Biophysical Chemistry, 35 (1990) 37–45
Elsevier

BIOCHE 01399

A method for the experimental study of DNA conformational transitions in fibers

S. Premilat, M. Harmouchi and G. Albiser

Laboratoire de Biophysique Moléculaire, U.A. CNRS 494, Faculté des Sciences, Université de Nancy I, BP 239, 54506 Vandoeuvre les Nancy, France

Received 1 May 1989
Revised manuscript received 8 September 1989
Accepted 11 September 1989

DNA; Conformational transition; Fiber dimensions; X-ray diffraction

The method proposed for the study of DNA conformational transitions is based on the proportionality, experimentally observed, between the length of a DNA fiber and the axial rise per nucleotide characterizing the molecular helix. Precise curves for the A-B and B-C transitions as a function of the relative humidity are obtained by using X-ray fiber data and measurements of fiber dimensions. It is thus shown that the A-B transition is a cooperative process between two different states, whereas the B-C transition can be considered as a progressive change of conformation. The present method is applied on two natural DNAs differing in base composition so that the effect of the nucleotide content on the conformational changes can be estimated.

1. Introduction

Recent single-crystal analyses [1–4] of oligonucleotides with well-defined nucleotide sequences have confirmed, in a more detailed way, the regular helical structures of DNA already established by X-ray fiber diffraction [5–8]. The local variations thus observed in the conformation of oligonucleotides depending on their precise base composition have given an accurate and perhaps more functional view of the DNA molecules. Moreover, single-crystal studies of oligonucleotides allowed the determination of new conformations, the Z double left-handed helices which are obtained from poly(C-G)-poly(C-G) in very high ionic content preparations [9,10]. We can add that results deduced from fiber or single-crystal studies of polynucleotides are frequently completed by the use of different spectroscopic techniques, mainly NMR [11–15] and infrared [16–20], which permit conformational information to be obtained on these same molecules in film and also in solution.

However, the dynamical aspects of the conformational transitions of DNA can hardly be studied by using single crystals. Fibers or films of DNA are actually more valuable for such approaches because they allow changes of the molecular conformation when subject to external variable and reversible constraints. Although they are well known in their general features, the conformational transitions of DNA are far from being well-determined experimentally [21–23] even if the main causes of these changes are firmly established. So, among the factors inducing transitions in DNA conformations, knowledge of the relative humidity of the fiber surroundings is surely essential, at least as concerns the type and content of salt associated to the DNA molecule [5,24–27]. The nucleotide composition and more precisely its

Correspondence address: S. Premilat, Laboratoire de Biophysique Moléculaire, U.A. CNRS 494, Faculté des Sciences, Université de Nancy 1, BP 239, 54506 Vandoeuvre les Nancy, France.

sequence are also important for the dynamics of conformational transition [8,28,29]. We could also include mechanical forces which can modify or even prevent conformational changes when applied to fibers [30,31].

Nevertheless, information on molecular structures and conformational transitions of DNA can also be obtained by the simple and direct observation of the fiber dimensions. Indeed, a parameter which characterizes very well the double helix form of DNA is the axial rise per nucleotide (or rise per base-pair), p. It represents the increase in length along the helix axis due to one pair of nucleotides in the antiparallel and complementary molecular chains. One should note that this relevant parameter is directly related to the length of DNA molecules. These long molecules can be well organized (by stretching a gel) into fibers where they are mainly lengthened and placed on end or side-by-side. The length of such a fiber should in principle be directly related to that of the DNA molecules it contains and hence to the type of helix they form. Moreover, changes of the molecular conformation, i.e., of the helix form, induced, for example, by variations of the relative humidity, can be performed keeping the structure of the fiber intact. Such changes modify the length of the DNA molecules and thus that of the fiber. Note that DNA helices could slide past one another during form transitions and the fiber length variations could therefore practically result from these movements. However, if such eventual molecular sliding is very small it should be possible to estimate changes of the molecular parameter p by simple measurements of variations of the sample length and to study, by this direct procedure, conformational changes. In the present work, it is shown that we can actually establish such an approach which allows us to propose accurate experimental curves for the A-B and B-C transitions in DNA.

