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# Conformational transitions and hydration of poly d(A-T) · poly d(A-T) in fibers

**A. Abouelkassimi, G. Albiser, and S. Premilat**

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

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**Abstract.** Conformational transitions of poly d(A-T) · poly d(A-T) have been studied by fiber X-ray diffraction and measurement of fiber dimensions. Results obtained for the D-A-B and D-B transitions are presented and analyzed. For all these form transitions, cooperativity effects are observed for the variation of the rise per nucleotide versus the relative humidity. Detailed information about hydration of the polynucleotide during form transitions and the numbers of water molecules per nucleotide necessary to stabilize the different helical conformations are presented.

**Key words:** poly d(A-T) · poly d(A-T) – D-A-B and D-B helical transitions – DNA hydration – X-ray fiber diffraction

## Introduction

Polynucleotides do present, as revealed by X-ray diffraction (Leslie et al. 1980), a polymorphism equivalent to that of natural DNA. Recent studies on oligonucleotides using different approaches such as NMR (Patel et al. 1987) and I.R. spectroscopy (Adam et al. 1986; Pilet et al. 1975) or crystallography (Kennard and Hunter 1989) have confirmed this polymorphism, which is observable for the family of right handed double helices (Wing et al. 1980) as well as for the left handed Z form (Wang et al. 1979).

The polymorphism of DNA and polynucleotides is associated with different conformational transitions which can be revealed by the study of well organized fibers (Lindsay et al. 1988; Mahendrasingam et al. 1983; Premilat et al. 1990). Actually, helical transitions depend on many physico-chemical parameters such as the type and concentration of salt, the relative humidity and also on the tension applied on fibers (Albiser et al. 1988). It should be noted that DNA conformations and form transitions are also dependent on the base composition or, more precisely, on the base sequence of polynucleotides (Arnott et al. 1974; Arnott et al. 1980; Leslie et al. 1980).

A recently proposed method for the experimental study of DNA conformational transitions in fibers allowed us to follow and analyze the A-B and B-C transitions by using, in a complementary way, fiber X-ray diffraction and measurement of fiber dimensions (Premilat et al. 1990). Moreover, it has been shown (Harmouchi et al. 1990) that one can also determine, by using results obtained from this method, the variations of the number of water molecules associated with the DNA base pairs during the form transitions.

In the present study, we used this experimental method in order to gain information on the conformational transitions in poly d(A-T) · poly d(A-T) which presents fiber X-ray patterns of the A, B or D double helical forms according to the sodium salt concentration and the relative humidity (Arnott et al. 1974; Davies et al. 1963; Mahendrasingam et al. 1983 and 1986; Leslie et al. 1980). Results of a detailed study of the D-B and D-A-B transitions which could have some biological importance in A+T rich sequences of DNA (Moreau et al. 1982), are presented.

## Material and methods

Lyophilized poly d(A-T) · poly d(A-T) associated with sodium chloride was purchased from Pharmacia and used without any further purification. Fibers were obtained from the stretching of a gel of polynucleotide humidified with water at pH 7 following a method already described (Fuller et al. 1967).

As the type of the helical transition observed depends mainly on the salt concentration, an empirical procedure was applied in order to get the appropriate amount of NaCl in the fiber. We proceeded as follows: the fiber was firstly tested by getting an X-ray pattern; the type of conformation and the degree of organization of the fiber was therefore determined. Then we added successive small

