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ARTICLE

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Helix-helix transitions in DNA: fibre X-ray study of the particular cases poly(dG-dC) · poly(dG-dC) and poly(dA) · 2poly(dT)

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Abstract The helix-helix transitions which occur in poly(dG-dC) · poly(dG-dC) and in poly (dG-m^{5}dC) · poly(dG-m^{5}dC) are commonly assumed to be changes between the right-handed A- or B-DNA double helices and the left-handed Z-DNA structure. The mechanisms for such transconformations are highly improbable, especially when they are supposed to be active in long polynucleotide chains organised in semicrystalline fibres. The present alternative possibility assumes that rather than the Z-DNA it is a right-handed double helix (S-DNA) which actually takes part in these form transitions. Two molecular models of this S form, in good agreement with X-ray measurements, are proposed. They present alternating C(2′)-*endo* and C(3′)-*endo* sugar puckering like the "alternating B-DNA" put forward some years ago. Dihedral angles, sets of atomic coordinates and stereo views of the two S-DNA structures are given, together with curves of calculated diffracted intensities. Furthermore, we question the possibility of obtaining semicrystalline fibres with triple helices of poly(dA) · 2poly(dT) in a way which renders X-ray diffraction efficient. It is suggested that, up to now, only double helices of poly(dA) · poly(dT) can actually be observed by fibre X-ray diffraction measurements.

Key words Poly(dG-dC) · poly(dG-dC) · Right-handed S-DNA models · Poly(dA) · 2poly(dT) · B′-B* DNA structural transition · X-ray fibre diffraction

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Introduction

Well-defined and precise molecular structures of oligonucleotides are now currently determined by single-crystal X-ray diffraction (Kennard and Hunter 1989). The Z structure of poly(dG-dC) · poly(dG-dC) was established this way from crystals of oligonucleotides (Wang et al. 1979, 1981). The precision of the data determined under such conditions leaves almost no doubt about the proposed left-handed double helical conformation. However, the precision in measurements one can expect from fibre X-ray patterns is much less. In these cases, the relative positions of the diffracted intensity spots allow, by using molecular models, establishment of structures which are globally in accordance with experimental results. However, the molecular conformations thus obtained are generally not unequivocal or, at least, their uniqueness is hardly demonstrable. The characteristic features of such molecular models, like the now classical A, B, C, D, . . . double helices of DNA (Saenger 1984), can possibly be reinforced when results obtained from experimental methods, other than X-ray, give complementary information.

Besides, the DNA right-handed double helical conformations are subject to different helix-helix transitions depending on the sequence of base pairs and on the physicochemical conditions. These order-order transitions are generally observed with DNA in solution or film and are thus well followed by various spectroscopic methods (Patel et al. 1987; Norden et al. 1992; Taillandier and Liquier 1992; Song et al. 1997). However, it appears to be very difficult and, as far as we know, not at present realised, to induce a helix-helix transition while keeping the organisation of oligonucleotide molecules in a crystal lattice. So one obtains, for instance, the B or Z form of oligo(dG-dC) duplexes, but no transition between these two structures can be observed in the crystal state. Conversely, these transitions are currently realised in semicrystalline DNA fibres by modifying the salt content or the relative humidity. We therefore proposed

and used an experimental approach which allowed us to study these structure transitions using, in a complementary way, X-ray diffraction and observation of DNA fibres with a microscope (Premilat et al. 1990). The advantage of this method lies mainly in the fact that there is no doubt about the type of helices involved in a given transition because X-ray patterns are fundamentally their signatures.

In the different form transitions thus observed, the handedness of the double helices is maintained but for the observations allocated to the A to Z and B to Z transitions. These two structural transitions, obtained with poly(dG-dC) · poly(dG-dC) in fibres and followed by X-ray diffraction measurements, do present tremendous mechanical difficulties. In fact, the movements necessary to achieve such changes in the handedness of long DNA double helices packed in a fibre appear to be practically insurmountable. One should note, for example, that such transitions imply relative rotations of the helix extremities around the helix axis of a very large number of turns while the molecular units remain well organised in a semicrystalline lattice. So, it is difficult to admit the reality of the currently assumed A $\rightarrow$ Z and B $\rightarrow$ Z transitions in poly(dG-dC) · poly(dG-dC) fibres and we therefore propose an interpretation of the experimental observations which avoids consideration of the transformation from a right- to a left-handed helix.