2. Materials and methods

In order to gain information about the effect of base composition on the DNA conformational transitions, two different natural DNAs were used. Namely, DNA from calf thymus containing 42% of G-C bases was purchased from Pharmacia and *Micrococcus lysodeikticus* DNA which contains 72% of G-C, was obtained from Sigma. The following method was used to realize, in a reproducible way, well oriented fibers from DNA gels: a few milligrams of dry DNA is put in solution in water at pH 7; its concentration is measured from the absorbance at 2600 Å and the NaCl content of the solution is fixed by dialysis. Two different preparations were made depending on the transition considered. For the A-B transition, an NaCl concentration of 0.005 M is achieved with DNA solutions of 3 mg/ml. However, to observe the B-C transition, a 10-fold lower NaCl concentration is necessary. These solutions are centrifuged at 40000 rpm and with the DNA gel thus obtained, fibers are obtained by stretching the gel between two glass rods. X-ray fiber diffraction patterns are obtained following the now classical method [5]. Details on the procedure we use have been given elsewhere [32] and, as before, a slight flux of hydrogen, passing through appropriate saturated salt solutions, allows us to obtain fixed relative humidities [33] in the X-ray camera where the fiber is placed.

The measurement of the fiber length is made using a binocular microscope. The sample is placed in a cell through which a small flux of humidified air is passed. This set-up, devised in order to maintain or modify the relative humidity in the fiber environment, is fixed on the microscope stage; it comprises two transparent bases through which the dimensions of the fiber can be measured. In the cell, the fiber is placed under the objective of the microscope with one end fixed to the holder while the other one remains free or is maintained by a very light tension (of the order of 1 g) which has no effect on the conformational changes [31] but permits one to avoid small deformations of the sample when it lengthens. An ocular micrometer is used for the determination of the fiber dimensions, i.e., its length (distance between two arbitrarily chosen points on the fiber) and diameter. It should be noted that for X-ray fiber diffraction as well as for measurements performed with the microscope, enough time must be allowed to ensure attainment of equilibrium of the

system at a given relative humidity [34]. This time depends on the thickness of the sample and also on the value of the fixed humidity. We noted that it takes less time to reach equilibrium when the relative humidity is reduced rather than when it is increased.

3. Results

3.1. The A-B transition

3.1.1. X-ray fiber diffraction

Samples of the two different DNAs, prepared from 0.005 M NaCl solutions, were studied by X-ray diffraction. The X-ray patterns thus obtained present the following features in common: the classical parameters of the B double helix are observed at a relative humidity (R.H.) of 95% and one obtains the A form at 66% R.H. (see tables 1 and 2). Between these two values of the relative humidity, a mixture of the two DNA conformations is observed on the X-ray patterns; this superposition is very clearly visible at 90 and 86% R.H. (an example of X-ray photographs is given in fig. 1). For lower values of the R.H. the more crystalline A form of DNA is preponderant on the X-ray patterns whereas the presence of the B form becomes gradually less observable. The A conformation disappears in turn at R.H. $< 60\%$. It is obviously difficult to obtain from X-ray patterns an estimation of the exact proportion of A and B forms in the mixture during the transition between the forms although their simultaneous presence is certain.

3.1.2. Fiber dimensions

Measurements of fiber dimensions, performed at fixed values of the relative humidity, with a binocular microscope, allowed us to draw the curves shown in fig. 2. These measurements of the length and diameter of fibers were made on the samples previously studied by X-ray diffraction under the same conditions of relative humidity. We can clearly ascertain that the length of DNA samples changes with the relative humidity according to sigmoidal curves, characteristic of cooperative effects, whereas the fiber diameter undergoes a steep diminution when the relative humidity is at about 95%. Thereafter, it decreases very slightly with lowering of the relative humidity. It should be noted that the large variation in fiber diameter appears before any change in its length can be observed. Moreover, a series of

Table 1

A-B transition: geometrical parameters of helical conformations; fiber length (L) and diameter (D) for calf thymus DNA