*Offprint requests to:* S. Premilat

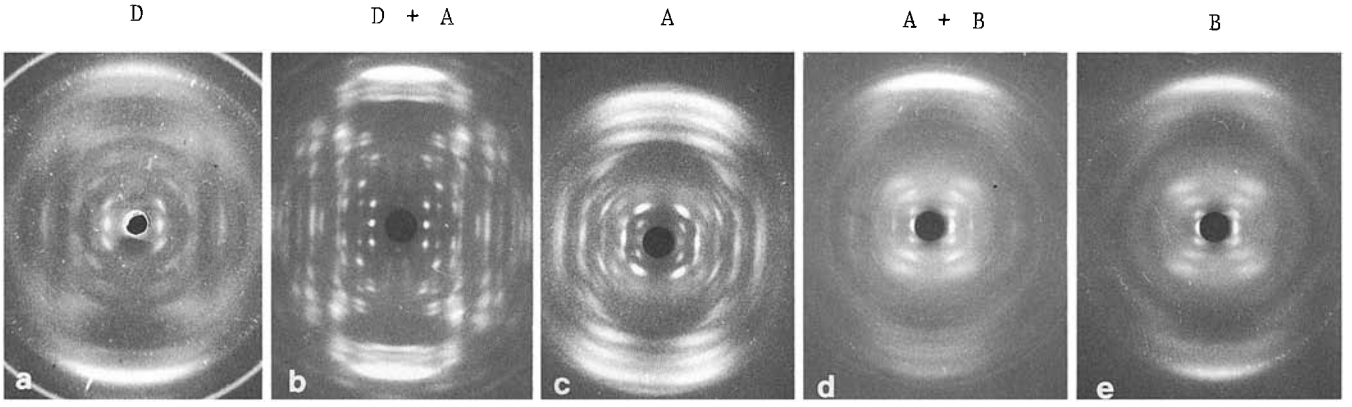


**Fig. 1.** X-ray patterns obtained at **a** 40% r.h. (D-form); **b** 60% r.h. (mixture of A and D forms); **c** 68% r.h. (A form); **d** 78% r.h. (mixture of A and B forms); **e** 88% r.h. (B form)

**Table 1.** D-A-B transition: geometrical parameter of helical conformations: $p$ (rise per nucleotide), $P$ (pitch)

| R.H. (%) | DNA form | Lattice type[a] | $a$ (A°) | $b$ (A°) | $c$ (A°) ($=P$) | $p$ (A°) | $P/p$ |
|---|---|---|---|---|---|---|---|
| 40 | D | T | 17.4 | 17.4 | 24.2 | 3.00 | 8.07 |
| 56 | D | T | 17.8 | 17.8 | 24.9 | 3.02 | 8.25 |
| 60 | D | T | 18.1 | 18.1 | 25.1 | 3.00 | 8.40 |
| | A | M | 21.4 | 40.2 | 27.6 | | |
| 68 | A | M | 21.8 | 40.6 | 27.8 | 2.54 | 10.96 |
| 74 | A | M | 22.5 | 41.0 | 28.1 | 2.56 | 10.98 |
| 78 | A | M | 23.1 | 41.9 | 28.5 | 2.56 | 11.05 |
| | B | H | 39.2 | | 32.4 | 3.32 | 9.76 |
| 88 | B | H | 44.2 | | 33.5 | 3.35 | 10 |
| 90 | B | H | 45.0 | | 33.6 | 3.36 | 10 |

[a] T: Tetragonal; M: Monoclinic; H: Hexagonal

amounts of a solution 0.01 $M$ NaCl on the humidified fiber the extremities of which were fixed to the holder. It is in this way that one can observe the D-B transition whereas the D-A-B transitions are obtained from fibers at lower salt concentrations. Conversely, fibers containing an excess of salt, as revealed by characteristic spots of NaCl powder on X-ray patterns, can be slightly "washed" by taking off the salt on the fiber surface (at low r.h.) with a few drops of water. Moreover it can be noted that a better organization of the fiber is obtained after it has been submitted to variations of the relative humidity (r.h.) while its extremities are maintained fixed to the holder. However, for the actual X-ray and dimension measurements, one extremity of the fiber is freed from the holder and this is done when equilibrium at low r.h. is realized (the diameter of the fiber then has its smallest value).

A special X-ray camera was realized in order to get the same physico-chemical conditions when X-ray or fiber dimension measurements are performed. The camera side facing the collimator is a transparent plastic sheet with a well on its centre to stop the direct X-ray beam. It allows us to observe the DNA fiber, with a binocular microscope, before and after every X-ray exposure. The photographic plate can be positioned at distances from the fiber equal or superior to 18 mm. One can also, without any opening the camera (the fixed value of the r.h. is not perturbed), modify the position of the fiber relative to the incident X-ray beam. The fiber is maintained vertical and the very slight tension applied by the glass rod fixed to its extremity prevents undulations of the fiber during form transitions. The r.h. in the fiber surroundings is given precise values according to the method already described (Premilat et al. 1990).