S is the designation firstly given to the structure of poly(dG-dC) associated with an original X-ray pattern (Arnott et al. 1980; Leslie et al. 1980) which, being given its similarity with the X-ray diffraction and parameters of the Z structure, was supposed to be due to that left-handed helical conformation. A helix-helix transition proposed as the A $\rightarrow$ Z transition has been observed with the potassium salt (Mahendrasingam et al. 1983; Fuller et al. 1984) of poly(dG-m^{5}dC) · poly(dG-m^{5}dC) (see results below) and the transition defined as the B $\rightarrow$ Z transition in poly(dG-dC) · poly(dG-dC) has been studied by fibre X-ray diffraction (Behe et al. 1981; Mahendrasingam et al. 1983, 1990; Fuller et al. 1984; Albiser and Premilat 1992). One can actually note that the order of occurrence of the structure transitions is A-Z-B or, as shown in what follows, A-S-B where S refers here to a double helix conformation halfway between the A and B structures. Such a conformation has actually already been proposed as the alternating B-DNA structure obtained from an X-ray study of a crystal of a short A-T oligonucleotide (Klug et al. 1979). This conformation exhibits an A-DNA type sugar pucker at the purine residues and a B-DNA sugar pucker at the pyrimidine residues. The alternating B form was also proposed by Klug and co-workers (Lomonossoff et al. 1981) for poly(dA-dT) in solution, based on the observation of the effect of the enzyme DNAse I on that polymer. Moreover, results obtained from NMR measurements, made at that same period, allowed the authors (Patel et al. 1979) to suggest that poly(dG-dC) · poly(dG-dC) could also adopt an alternating B-DNA structure in solution. So, the alternating B-DNA form is here revisited but with the helical parameters of the 12-fold Z (or S) form rather than those of the B-DNA structure. The molecular model thus obtained exhibits alternating C(2′)-*endo* and C(3′)-*endo* sugar puckering. However, the presently proposed S-DNA structure is a right-handed double helix which gives calculated diffracted intensities in good agreement with the fibre X-ray pattern of poly(dG-dC) · poly(dG-dC).

Beside the problem raised by the transition from a right- to a left-handed double helix, we also consider another very difficult mechanical problem related to a supposedly possible transition from a double to a triple helical conformation of poly(dA) · 2poly(dT) in fibre. Actually, we reproduced the results presented by Arnott and Selsing (1974) when poly(dT) is added to poly(dA) · poly(dT) in order to obtain the X-ray pattern attributed to the triple helix of poly(dA) · 2 poly(dT). However, it is the X-ray pattern of the B* double helical conformation of poly (dA) · poly(dT) (Premilat and Albiser 1997) which is in fact obtained in that case and the implications of this experimental observation are discussed below.

Materials and methods

The lyophilised polynucleotides, obtained from preparations with sodium chloride, were purchased from Pharmacia and used without any further purification. Well oriented fibres were obtained, following a method already described (Langridge et al. 1960a; Fuller et al. 1965), from the stretching of a gel of polynucleotide humidified with water at pH 7. The gel of poly(dA) · 2poly(dT) was obtained from solutions of polynucleotides made up in the 1:2 stoichiometry. The homopolymers poly(dA) and poly(dT) were dissolved in 0.1 M NaCl, 0.01 M EDTA at pH 7.0 (Arnott and Selsing 1974). The appropriate stoichiometric ratio was determined by the measurement of the absorptivities of 26.5 O.D./mg for poly(dA) and 26.3 O.D./mg for poly(dT).

The X-ray experimental device used is that described previously (Premilat and Albiser 1997). The relative humidity (r.h.) in the fibre surroundings is fixed at precise values by passing hydrogen through saturated salt solutions. The specimen-to-film distance, of about 15 mm, is determined precisely for each fibre lightly dusted with quartz or calcite powder. The photographs obtained from nickel-filtered. Cu Kα radiation are enlarged for measurement of the helical parameters. The intensities diffracted by a single double-helix in the lattice are determined (Langridge et al. 1960a) from experimental data with a Zeiss microdensitometer and are compared with intensities calculated using a molecular model.

The procedure followed to compute atomic coordinates of nucleotides in a helical conformation is the one previously used (Premilat and Albiser 1983, 1997). The bond lengths and bond angles are taken from the listing given by Seeman et al. (1976). They can be very slightly varied but they are always maintained in intervals of values determined by X-ray studies on single crystals (Gelbin

et al. 1996). Hence, the geometry of the sugar-phosphate backbone is completely defined by the set of values given to its dihedral angles. The model building program allows us to modify the dihedral angles of the molecular model starting from angle values and atomic coordinates corresponding to the A- and B-DNA double helical structures we determined previously (Premilat and Albiser 1983). As sugar rings present some flexibility, perturbations of their conformations are allowed during the process of improvement of the chain conformation. Nevertheless, these perturbations do not permit the sugar rings to obtain angle values out of the intervals characterising respectively the A and B sugar puckers. The other torsional angles of the phosphate chain are subject to much larger modifications of their initial values in order for the molecular model to be in accordance with the experimental data. Bases will be maintained almost perpendicular to the helix axis as far as atomic overlaps do not impose the introduction of tilt and twist.

