So, $V_f = V_w + V_0$, where V_w is the water volume and V_0 the DNA volume at 0% r.h. The number of water moles in the fiber is then given by $m \cdot V_w/M_w$, with m the density and M_w the molecular weight of water. Hence, the number, G, of water molecules per molecule of nucleotide in the fiber is:

$$G = (m \cdot V_w/M_w)/(2N \cdot n/\mathcal{N})$$

$$= (m \cdot \mathcal{N}/2M_w)\,(V_w/N \cdot n) = K \cdot V_w/N \cdot n$$

with V_w given in mm^3, the constant $K = 1.67 \cdot 10^{19}$ mm^{-3}). Note that water molecules bound to DNA at 0% r.h. (Tao et al. 1989) are not included.

Table 1. A–B transition of Na DNA. Fiber length (l), diameter (D) and geometrical parameters of helical conformations, p (rise per nucleotide), unit cell constants a, b

r.h. %	l (mm)	D (mm)	p (Å)	a (Å)	b (Å)	Lattice	DNA form
95	4.000	0.214	3.37	44.6	–	hexag.	B
92	3.920	0.182	3.36	41.0	–	hexag.	B
90	3.629	0.173	3.36 2.58	22.8	41.3	mono.	B–A
86	3.364	0.168	3.34 2.57	22.6	41.2	mono.	B–A
75	3.152	0.158	2.56	22.2	41.0	mono.	A
66	3.046	0.154	2.55	21.8	39.8	mono.	A
58	2.980	0.152	–	21.4	39.2	mono.	
45	2.864	0.151	–	21.4	38.2	mono.	
0	2.543	0.148					

Estimated standard deviations:

l, D: $3 \cdot 10^{-3}$ mm; p: 0.01 Å; a, b: 0.1 Å; r.h.: 1%

Results

I. The A–B transition

DNA fibers used for the study of the A–B transition were prepared from solutions with 0.5 Na^+ per nucleotide. The cooperativity of the A–B transition in DNA is well established; it is therefore interesting to analyze, in this case, the variations of the parameter G with the fraction X_B of nucleotides in the B form. We have shown (Premilat et al. 1990) that the variation of X_B with the r.h. can be determined by measurements of the fiber length and we have:

$$X_B = (l - l_A)/(l_B - l_A)$$

where l_B, l_A and l represent, respectively, the length of the fiber in the conformations B, A and intermediate states appearing during the conformational change (mixtures of A and B).

As an example of experimental results, note that the fiber under study adopts the B form at 95% r.h. and the A form at 66% with the corresponding rises per nucleotide $p_B = 3.37$ A and $p_A = 2.55$ A. For these well defined conformational states of the DNA, the fiber lengths $l_B = 4.000$ mm and $l_A = 3.046$ mm were measured (Table 1). The number N of base pairs along the fiber axis is then given by:

$$N = l_B/p_B = l_A/p_A = 1.19 \cdot 10^7$$

We noted that the ratio S/s of the fiber section to the section of a DNA double helix remains constant for r.h. lower than 80% (Fig. 1). This constant value represents the average number, n, of pairs of nucleotides contained in a section of the fiber and is, for the present fiber, equal to $43 \cdot 10^8$. This set of data allows us to determine values of G as a function of the relative humidity (Fig. 2). The present results are in accordance with previous observations (Tao et al. 1989). By using the set of values of X_B as a function of the r.h. (Premilat et al. 1990) and the data for G as a function of r.h. (Fig. 2) one can establish the curve