	R.H. (%)					
	95	92	90	86	75	66
Pitch P (A)						
B-form	33.7	33.6	33.0			
A-form			28.4	28.06	27.8	27.4
p(A)						
B-form	3.37	3.36	3.36	3.34		
A-form			2.58	2.57	2.56	2.55
P/p						
B-form	10.00	10.00	9.82			
A-form			11.00	10.92	10.86	10.74
L (mm)	3.775	3.700	3.425	3.175	2.975	2.875
D (mm)	0.375	0.317	0.307	0.293	0.281	0.275
L/p ($\times 10^{7}$)	1.12	1.10			1.16	1.12
X(B-form)	1	0.92	0.61	0.33	0.11	0
m (A)	3.37	3.30	3.05	2.82	2.64	2.55
L/m ($\times 10^{-7}$)	1.12	1.12	1.12	1.12	1.12	1.12

Table 2

A-B transition: geometrical parameters of helical conformations; fiber length (L) and diameter (D) for micrococcal DNA

	R.H. (%)					
	95	92	90	86	75	66
Pitch P (A)						
B-form	33.7					
A-form			28.08	27.9	27.7	27.2
p(A)						
B-form	3.37		3.35	3.34		
A-form			2.58	2.57	2.56	2.55
P/p						
B-form	10					
A-form			10.88	10.86	10.82	10.70
L (mm)	3.531	3.203	3.028	2.921	2.771	2.689
D (mm)	0.357	0.299	0.289	0.277	0.266	0.260
L/p ($\times 10^{-7}$)	1.05				1.08	1.05
X (B-form)	1	0.61	0.40	0.28	0.10	0
m (A)	3.37	3.05	2.88	2.78	2.63	2.55
L/m ($\times 10^{-7}$)	1.05	1.05	1.05	1.05	1.05	1.05

At 93% R.H., $L = 3.460$ mm, $D = 0.309$ mm.

dimension measurements, performed in the two senses of variation of the relative humidity, indicates the perfect reversibility of the transition. It is important, however, to observe that we actually obtain the same fiber length at a given relative humidity irrespective of the sense of variation, provided that enough time has been taken to establish a good equilibrium (stability of the fiber length). We noted that such equilibrium conditions take longer to reach when the transition goes from A to B as compared to the reverse direction. Fig. 3 shows, as an example, a DNA fiber at 95 and 75% R.H. The length variation is obvious as well as the modification of the sample volume.

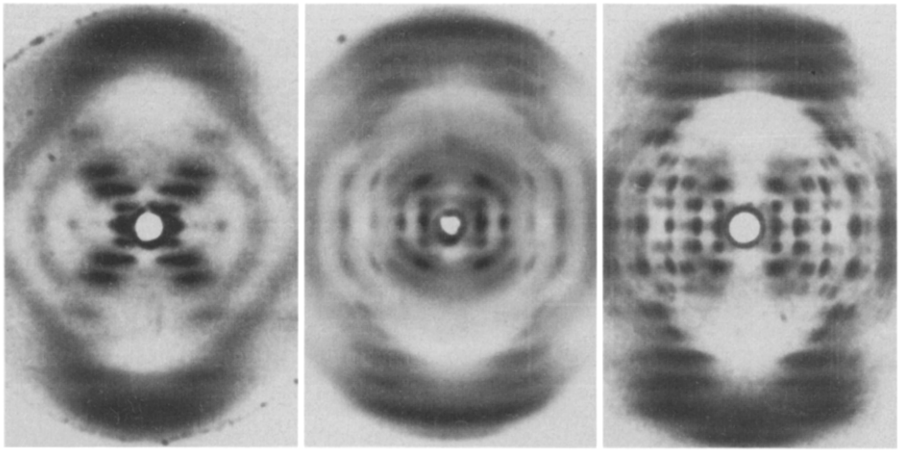

Fig. 1. X-ray fiber patterns obtained at (B) 95% R.H. (B-form), (B-A) 90% R.H. (mixture of A- and B-forms) and (A) 75% R.H. (A-form).

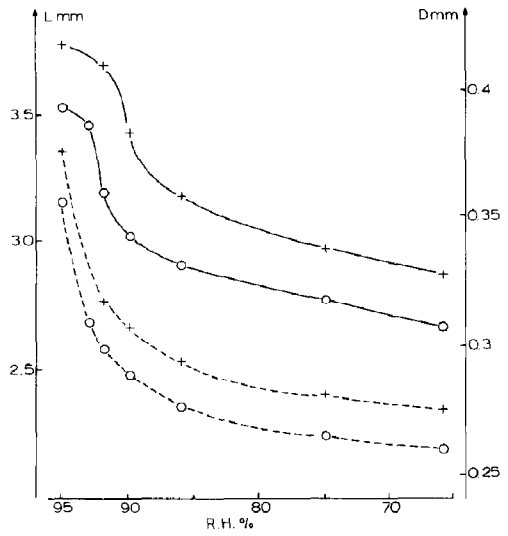

Fig. 2. A-B transition: DNA fiber dimensions as a function of the relative humidity. Length (+ ——— +) and diameter (+ - - - - - - +) for calf thymus DNA; length (○ ——— ○) and diameter (○ - - - - - - ○) for micrococcal DNA.