Moreover, the DNA molecules being composed of two antiparallel and complementary chains with bases paired as proposed by Watson and Crick, the calculated conformations must present a dyadic symmetry and good hydrogen bonds between associated bases. The stereochemistry of the molecular models is also tested and possible too short interatomic distances are eliminated by very small variations of the dihedral angles. When a chain is built in a proper helical form, the antiparallel and complementary one is adjusted, by a rotation around the common helical axis, to a position that realises a good fit for hydrogen bonds between bases associated according to the Watson-Crick pairing. Then, the cylindrical coordinates of the atoms are calculated in a reference frame that uses a dyad axis and the screw axis. In such a frame the dyadic symmetry of the system is used so that the cylindrical coordinates (ϕ, z, R) of a given atom of the main sugar-phosphate chain [and also the bases for poly(dG-dC) · poly(dG-dC)] on one helix correspond to the coordinates ($-\phi$, $-z$, R) for the homologous atom on the other helix.

The diffracted intensities curves are computed following the procedure previously described (Premilat and Albiser 1983, 1997) and using the atomic cylindrical coordinates of the best calculated conformations. Thus, with

$$A_n = \sum_j f_j J_n(2\pi R_j \xi) \cos\left(-n\phi_j + \frac{2\pi l z_j}{c}\right)$$

and

$$B_n = \sum_j f_j J_n(2\pi R_j \xi) \sin\left(-n\phi_j + \frac{2\pi l z_j}{c}\right)$$

the structure factor of a molecule in helical conformation is given by (Cochran et al. 1952):

$$F(l, \psi, \xi) = \sum_n (A_n + iB_n) \exp in \left(\psi + \frac{\pi}{2}\right)$$

where l is the layer line number; R_j, ϕ_j, z_j are the cylindrical coordinates of the jth atom in the repeating unit and f_j its scattering factor; c is the length of the unit cell along the helix axis; ψ and ξ are, respectively, the angular and the radial coordinates of a lattice point in reciprocal space; J_n is the first-order Bessel function of n, and n is defined as the integral solution (positive or negative) of the expression $n/P = (l/c) - (m/p)$, where P is the helix pitch, p the axial separation of the repeating units and m any integer. Hence, for poly (dG-dC) · poly(dG-dC) the diffracted intensities are calculated with values of n verifying the relation $l = n + 6m$ (n was presently limited by $|n| < 13$ for every value of l). The atomic scattering factors f_j are those given by Langridge et al. (1960b) and Fraser et al. (1978); they take into account the water in the structure and include a temperature factor. Practically, one calculates the diffracted intensities averaged over the rotation ψ. Hence, the diffracted intensities are given by:

$$I(l, \xi) = F^2(l, \xi) = \sum_n (A_n^2 + B_n^2)$$

However, note that for poly(dG-dC) · poly(dG-dC) the dyadic symmetry can be used. The B_n factors are then zero and calculations are performed with a CG or GC nucleotide pair of one strand. Improvements of the calculated intensities, until a good agreement with the experimental data is realised, are obtained by modifying the geometry of the main chain, the tilt and twist of the bases, whereas the helical parameters of the model are always constrained to preserve their experimental values. In fact a conformation verifying the imposed constraints and presenting an acceptable stereochemistry is progressively obtained by going back and forth between the molecular model and the calculated diffracted intensities.

Results

Poly(dG-dC) · poly(dG-dC) in fibre

The helix-helix transitions of poly(dG-dC) · poly (dG-dC) are very well followed by observation of the changes in the fibre X-ray diffraction patterns (Fig. 1a, b). Actually, as the r.h. rises, one observes the progressive transition from the S_I to the S_{II} form and at 96% r.h. a very fast change from S_{II} to the B-DNA structure (Albiser and Premilat 1992). The reverse transition from B to S_{II} occurs at about 90% r.h. and that of S_{II} to S_I for a r.h. near 65%. The A-DNA X-ray pattern has been observed with a potassium salt preparation of poly (dG-dC) · poly(dG-dC) fibres (Mahendrasingam et al. 1983, 1990). The A-DNA form is also observed with poly(dG-m^5 dC) · poly(dG-m^5dC) (Fig. 1c) as it was with a crystal of methylated (G-C) oligonucleotide (Tippin et al. 1997). For the present experimental result obtained with the methylated polymer, the transition from A to S form is completely achieved at 90% r.h. (Fig. 1c, d). However, we could not obtain the reverse