The evaluation of the number of water molecules associated with a nucleotide as well as its variation during a conformational transition is made following the method previously used for the study of the A-B and B-C transitions (Harmouchi et al. 1990).

## Results

### *A. The D-A-B transitions*

*X-ray fiber diffraction.* At 40% r.h. the poly d(A-T) · poly d(A-T) presents the D helical conformation (Fig. 1 a) with parameters given in Table 1. These experimental data show some variations of the unit cell with increasing r.h. We note that the unit cell parameters at low r.h. do not differ from values given in recent X-ray diffraction studies of the D form (Millane et al. 1984; Forsyth et al. 1989). The A form is observed at 68% r.h. (Fig. 1 c) and a mixture of D and A forms is obtained for r.h. values between 40 and 68% (Fig. 1 b). When the r.h. is increased from 68%, a mixture of A and B forms is observed (Fig. 1 d) on X-ray patterns (easily observable at 78% r.h.). At 88% r.h. X-ray patterns of the B-form alone are obtained (Fig. 1 e). By decreasing the r.h. from 88%, we could only observe the reversible B-A transition; the transition from A to D could not be realized (Mahendrasingam et al. 1983) when the r.h. was lowered in the range of 68 to 40% and the poor X-ray patterns then obtained indicate the disorganization of the A form of the polynucleotide in the fiber. However, transitions from the D (or F) to A and then to the B form of poly d(A-T) · poly d(A-T) were already observed from X-ray diffraction studies (Fuller et al. 1984) and actually, one can get the D form again by a complete rehydration of the fiber

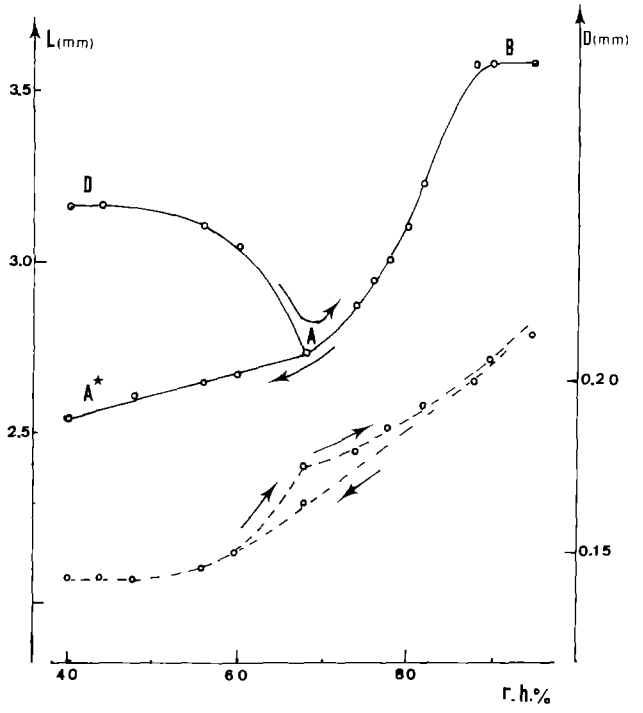


**Fig. 2.** D-A-B transition: variations of the length (—) and diameter (- - -) as a function of the r.h.

(we then have the B form) followed by a decrease of the r.h. until 40%. However, it is necessary to keep the two ends of the fiber fixed to the holder during this last operation. Therefore the important effect of a tension applied to the fiber (Albiser et al. 1988) must be taken into account in order to realize reversible transitions. The present result confirms, in a complementary way, those given in a recent study (Loprete and Hartman 1990) of non-oriented gels of poly d(A-T) · poly d(A-T) where the D form could not be observed.

*Fiber dimensions.* Results of measurement of the fiber dimensions, made as explained above, are presented in Fig. 2. We can thus see that the variations of the fiber length with the r.h. is characterized by cooperative effects for the D-A and A-B transitions. The D-A transition is not reversible under the present conditions but the A-B transition is perfectly reversible as noted before for natural DNA (Lindsay et al. 1988; Premilat et al. 1990). The part A-A* of the transition curve (Fig. 2), corresponding to the non-cooperative disorganization of the A form, is linear with the r.h. (values of the helical parameters can then no longer be obtained from X-ray patterns).