For instance, we observed that the ratio between the volumes of the fiber at 95 and 66% R.H., respectively, is about 2.5 (values of length and diameter listed in table 1).

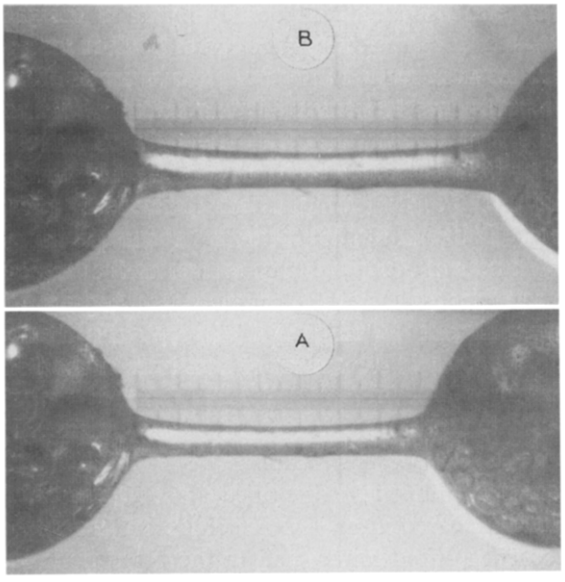

Fig. 3. Example of a DNA fiber at 95% R.H. (B) and 75% R.H. (A).

3.2. Relation between X-ray results and fiber dimensions

In tables 1 and 2, examples of values of the length L and diameter of fibers made from the two different DNAs are listed as a function of the relative humidity. In these tables are also indicated values of the parameter p (axial rise per nucleotide) obtained from X-ray diffraction of the same samples under identical conditions. We can note that the ratio L/p has the same value at 95 and 66% R.H. Between these relative humidity values, this ratio cannot be well defined because of the low precision of the X-ray data obtained when conformations A and B coexist in DNA fibers. Note that the ratio L/p represents the constant number of base pairs located along the fiber axis, between the two points chosen for the length measurement. Therefore, we can estimate the percentage of nucleotides respectively in the A or B conformation by simple measurement of the fiber length. Indeed, with one among these fibers taken as an example, we obtained X-ray patterns of the B form at 95% R.H. and then determined a value of $p(\mathrm{B}) = 3.37$ Å for the parameter p whereas at 66% R.H., we found the corresponding value for conformation A to be $p(\mathrm{A}) = 2.55$ Å. The values of the fiber length corresponding to these two situations were respectively $L(\mathrm{B}) = 3.775$ mm and $L(\mathrm{A}) = 2.875$ mm. Therefore, we have $L(\mathrm{B})/L(\mathrm{A}) = 1.31$ and $p(\mathrm{B})/p(\mathrm{A}) = 1.32$; hence, we can practically write $L(\mathrm{B})/L(\mathrm{A}) = p(\mathrm{B})/p(\mathrm{A})$, i.e., there is a very good proportionality between the length of the fiber and the helical parameter p.

Note that such behavior is actually general and perfectly reversible; it is observed with any homogeneous and well-oriented DNA fiber (i.e., giving the A and B X-ray patterns according to the relative humidity). Consequently, it appears that the fiber length variations are practically only due to changes of the DNA helical form. Molecular sliding may exist in the fiber but has no perceptible effect and can thus be neglected in the present case. For intermediate values of the relative humidity a mixture of A and B forms is present in the fiber. Very recently, it has been shown that the A and B forms can also coexist in a single crystal [35]. However, the values then determined must be