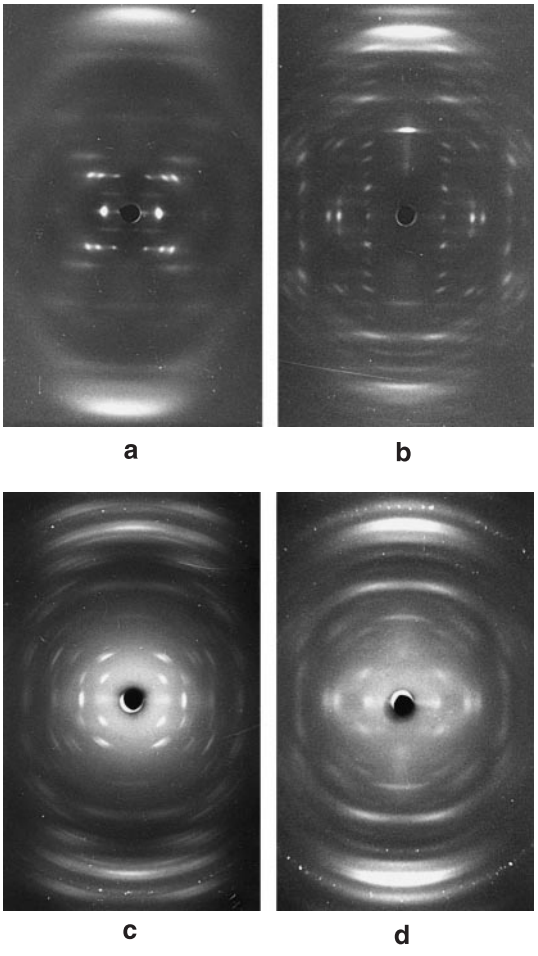

Fig. 1a–d X-ray fibre diffraction patterns of poly(dG-dC) · poly(dG-dC): **a** the B-DNA form obtained at high r.h.; **b** the S_I form obtained at low r.h. with the same fibre; **c** the A-DNA pattern obtained with poly(dG-m^{5}dC) · poly(dG-m^{5}dC) at 70% r.h.; **d** the S form obtained with the same fibre of poly(dG-m^{5}dC) · poly(dG-m^{5}dC) at 90% r.h.

change from S to A as previously remarked (Mahendrasingam et al. 1983). The DNA remains tightened in the S structure and it seems that helices are then so interwoven that the transition to the A form, which should occur at low r.h., cannot be mechanically realised in the fibre.

The calculation of S-DNA helical structures is performed taking into account the geometrical constraints deduced from X-ray measurements. The X-ray pattern presented in Fig. 1a was obtained at 75% r.h. and it is of the S_I type. One can see that diffraction patterns of the S-DNA structure of poly(dG-dC) · poly(dG-dC) show very clearly defined Bragg spots and that important offmeridional reflections are present on all the layer lines (Fig. 1b). This reveals that the double helices are very well packed in the crystalline lattice. The observed Bragg reflections correspond to a hexagonal lattice; they can be indexed with the unit cell dimensions $a = b = 31.5$ Å and $c = 43.2$ Å. Moreover, there are three double helices per unit cell since Bragg reflections on the equatorial line ($hk0$) are only observed for $-h + k = 3n$ (n is an integer). Modifications of the unit cell dimensions with variations of the r.h. can be observed, as remarked previously (Albiser and Premilat 1992), during the form transitions. However, the lattice remains always hexagonal while its parameter a varies from 29 Å for S_I to more than 43 Å for S_{II} and even more than 48 Å for the B-DNA form.

The strong meridional reflection on the sixth layer line (Fig. 1b) is due to the dinucleotide repeat unit, whereas the meridional reflection on layer line 12 corresponds to 12 nucleotide pairs per helix turn. Therefore, the rotation per symmetry unit composed of a C-G and a G-C base pair must be of 60° and there are 6 such units per helix turn. Moreover, the helix pitch $P = c = 43.2$ Å. One should note that the 7.2 Å distance along the axis, here observed for the rise per unit p ($= c/6$), is a well-known characteristic parameter for the S- and Z-DNA forms revealed by X-ray diffraction (Wang et al. 1979, 1981). In the proposed conformation, that distance should be found in the distance along the helix axis between successive symmetry units. However, there is no reason to suppose that within a unit (a C-G and a G-C base pair) the two rises, CG and GC in a DNA strand, should be equal.