We noted that when the r.h. is increased starting from a low value (D form), the fiber diameter presents two types of variations corresponding respectively to the D-A and A-B transitions (Fig. 2) with a marked point of inflexion between these two transitions. When the r.h. is decreased from the high value corresponding to the B form, the route followed by the diameter value is not reversible as for the fiber length; the diameter decrease is then uniform even in the A-A* portion of the curve.

**Table 2.** D-B transition: geometrical parameters of helical conformations: $p$ (rise per nucleotide), $P$ (pitch)

| R.H. (%) | DNA form | Lattice type[a] | $a$ (A°) | $b$ (A°) | $c$ (A°) ($=P$) | $p$ (A°) | $P/p$ |
|---|---|---|---|---|---|---|---|
| 40 | D | T | 17.3 | 17.3 | 24.0 | 2.97 | 8.08 |
| 48 | D | T | 17.5 | 17.5 | 24.3 | 2.97 | 8.18 |
| 56 | D | T | 17.8 | 17.8 | 24.6 | 2.99 | 8.22 |
| 60 | D | T | 18.2 | 18.2 | 24.7 | 3.00 | 8.23 |
| 64 | D | T | 19.2 | 19.2 | 25.3 | 3.00 | 8.43 |
| | B | H | 37.4 | | 31.6 | 3.33 | 9.49 |
| 68 | D | T | 20.2 | 20.2 | 27.1 | | |
| | B | H | 37.7 | | 32.3 | 3.33 | 9.70 |
| 76 | B | H | 40.2 | | 33.4 | 3.33 | 10.03 |
| 80 | B | H | 41.0 | | 33.4 | 3.34 | 10 |
| 86 | B | H | 43.0 | | 33.5 | 3.35 | 10 |

[a] T: Tetragonal; H: Hexagonal

### *B. The D-B transition*

We proceeded as for the preceding study but salt was added to the fiber progressively until the A form could no longer be observed on X-ray patterns. The perfectly reversible B-D transition can then be analysed from X-ray patterns. Note that it is not necessary in the present case to maintain the fiber fixed to the holder in order to get the D form.

Results thus obtained are given in Table 2. One can see that for r.h. lower than 60%, the D form is obtained (Fig. 3a); its helical parameters vary very slightly with the relative humidity. As soon as the r.h. is 76%, only the B form appears on X-ray patterns (Table 2 and Fig. 3c).

Between 60% and 76% r.h. (i.e. the range of D-B transition) no continuous variation of the parameter p (rise per nucleotide) is observed as was the case for the B-C transition (Premilat et al. 1990). A mixture of D and B forms is rather clearly observed on the X-ray patterns (Fig. 3b). Measurements of fiber dimensions give values presented in Fig. 4. The fiber length remains practically constant for r.h. lower then 60% (D form) and a steep increase of length is then observed until 80% r.h. The length variations with the r.h. are perfectly reversible and in complete agreement with observations made on X-ray patterns. Actually, we noted that the ratio of the length values at 80 and 56% r.h. is indeed equal to the ratio of the corresponding values of the parameter $p$ measured at the same r.h.; this is in accordance with the direct relation existing between the fiber length and the rise per base pair in the molecular double helix (Premilat et al. 1990).

### *C. Hydration of poly d(A-T) · poly d(A-T)*

The important role played by water during form transitions of the poly d(A-T) · poly d(A-T) can be well appreciated in Figs. 2 and 4 where the variation of the fiber diameter is represented as a function of the r.h.

The double helical conformations adopted by natural DNA as well as by synthetic polynucleotides are directly

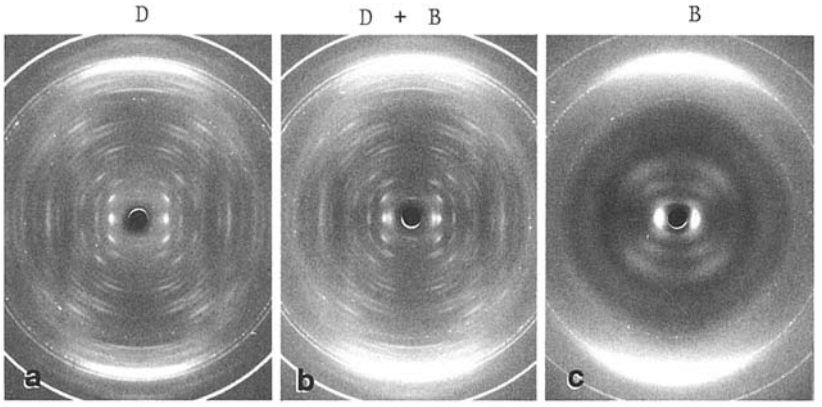


**Fig. 3.** X-ray patterns obtained at **a** 54% r.h. (D-form), **b** 64% r.h. (mixture of D and B forms) and **c** 76% r.h. (B form)