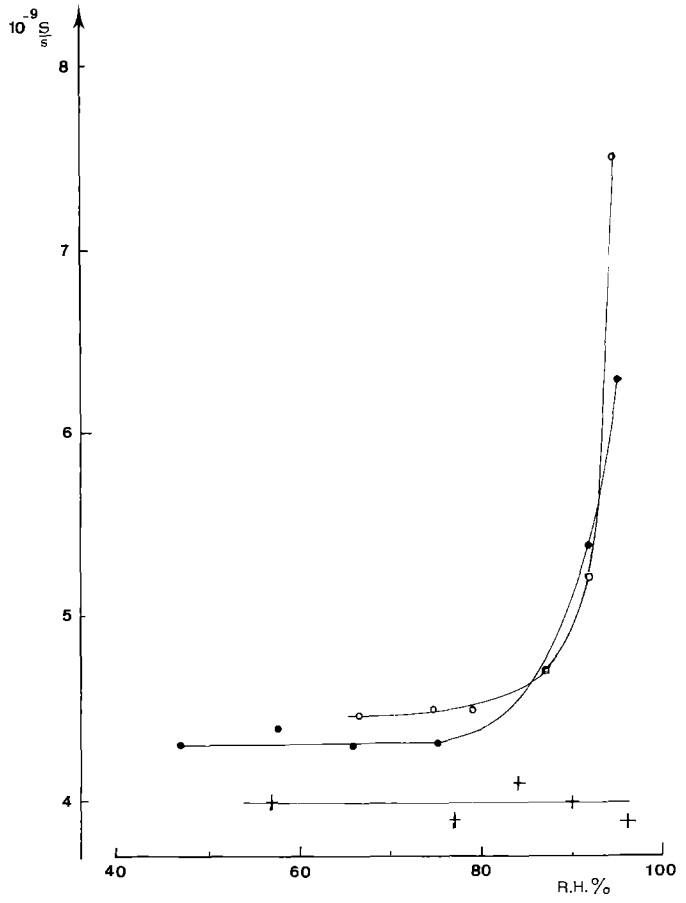

Fig. 1. Ratio of the fiber section, S, to a section, s, of the DNA double helix as a function of the relative humidity. A–B transition with Na DNA (–●–); B–C transition: Na DNA (–o–); Li DNA (–+–)

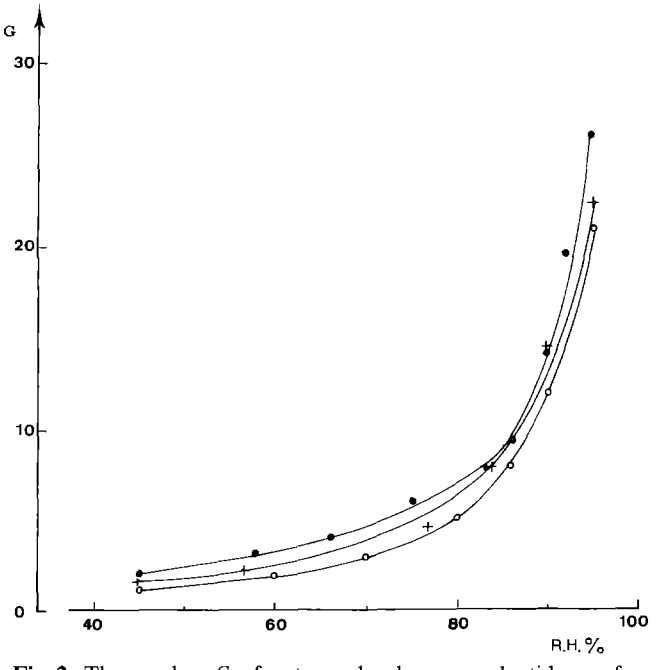

Fig. 2. The number, G, of water molecules per nucleotide as a function of the r.h. for Na DNA: A–B transition (–●–); B–C transition (–o–). Li DNA: B–C transition (–+–)

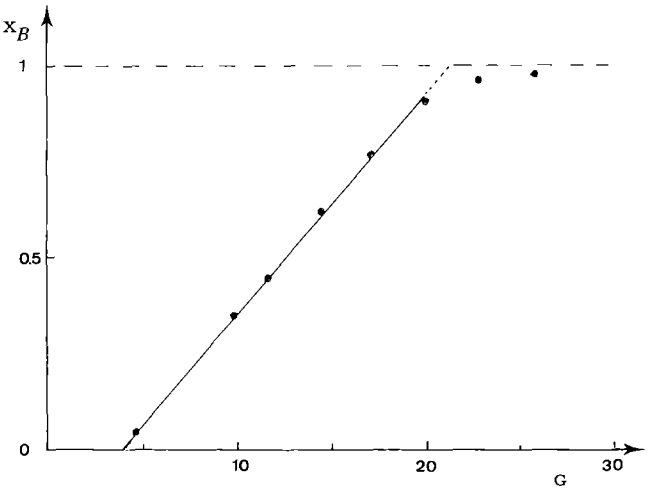

Fig. 3. A–B transition: fraction X_B of nucleotides in the B form versus G

of X_B versus G given in Fig. 3 where linear variations of X_B are observed until $G=20$. This last value is obtained at 92% r.h. (Fig. 2) and corresponds to $X_B=0.92$. When G is larger than 20, a saturation effect appears and X_B is no longer proportional to G. Practically, the extrapolation of the linear part of the curve $X_B=f(G)$ until its intersection with the line $X_B=1$ (Fig. 3) gives a number $G_B=21$ molecules of water per molecule of nucleotide necessary to get the DNA completely in its B form. When G is larger than G_B, the DNA helices remain in the B form but the lattice dimensions are increased. In addition, the linear part of the curve $X_B=f(G)$ intersects the line $X_B=0$ at $G_A=4$ (Fig. 3) and this value represents the number of water molecules per molecule of nucleotide in the A form. Between these two extreme values of G, we have the fraction X_B of nucleotides in the B form and $(1-X_B)$ in A. The linear variation of G with X_B can therefore be expressed by:

$$G = X_B(G_B - G_A) + G_A \ .$$

II. The B–C transition

The B–C transition of DNA was studied with Na and Li DNA fibers. In order to get the B–C rather than the A–B transition with Na DNA, one has to use very low salt concentrations. We prepared solutions with 0.05 Na^+ per nucleotide in the DNA solution. With LiCl there is no such limitation as only the B–C transition is observed with that salt.