One can see that the X-ray pattern of the S form of poly(dG-dC) · poly(dG-dC) corresponds to helical parameters of the Z-DNA structure except for the handedness of the double helix, which cannot be directly deduced from fibre X-ray data. Nevertheless, the experimental observations about the helix-helix transitions described above led us to represent the S form as a right-handed double helix. In fact, two possible molecular models were considered. They can be designated as C_AG_B or C_BG_A depending on the two possible structures made with the base C respectively associated with an A-DNA sugar pucker or a B-DNA one (and vice versa for G). Note that it is not sufficient to permute the C and G bases to obtain C_BG_A from C_AG_B because the relative positions of the sugar-phosphate strands and thus the orientation of the dyad axis must also be modified in order to obtain good H-bonds between paired bases. Curves of the calculated intensities corresponding to the molecular models giving the best agreement with the experimental diffraction data are presented in Fig. 2. It can be seen that the calculated intensities are similar for the two S-DNA forms. The two families of curves actually have the same shape and maxima are located at almost the same positions. So, fibre X-ray diffraction does not allow us to discriminate between these two models. Such a point could possibly be resolved using other experimental methods.

The two right-handed double helical conformations do incorporate significant propeller twist between bases, since calculations which introduce modifications in the base orientation show that tilt and twist of the bases have an important effect on the relative values of calculated intensities. The furanose puckering is alternately C3′-*endo* and C2′-*endo* in both antiparallel and geometrically identical sugar-phosphate strands. Figure 3

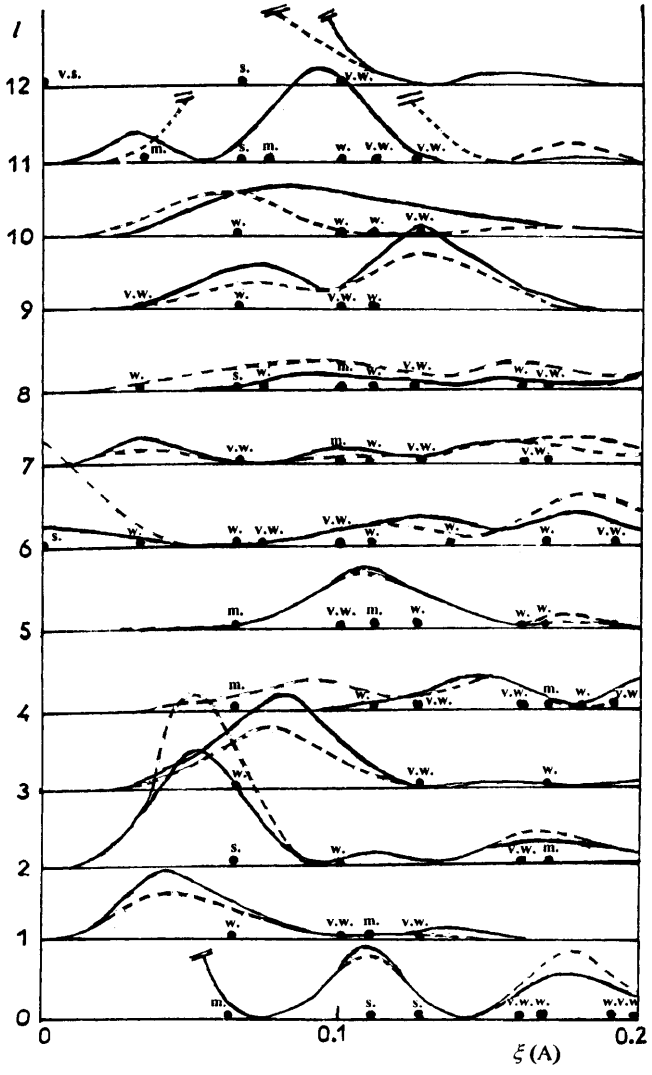

Fig. 2 Square of the cylindrically averaged Fourier transform of the S molecular model of the two right-handed double helices of poly(dG-dC)·poly(dG-dC), compared with observed intensities of X-ray diffraction. For these last, the positions of the Bragg reflections are indicated by *dots* and are labelled: *v.s.* very strong; *s.* strong; *m.* medium; *w.* weak; *v.w.* very weak. *Continuous curves* for C_AG_B and *broken lines* for C_BG_A