understood as being averages which could result from a mixed population of molecules of each form as well as from DNA molecules containing fractions of the two forms. The distinction between these two possibilities is of little importance and can scarcely be made experimentally. We, therefore proceed in the following to deal with the situation according to the second hypothesis which is generally used in theoretical approaches to the problem of conformational transitions in polypeptides [36] and DNA [37]. Accordingly, the length L, measured at any given value of the relative humidity between 66 and 95%, can be written as follows. $L = X(\mathrm{B})L(\mathrm{B}) + X(\mathrm{A})L(\mathrm{A})$, where $X(\mathrm{B})$ and $X(\mathrm{A})$ denote the fractions of nucleotides in conformation B and A, respectively. So, with $X(\mathrm{B}) + X(\mathrm{A}) = 1$, we have $X(\mathrm{B}) = (L - L(\mathrm{A}))/(L(\mathrm{B}) - L(\mathrm{A}))$. An example of $X(\mathrm{B})$ values thus obtained with calf thymus DNA (42% of G-C) is given in table 1 and corresponding values for micrococcal DNA (72% of G-C) are listed in table 2. In these tables, we can also see that, when the mean parameter $m = 3.37X(\mathrm{B}) + 2.55X(\mathrm{A})$ is used in a complementary way, the ratio L/m remains practically constant in the relative humidity interval between 66 and 95%. In contrast, we observe in table 1, that at 92% R.H., the ratio L/p has a value of 1.10 instead of 1.12; this means that a small fraction of nucleotides already have adopted conformation A. In a symmetrical way, the excessively high value of 1.16 obtained for L/p shows that some fraction of the DNA is still in the B conformation at 75% R.H.

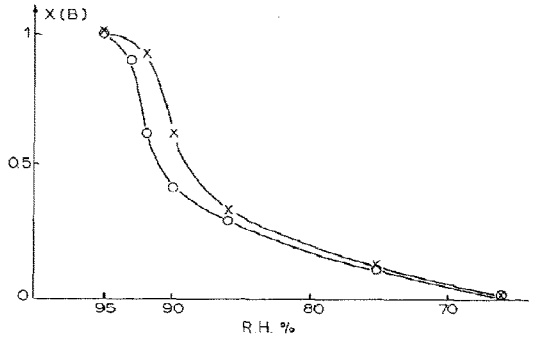

Fig. 4. A-B transition: fraction of nucleotides in the B form as a function of the relative humidity. (× ——— ×) Calf thymus DNA (42% C-G); (○ ——— ○) micrococcal DNA (72% C-G).

The same kind of behavior is observed with samples derived from the micrococcal DNA. However, we can see, in table 2, that such variations appear at shifted values of the relative humidity, expressing the effect of the base composition on the transition.

More precisely, in fig. 4 are shown, for the two DNA types presently studied, the curves of the fraction $X(\mathrm{B})$ of nucleotides in the B-form vs. relative humidity. We can observe that a higher percentage of G-C facilitates the transition to the A-form when the relative humidity decreases [28]. So at 90% R.H., the DNA containing 42% of G-C is at 40% in the A conformation (mid-point at 89% R.H.) whereas, under these conditions, 60% of the DNA which contains 72% of G-C is already in that form (mid-point at 92% R.H.).

We pointed out above that the ratio of the volumes of the fiber in respectively the B- and A-forms can be deduced directly from measurements of dimensions made with a microscope. However, the ratio of the volumes actually occupied by the DNA molecule in these two conformations can also be determined from X-ray data. Indeed, the equatorial spot permits one to determine the average distance between helices in the lattice (22.3 Å in the B-form and 18 Å in the A-form) and thus with values of $p(\mathrm{B})$ and $p(\mathrm{A})$, we can evaluate the order of magnitude of the volumes occupied in the crystal lattice by the DNA and the water associated with the molecular conformations. The volume ratio thus obtained is about 2 as compared to 2.5 determined by using fiber dimensions. The difference is mainly due to the fact that the sample is swollen with water at high relative humidity; it then contains highly hydrated disorganized parts [34] between crystallized regions where DNA molecules are well positioned.