NaDNA fibers. X-ray fiber diffraction shows clearly the B or C conformation adopted by the DNA helices depending on the value of the relative humidity. We also noted that for r.h. lower than 75%, DNA fibers are largely disorganized. The experimental curve corresponding to the B–C transition as a function of the r.h. is linear and shows a change of slope in the vicinity of 75% r.h. (Premilat et al. 1990). It corresponds to a progressive and not a cooperative change of conformation: we thus deter-

Table 2. B–C transition of Na DNA. Fiber length (l), diameter (D) and geometrical parameters of helical conformations, p (rise per nucleotide), hexagonal unit cell a

r.h. %	l (mm)	D (mm)	p (Å)	a (Å)	DNA form
95	3.375	0.214	3.35	40.8	B
92	3.341	0.178	3.34	39.8	B
87	3.285	0.160	3.33	38.2	C
82	3.228	0.152	3.32	37.4	C
79	3.195	0.149	3.31	36.6	C
75	3.150	0.143	3.30	35.2	C
64	3.087	0.141			
58	3.062	0.139			
45	3.017	0.137			
0	2.870	0.135			

Estimated standard deviations:

l, D: $3 \cdot 10^{-3}$ mm; p: 0.1 Å; a: 0.1 Å; r.h.: 1%

Table 3. B–C transition of Li DNA. Fiber length (l), diameter (D) and geometrical parameters of helical conformations, p (rise per nucleotide), hexagonal unit cell a

r.h. %	l (mm)	D (mm)	p (Å)	a (Å)	DNA form
95	1.160	0.186	3.37	49.0	B
90	1.125	0.161	3.36	41.9	B
84	1.085	0.143	3.35	35.8	B
77	1.050	0.136	3.33	35.1	C
57	1.005	0.128	3.31	33.2	C
45	0.990	0.126	3.30	32.5	C
34	0.980	0.125	3.28	32.0	C
0	0.950	0.121			

Estimated standard deviations:

l, D: $3 \cdot 10^{-3}$ mm; p: 0.1 Å; a: 0.1 Å; r.h.: 1%

mined a progression rate R for the transition from C to B as a function of the number of water molecules per nucleotide. Values of this factor R at different r.h. were obtained from measurements of the fiber length l. We actually have:

$$R = (l - l_c)/(l_B - l_C),$$

where l_B and l_C are the lengths of the fiber in the B (r.h. higher than 95%) and the C (75% r.h.) forms. Different parameters characterizing the DNA fiber during the conformational change are given in Table 2. We determined

$$P_B = 3.35 \text{ A} \quad \text{and} \quad P_C = 3.30 \text{ A}$$

from X-ray patterns.

As for the A–B transition, the ratio S/s remains practically constant during the transition as long as the r.h. is lower than or equal to 80%. We can see in Fig. 1 that the variations of this ratio are very similar to those observed for the A–B transition.

By using the values of l as a function of the r.h. (Table 2) and combining them with the variations of G with the r.h. (Fig. 2) one can establish a curve representing R as a function of G. We can see in Fig. 4, that the relation between these two quantities corresponds to a saturation effect as the function associated with the curve can be written as:

$$R = 1 - \exp(-u(G - G_c)) \quad \text{or} \quad \ln(1-R) = -u(G - G_c)$$

with a positive constant u; $G \to G_c$ when $R \to 0$ and G_c the number of water molecules per molecule of nucleotide necessary to get the DNA completely in the C conformation (at 75% r.h.). The experimental curve presented in Fig. 4 corresponds to $u = 0.16$ and $G_c = 3.5$.