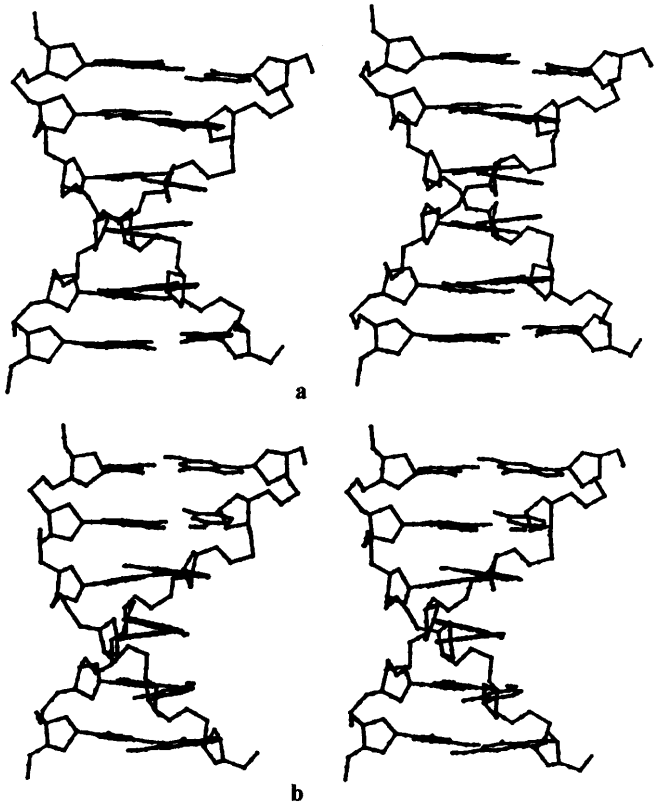

Fig. 3a,b The two right-handed double-helical structures of poly(dG-dC)·poly(dG-dC): stereo drawings of **a** the C_BG_A molecular model and **b** the C_AG_B molecular model

shows a stereo drawing of the two optimised models of the S duplex structures of poly(dG-dC) · poly(dG-dC). These two molecular conformations present no inter-strand overlap, they are free from too short contacts between non-bonded atoms and the hydrogen bonds between Watson-Crick base pairs correspond to generally accepted atomic distances (Fuller 1959; Taylor and Kennard 1982; Gelbin et al. 1996). The stacking of the bases and their positions on a sugar-phosphate strand are represented in Fig. 4. The chain dihedral angles and the geometrical parameters characterising these new helical conformations are presented in Tables 1 and 2. The atomic coordinates of the optimised C_BG_A and C_AG_B models of the S-DNA right handed double helical structures are given in Tables 3 and 4.

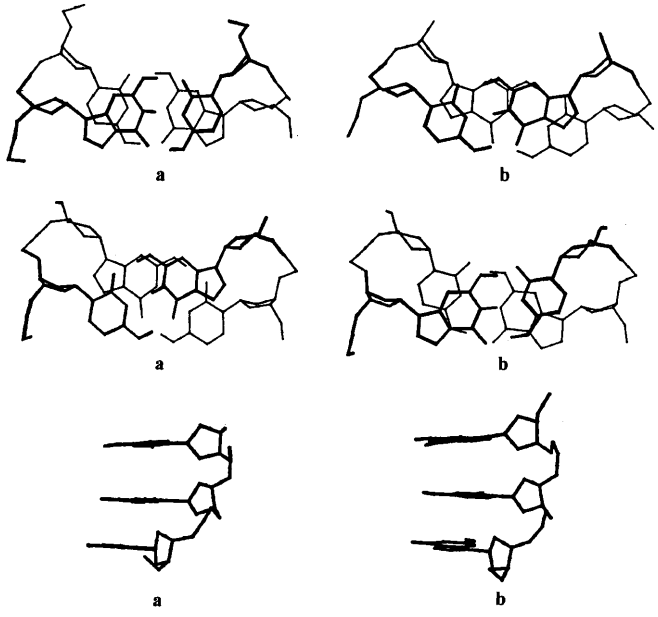

Fig. 4 Drawings showing the relative positions along one strand of bases as well as their stacking and the hydrogen bonding between cytosine and guanine in the right-handed S form of poly(dG-dC)·poly(dG-dC): **a** the C_BG_A model; **b** the C_AG_B model

Table 1 Dihedral angles of the right-handed S-DNA conformation of poly(dG-dC) · poly(dG-dC). Angle values (Premilat and Albiser 1983) for A- and B-DNA are also indicated. χ is the angle about the glycosidic bond

Dihedral angles (°)	A-DNA	S-DNA		B-DNA
		C_A or G_A	C_B or G_B	
α[O3′-P-O5′-C5′]	−59	−46.0	−7.5	−31
β[P-O5′-C5′-C4′]	163	147.0	150.8	148
γ[O5′-C5′-C4′-C3′]	51	29.8	37.0	37
δ[C5′-C4′-C3-O3′]	81	85.0	132.3	132
ε[C4′-C3′-O3′-P]	−136	−172.7	−144.4	−156
ξ[C3′-O3′-P-O5′]	−87	−96.8	−127.0	−133
χ	−165	−137.0	−120.0	−123

Table 2 Morphological parameters for the two right-handed S-DNA double helices of poly(dG-dC) · poly(dG-dC). *P*: helix pitch. *D*: base-pair displacement from the helix axis (positive if the helix axis is on the major groove side and negative if on the minor groove side) θ_p: the propeller twist of the bases. Groove widths and depths are defined from Park et al. (1987)