3.3. The B-C transition

3.3.1. X-ray fiber diffraction

We continued as described above but samples were prepared from DNA solutions with a salt content 10-fold lower than in the preceding case. Only results obtained with DNA from calf thymus are presented, since, under these conditions, no

Table 3

B-C transition: geometrical parameters and DNA fiber length (L) and diameter (D)

	R.H. (%)						
	95	92	87	82	79	75	64
Pitch							
P (A)	33.5	33.0	32.0	31.0	29.4	28.9	
p (A)	3.35	3.34	3.33	3.32	3.31	3.30	
P/p	10.00	9.90	9.60	9.33	8.88	8.75	
L (mm)	3.375	3.341	3.285	3.228	3.195	3.150	3.087
D (mm)	0.214	0.178	0.160	0.152	0.149	0.143	0.142
L/p ($\times 10^{-7}$)	1.007	1.000	0.986	0.972	0.965	0.955	
DNA form	B	B	C	C	C	C	

difference in the behavior of the two DNAs can be clearly discerned. Fiber X-ray diffraction therefore gives patterns which, according to the relative humidity, correspond to the B-C transition of DNA. The results thus obtained, with the relative humidity fixed at values between 75 and 95%, are presented in table 3. For R.H. < 75%, DNA fibers are disorganized and X-ray patterns are so poor that the determination of helical parameters becomes practically impossible.

In the present case, variations in the helical parameters of the DNA molecules do not have the same features as those of the A-B transition; the conformational change is now rather progressive and slow. Characteristic X-ray patterns of the C-form, with a supplementary spot at 0.1 A-1 on the first layer line, are obtained at R.H. $\leqslant$ 87%. Moreover, the crystal lattice remains hexagonal and the helical parameter p (see table 3) decreases linearly with the relative humidity.

3.3.2. *Fiber dimensions*

Measurements of fiber dimensions, at different fixed values of the relative humidity, made with the binocular microscope, allowed us to draw the experimental curves presented in fig. 5. We can observe linear variations of the fiber length with a change of slope at 75% R.H. (no X-ray pattern can then be used). Moreover, it can be noted that the fiber diameter decreases very rapidly as far as 75% R.H., subsequently decreasing more slowly. This latter fact can be used to explain, qualitatively at least, the change in slope of the plot for length vs. relative humidity. Indeed, it appears that up to 75% R.H., there is sufficient water around the DNA helices to facilitate changes being induced in their conformation than at lower relative humidity where almost all excess water has been removed. A series of length and diameter measurements have shown the perfect reversibility of this progressive process of DNA conformational change which, now, does not show any cooperative effect.

3.4. *Relation between X-ray results and fiber dimensions*

The ratio of the sample length L divided by the helical parameter p, determined at different relative humidities (in the interval 75–95%), is listed in table 3. This ratio which represents, as stated

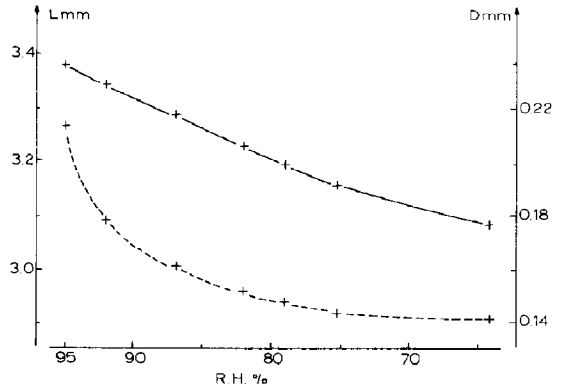

Fig. 5. B-C transition: variations of the length (+ ——— +) and diameter (+ - - - - - - +) as a function of relative humidity.

above, the constant number of base-pairs along the fiber axis, does not remain exactly constant; it diminishes by a few percent when the relative humidity decreases.

Furthermore, we also observed that the ratio of the fiber volume divided by the mean volume occupied by a DNA double helix in the crystal lattice (X-ray data) becomes constant at R.H. $\leqslant 82\%$. Therefore, in the present case, as in that of the A-B transition, the DNA fiber is swollen at high values of the relative humidity when in the B form; it then includes disorganized and highly hydrated parts. At the other limit of relative humidity, the very small decrease in length when R.H. $< 75\%$ (see table 3) corresponds to the disorganization of DNA helices as concluded from the very poor X-ray patterns we obtained under these conditions.

4. Discussion

Fiber X-ray diffraction results compared to values obtained from measurements of dimensions made on the same samples at the same relative humidity allowed us to establish a simple and precise method for the study of DNA conformational transitions. We observed experimentally that the main factor responsible for the fiber length variations is the change, with relative humidity, of the DNA helical parameters. We therefore used the proportionality actually existing between the length of a DNA fiber and the helical parameter p (axial rise per base-pair) of the DNA molecular conformation.