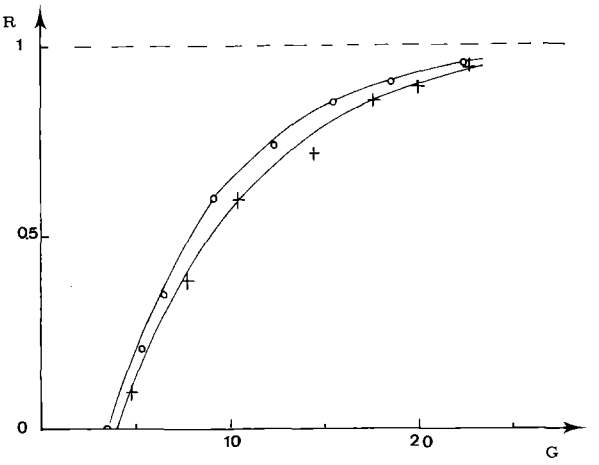

Fig. 4. Progression rate R for the B–C transition as a function of the number G of water molecules per nucleotide. Na DNA (-o-); Li DNA (-+-)

Li DNA fibers. DNA fibers prepared from Li DNA solutions give better defined X-ray patterns than those obtained from NaDNA fibers. The fibers are homogeneous and very well organized. The crystal lattice remains hexagonal with practically the value of the a parameter obtained with Na DNA at r.h. lower than 80%. For higher values of the r.h. the lattice parameter a increases much more with Li DNA fibers than with Na DNA (Tables 2, 3).

We also noted that the X-ray reflection on the first layer line at 0.1 A^{-1}, which is characteristic for the C conformation, disappears when the r.h. is in the range of 80 to 86%. It is important to observe that it takes much more time to stabilize a Li DNA fiber at a given r.h. than a Na DNA one; the withdrawal of water is more difficult to achieve when the Li salt is present. Nevertheless the same kind of curve of R versus G is obtained (Fig. 4) with $u = 0.14$ and $G_c = 4.0$.

In Table 3 different fiber data are given to show their variations during the B–C transition of Li DNA. An important difference in the behaviour of the Li DNA compared to the Na DNA is that the ratio S/s remains constant when the r.h. is varied (see Fig. 1). This may be related to a better organization of Li DNA molecules in the fibers and also to the higher conformational stability given to the DNA by the Li salt. This is the reason why the B–C transition is the only possible one with the Li salt; it actually represents a relatively slight change of conformation compared to the more substantial A–B transition.

Discussion

Measurements of DNA fiber dimensions associated with X-ray fiber diffraction give precise information on the polymorphism of the DNA and on the role of water during conformational transitions (Premilat et al. 1990). The determination of the fiber volume and its variation with r.h. also allows one to determine the evolution of the number of water molecules associated with a nucleotide during the A–B and B–C transitions.

For the A–B transition of DNA, the results presently obtained show that every nucleotide in the A form is associated with an average of 4 water molecules (water molecules still present at 0% r.h. are not included) whereas 20 are necessary to stabilize the B conformation (Saenger et al. 1986; Tao et al. 1989). Mixtures of these two stable forms correspond to intermediate states of the DNA which can be defined by the fraction X_B of nucleotides in the B form. We observed that this fraction varies linearly with the average number of water molecules per nucleotide. This experimental fact confirms that no other conformation (different from A or B) appears during the A–B transition. Moreover, we can note that a nucleotide remains in the A form as long as the number of water molecules in its vicinity is lower than $G_B = 20$.

For the B–C transition, obtained with Li as well as with Na DNA, we observed exponential variations of the progression rate of the transition as a function of G. Such an effect of saturation shows clearly the progressive deformation of the DNA conformation which changes from the C to the B form by addition of water molecules to the double helices. When the average number of water molecules per nucleotide is larger than 9, the B form is stabilized. This happens for relative humidities equal to or higher than 85%.

Moreover, we observed that only four water molecules are associated with one nucleotide when the DNA is in the C form at 75% r.h.; surprisingly, this is the same amount as for the A form. However, as noted before, whereas 20 water molecules per nucleotide are necessary in order to get all the DNA in the B form during the A–B transition, only 10 are sufficient to obtain it from the C–B transition (with Na or Li salt). These minimum amounts of water stabilizing the B form are obtained at 92% r.h. (A–B transition) and 86% r.h. (B–C transition).

The ratio of the fiber section to the section occupied by a DNA double helix in the lattice is constant when the r.h. is lower than 80%. At higher values of the r.h., this ratio increases for Na DNA fibers whereas it remains constant during the B–C transition of Li DNA. Note that similar results, showing differences in the behaviour of Na and Li DNA, have recently been presented (Lindsay et al. 1988). The swelling of Na DNA fibers thus observed indicates the enlargement of highly hydrated and disorganized parts of the fiber. As Li DNA fibers do not show such behaviour, it seems that the presence of Li salt induces a good organization of the DNA in the fiber (X-ray patterns are better defined) and also a stabilization of the B-form as only the small conformational changes necessary for the B–C transition are then possible.

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