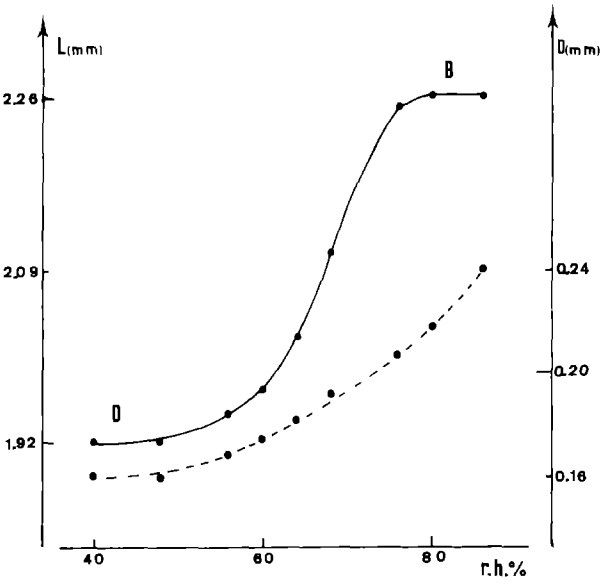


**Fig. 4.** D-B transition: variations of the fiber length (—) and diameter (- - -) as a function of the r.h.

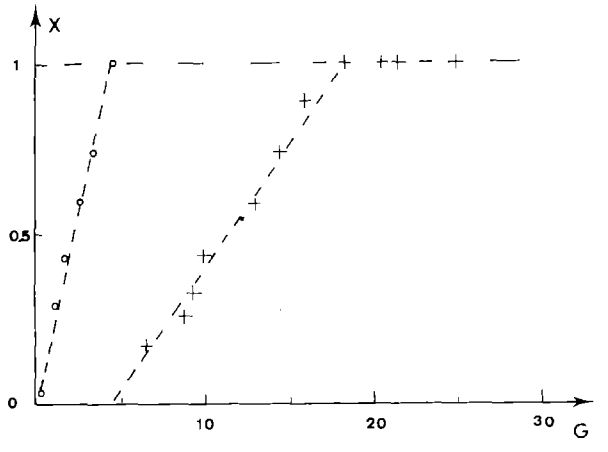


**Fig. 5.** D-A-B transition: (o) fraction $X_A$ of nucleotides in the A form, (+) fraction $X_B$ of nucleotides in the B form versus the number $G$ of water molecules per nucleotide

related to the degree of hydration of the molecules. Hydration can be defined by the averaged number of water molecules associated with a nucleotide of the molecular helix. This number, $G$, can be determined by the following expression previously established (Harmouchi et al. 1990):

$$G = K(V_f - V_0)/N \cdot n$$

where $V_f$ is the volume of the fiber at a given r.h., $V_0$ that volume at 40% r.h.; $N$ is the number of nucleotides situated along the fiber axis, $n$ is the number of nucleotide pairs in a section of the fiber and $K$ is a constant equal to $1.67 \cdot 10^{19}$ mm$^{-3}$.

*1. Variations of G during the D-A transition.* By using the values of the fraction $X_A$ of nucleotides in the A form as a function of the r.h. as well as the values of $G$ corresponding to the D to A transition, we established the curve of $X_A$ versus $G$ presented in Fig. 5. The linear variation of $X_A$ until $G=5$ (r.h. of 68%) corresponds to the transition D-A. Results thus obtained show that only one water molecule (an average) is associated with a nucleotide in the D form at 56% r.h. while 5 water molecules per nucleotide stabilize the A form.