Parameters	S-DNA	
	C_BG_A	C_AG_B
Rise/residue (Å)	7.2	7.2
Rotation/residue (°)	60	60
P (Å)	43.2	43.2
D (Å)	0.6	0.7
θ_p (°)	13.8	18.5
Deoxyribose with C	C2′ *endo*	C3′ *endo*
Deoxyribose with G	C3′ *endo*	C2′ *endo*
Hydrogen bond (A)		
H2···O2	2.84	2.75
H1···N3	2.84	2.73
O6···H4	2.84	2.80
Groove width (Å)		
Major	13.5	13.8
Minor	9.5	8.5
Groove depth (Å)		
Major	10.0	10.1
Minor	7.2	6.7

Table 3 S-DNA structure of poly(dG-dC) · poly(dG-dC). Cylindrical coordinates of the C_BG_A right-handed molecular model. The atomic coordinates corresponding to the complementary chain are simply obtained by changing the sign of ϕ and *z*. The following CG unit in the double helix is obtained by replacing ϕ by $(\phi + 60)$ and *z* by $(z + 7.2)$

	R (Å)	ϕ (°)	*z* (Å)
Cytosine			
N1	4.76	−85.78	−1.28
C6	4.95	−101.56	−1.08
C5	4.14	−115.41	−1.00
C4	2.72	−114.44	−1.14
N4	2.27	−143.37	−1.07
N3	2.39	−85.24	−1.34
C2	3.64	−74.64	−1.42
O2	4.10	−57.71	−1.60
Guanine			
N9	4.85	−48.25	1.90
C8	4.80	−64.54	2.07
N7	3.72	−74.57	2.16
C5	2.73	−57.20	2.04
C4	3.66	−38.94	1.88
N3	3.89	−18.63	1.73
C2	3.05	−1.53	1.75
N2	3.87	16.11	1.62
N1	1.68	0.89	1.90
C6	1.32	−52.87	2.06
O6	0.93	−116.15	2.19
Phosphate			
O3′	9.40	−104.85	−3.76
P	9.57	−105.48	−2.18
O1	11.01	−106.40	−1.87
O2	8.88	−112.94	−1.65
O5′	9.09	−96.57	−1.71
Deoxyribose			
C5′	8.97	−90.00	−2.70
C4′	8.20	−81.99	−2.20
C3′	8.35	−79.83	−0.74
C2′	6.95	−81.48	−0.17
C1′	6.05	−78.36	−1.37
O1′	6.79	−83.21	−2.46
Phosphate			
O3′	8.86	−71.10	−0.55
P	9.91	−70.05	0.65
O1	11.27	−68.98	0.09
O2	10.01	−76.94	1.51
O5′	9.43	−62.22	1.42
Deoxyribose			
C5′	9.28	−54.94	0.63
C4′	8.41	−48.37	1.29
C3′	8.29	−48.79	2.79
C2′	7.07	−42.16	3.05
C1′	6.22	−42.11	1.76
O1′	7.05	−49.37	0.86

Poly(dA) · 2poly(dT) in fibre

X-ray diffraction patterns of poly(dA) · 2poly(dT) were obtained following the usual experimental procedure (see Materials and methods). Results thus obtained are presented in Fig. 5 together with the patterns of the B′ and B* forms of poly(dA) · poly(dT) (Premilat and Albiser 1997). X-ray patterns show that the polynucleotide in fibre is only partially ordered since one observes a certain number of Bragg reflection spots and important diffraction streaks. There are significant diffracted intensities on the equatorial, the three first layer lines and two higher lines. The Bragg reflections correspond to a hexagonal lattice; they can be indexed with the unit cell dimensions $a = b = 47$ Å and $c = 183.5$ Å. Moreover, since Bragg reflections on the equatorial line (*hk*0) are only observed for $-h + k = 3n$ (*n* is an integer), there are three helices per unit cell. The lateral spacing of the DNA helices in the lattice, obtained from the positioning of Bragg spots on the equatorial line, is 27 Å. So, analysis of X-ray patterns of poly(A) · 2poly(T) in fibre gives a helix pitch $P = 36.7$ Å and an axial rise per nucleotide $p = 3.22$ Å. There are therefore 11.4 nucleotide pairs per helix turn, an angle of 31.6° for the rotation per nucleotide around the helix axis and the above-mentioned axial dimension for the unit cell $c = 5P = 183.5$ Å.