Consequently, a most significant variation in the fiber length can easily be observed during the perfectly reversible A-B transition. The presence of cooperative effects in this conformational change is well characterized by the sigmoidal form of the transition curve of the length (or the fraction of nucleotides in the B-form) vs. the relative humidity. In contrast, the B-C transition shows very progressive variations of the fiber length, indicating a continuous deformation of the B conformation of DNA which takes the C-form when the relative humidity is diminished. The latter conformational transition is also perfectly reversible. However, in this case, the proportionality between the fiber length and the helical parameter p is somewhat perturbed by disorganization of the fiber (X-ray patterns become poor when the relative humidity is lowered). The excessively large diminution in fiber length thereby observed (table 3) may be due to slight sliding of DNA helices past one another or to some undulations of DNA molecules which are superimposed on conformational changes as described previously in oligonucleotide single-crystal studies [38].

Moreover, it is interesting to note that even with samples made from very low salt concentration solutions, we were unable to observe any transitions of the type C-A-B [25] which should be characterized by a significant decrease followed by an almost equal increase in fiber length when the relative humidity undergoes a steady decrease.

The determination of variations in fiber diameter and hence of the volume also provides valuable information on the importance of water in the process of DNA conformational changes. The particularly large decrease in fiber diameter during the B-A transition, a decrease which takes place before any variation of the fiber length occurs, may be due to the loss of a significant amount of water located between the organized parts of the fiber as well as around the double helices. It is only after this release of excess water has been achieved that a decrease in relative humidity induces changes of the DNA conformation by removing water molecules from the inside of the molecular structure. For the reverse A-B transition, we observed that, from an increase of the relative humidity, it firstly results in a change of the DNA conformation due to the penetration of water into the molecular structure. This is clearly associated with lengthening of the fiber. The swelling of the lattice of helices and thus of the sample is only observed after.

During the B-C transition, the fiber diameter also diminishes very rapidly with a decrease in the relative humidity. In this case, however, the fiber length decreases slightly and regularly at the very beginning of the process. We can thus suppose that the withdrawal of water from the DNA environment induces constraints on organized parts of the fiber which modify the double helix confor-

mation. The low NaCl content of the DNA preparations does not allow, in this case, the transition to the A-form.

To conclude, let us add that we also began the study of the B-Z transition, i.e., the change between a right-handed and a left-handed helix [39,40]. Such a transition which is observed with samples of poly(G-C)-poly(G-C) made from solutions under unusual chemical conditions [9] may also correspond to variations in the fiber dimensions. Observations with the microscope are, however, very difficult in this case because the conformational change occurs at relative humidities between 100% (for B) and 95% (for Z). For such values of the relative humidity, the fibers are unstable and become distorted when the slightest tension is applied. We are currently attempting to modify these unfavorable conditions in order to be able to apply the present experimental method to the study of this singular conformational transition.