*2. Variations of G during the A-B transition.* The variation of the fraction $X_B$ of nucleotides in the B form versus $G$ is given in Fig. 5. Here also, the variation is linear until $G=18$ (r.h. of 88%). This last value of $G$ is the average number of water molecules necessary to transform all the poly d(A-T) · poly d(A-T) into its B form. For larger values of $G$, there is a saturation which mainly affects the fiber diameter.

*3. Variations of G during the D-B transition.* In Fig. 6 one can see the variation of $X_B$ as a function of $G$. The variation is linear until $G=13$, a value corresponding to $X_B=1$. This value of $G$ represents the average number of water molecules associated with every nucleotide in the B form during the D to B transition. The present results show that there are now 3 water molecules per nucleotide in the D form at 60% r.h. (Forsyth et al. 1989). This

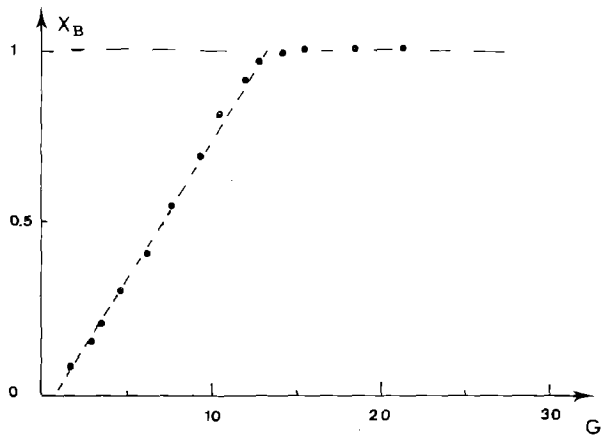


**Fig. 6.** D-B transition: fraction $X_B$ of nucleotides in the B form versus the number $G$ of water molecules per nucleotide

figure, much larger than that for the D-A transition, could be a consequence of the larger amount of salt used, in the present case, in order to avoid the A form.

## Discussion

The experimental method which associates fiber dimension measurements with X-ray diffraction, allows one to gain information on the role played by water during the different conformational transitions of poly d(A-T) · poly d(A-T). Actually, the present study gives the evolution of the average number of water molecules associated with a nucleotide during the D-A-B and D-B transitions. It is also shown that the A form is stabilized with an average of 5 water molecules per nucleotide when the D to A transition is performed in the poly d(A-T) · poly d(A-T) (one should note that water present in the fiber at 40% r.h. is not included in these estimations). For the A-B transition, an average of 18 water molecules per nucleotide is necessary to get the polynucleotide in the B conformation. This figure is indeed very near to the value obtained for natural DNA (Saenger 1984; Brandes et al. 1989; Harmouchi et al. 1990). However, when the B form is obtained from the D-B transition, an average of 13 water molecules per nucleotide are then sufficient to stabilize the B form. This value is intermediate between that obtained ($G=9$) for the C-B transition in natural DNA (Harmouchi et al. 1990) and the value of 18 presently observed for the B form resulting from the A to B transition. One should note that these different numbers of water molecules necessary to stabilize the B form result from the different salt concentrations used in order to obtain specific DNA transitions.

The present method of analysis does not allow one to determine the precise position of water molecules in the double helical structure as it is realized in single crystal X-ray studies on oligonucleotides (Drew et al. 1981, Conner et al. 1984). Nevertheless, our approach permits one to discriminate between helical deformations and actual transitions between distinct structural families of double helices. For instance, one can draw important information from the observation of the linearity of the curves representing the fraction $X$ of nucleotides in a given helical form as a function of the water content $G$ (Figs. 5 and 6). Such behaviour is characteristic of transitions between different forms of the double helices while a progressive deformation of one conformation would result in non-linear variations of $X$ versus $G$ (Harmouchi et al. 1990). This linearity, clearly observed for the D-B as well as for the D-A-B transitions, is in accordance with the sigmoidal variations of the fiber length as a function of the r.h. (Figs. 2 and 4); this indeed characterizes cooperative conformational transitions between distinct helical forms of polynucleotides or natural DNA (Premilat et al. 1990). Moreover, mixtures of X-ray patterns corresponding respectively to A, B and D forms are actually observed when the r.h. is given values located in the transition intervals. It appears therefore that the D form of poly d(A-T) · poly d(A-T) is not an element of the B family but rather a stable distinct conformation of polynucleotides or DNA.

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