References

1 R.M. Wing, H.R. Drew, T. Tanako, C. Broka, S. Tanaka, K. Itakura and R.E. Dickerson, Nature 287 (1980) 755.
2 Z. Shakked, D. Rabinovich, W.B.T. Cruse, E. Egert, O. Kennard, G. Sala, S.A. Salisbury and M.A. Viswamitra, Proc. R. Soc. Ser. B 213 (1981) 479.
3 B.N. Conner, T. Tanako, J. Tanaka, K. Itakura and R.E. Dickerson, Nature 295 (1982) 294.
4 A.H.J. Wang, S. Fujii, J.H. van Boom and A. Rich, Proc. Natl. Acad. Sci. U.S.A. 79 (1982) 3968.
5 R. Langridge, H.R. Wilson, C.W. Hooper, M.H.F. Wilkins and L.D. Hamilton, J. Mol. Biol. 2 (1960) 19.
6 W. Fuller, M.H.F. Wilkins, H.R. Wilson and L.D. Hamilton, J. Mol. Biol. 12 (1965) 60.
7 D.A. Marvin, M. Spencer, M.H.F. Wilkins and L.D. Hamilton, J. Mol. Biol. 3 (1961) 547.
8 A.G.W. Leslie, S. Arnott, R. Chandrasekaran and R.L. Ratliff, J. Mol. Biol. 143 (1980) 49.
9 A.H.J. Wang, G.J. Quigley, F.J. Kolpak, J.L. Crawford, J.H. van Boom, G. van der Marel and A. Rich, Nature 282 (1979) 680.
10 A.H.J. Wang, G.J. Quigley, F.J. Kolpak, G. van der Marel, J.H. van Boom and A. Rich, Science 211 (1981) 171.
11 H. Shindo, J.B. Wooten, B.H. Pheiffer and S.B. Zimmerman, Biochemistry 19 (1980) 518.
12 D.J. Patel and L. Shapiro, Annu. Rev. Biophys. Biophys. Chem. 16 (1987) 423.
13 M.H. Sarma, G. Gupta and R.H. Sarma, Biochemistry 27 (1988) 3423.
14 R. Brandes, R.R. Vold, D.R. Kearns and A. Rupprecht, Biopolymers 27 (1988) 1159.
15 C.A.G. Haasnoot, H.P. Westerink, G. van der Marel and J. van Boom, J. Biomol. Struct. Dyn. 2 (1984) 345.
16 W. Pohle and H. Fritzsche, Nucleic Acids Res. 8 (1980) 2527.
17 J. Pilet and J. Brahms, Biopolymers 12 (1973) 387.
18 J. Pilet and M. Leng, Proc. Natl. Acad. Sci. U.S.A. 79 (1982) 26.
19 J.A. Taboury and E. Taillandier, Nucleic Acids Res. 13 (1985) 4469.
20 E. Taillandier, S. Adam, J.P. Ridoux and J. Liquier, Nucleic Acids Res. 16 (1988) 5621.
21 W. Saenger, in: Principles of nucleic acid structure, ed. C.R. Cantor (Springer-Verlag, New York, 1984) p. 228.
22 S.C. Erfurth, P.J. Bond and W.L. Peticolas, Biopolymers 14 (1975) 1245.
23 V.N. Potaman, Yu.A. Bannikov and L.S. Shlyachtenko, Nucleic Acids Res. 8 (1980) 635.
24 P.J. Cooper and L.D. Hamilton, J. Mol. Biol. 16 (1966) 562.
25 N.J. Rhodes, A. Mahendrasingam, W.J. Pigram, W. Fuller, J. Brahms, J. Vergne and R.A.J. Warren, Nature 296 (1982) 267.
26 J. Portugal and J.A. Subirana, EMBO J. 4 (1985) 2403.
27 P.K. Parrack, S. Dutta and V. Sasisekharan, J. Biomol. Struct. Dyn. 2 (1984) 149.
28 J. Pilet and J. Brahms, Nat. New Biol. 236 (1972) 136.
29 R.E. Dickerson, J. Mol. Biol. 166 (1983) 419.
30 M. Fornells, J.L. Campos and J.A. Subirana, J. Mol. Biol. 166 (1983) 249.
31 G. Albiser, M. Harmouchi and S. Premilat, J. Biomol. Struct. Dyn. 6 (1988) 359.
32 S. Premilat and G. Albiser, J. Mol. Biol. 99 (1975) 27.
33 M.A. O'Brien, J. Sci. Instrum. 25 (1948) 73.
34 S.M. Lindsay, S.A. Lee, J.W. Powell, T. Weidlich, C. Demarco, G.D. Lewen, N.J. Tao and A. Rupprecht, Biopolymers 27 (1988) 1015.
35 J. Doucet, J.P. Benoit, W.B.T. Cruse, T. Prange and O. Kennard, Nature 337 (1989) 190.
36 D. Poland and H.A. Scheraga, Theory of helix-coil transition in biopolymers (Academic Press, New York 1970).
37 T.M. Birshtein and O.B. Ptitsyn, Conformations of macromolecules (Interscience, New York, 1966).
38 R.E. Dickerson, D.S. Goodsell, M.L. Kopka and P.E. Pjura, J. Biomol. Struct. Dyn. 5 (1987) 557.
39 S. Arnott, R. Chandrasekaran, D.L. Birdsall, A.G.W. Leslie and R.L. Ratliff, Nature 283 (1980) 743.
40 V. Sasisekharan and S.K. Brahmachari, Curr. Sci. 50 (1981) 10.