The noteworthy fact is that the X-ray pattern of poly(dA) · 2poly(dT) we obtained (Fig. 5c) is closely similar to the one reported in the literature (Arnott and

Table 4 S-DNA structure of poly(dG-dC)·poly(dG-dC). Cylindrical coordinates of the C_AG_B right-handed molecular model. The atomic coordinates corresponding to the complementary chain are simply obtained by changing the sign of ϕ and z. The following GC unit in the double helix is obtained by replacing ϕ by $(\phi + 60)$ and z by $(z + 7.2)$

	R (Å)	ϕ (°)	z (Å)
Guanine			
N9	4.76	−83.78	−1.78
C8	4.79	−100.23	−1.59
N7	3.77	−111.30	−1.56
C5	2.69	−95.89	−1.75
C4	3.52	−75.50	−1.89
N3	3.66	−54.03	−2.09
C2	2.75	−36.47	−2.15
N2	3.53	−16.71	−2.34
N1	1.38	−36.36	−2.02
C6	1.27	−99.19	−1.81
O6	1.20	−158.67	−1.71
Cytosine			
N1	4.90	−46.95	1.49
C6	5.08	−62.25	1.73
C5	4.24	−75.28	1.94
C4	2.82	−73.23	1.90
N4	2.29	−100.41	2.10
N3	2.54	−45.33	1.67
C2	3.79	−35.91	1.46
O2	4.24	−19.67	1.24
Phosphate			
O3′	9.40	−102.85	−4.26
P	9.57	−103.28	−2.68
O1	11.01	−104.40	−2.37
O2	8.88	−110.94	−2.15
O5′	9.09	−94.57	−2.21
Deoxyribose			
C5′	8.97	−88.00	−3.20
C4′	8.20	−79.99	−2.70
C3′	8.35	−77.83	−1.24
C2′	6.95	−79.48	−0.67
C1′	6.05	−76.36	−1.87
O1′	6.79	−81.21	−2.96
Phosphate			
O3′	8.86	−69.10	−1.05
P	9.91	−68.05	0.15
O1	11.27	−66.98	−0.41
O2	10.01	−74.94	1.01
O5′	9.43	−60.22	0.92
Deoxyribose			
C5′	9.28	−52.94	0.13
C4′	8.41	−46.37	0.79
C3′	8.29	−46.79	2.29
C2′	7.07	−40.16	2.55
C1′	6.22	−40.11	1.26
O1′	7.05	−47.37	0.36

Selsing 1974), for which they proposed a triple helix molecular model. However, Fig. 5b shows that this pattern, and the measured helical parameters (see Results above), are exactly those of the B* double helix of poly(dA) · poly(dT) at 30 °C. For that B* helical structure, morphological parameters and a molecular model, giving a good agreement with fibre X-ray diffraction results, are already presented in a previous work (Premilat and Albiser 1997). In this last study, we also observed that the B* structure remains stable at high temperature (up to 70 °C). Such behaviour also argues against the poly(dA) · poly(dT) having converted to

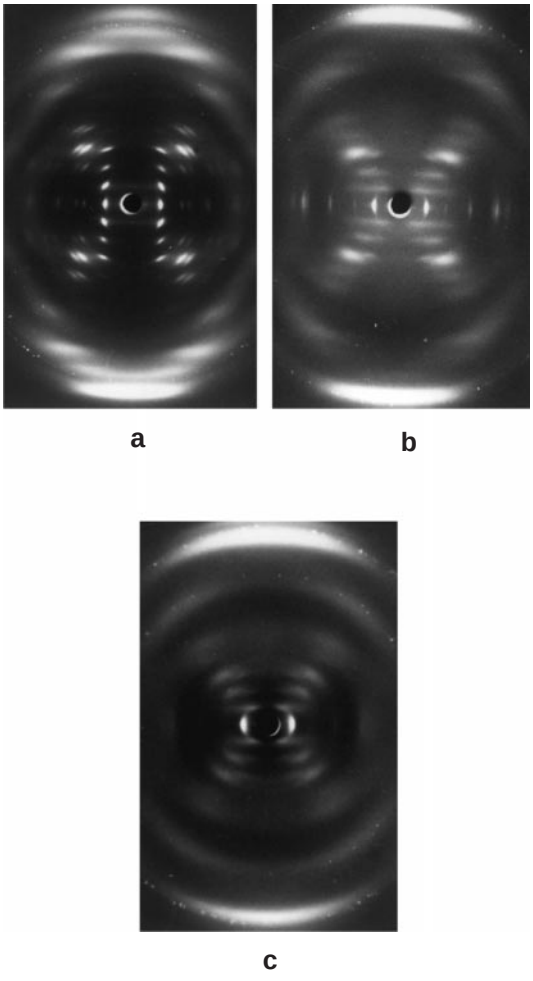

Fig. 5a–c X-ray fibre diffraction patterns of poly(dA)·poly(dT): **a** the αB′ form obtained at 20 °C and high r.h.; **b** the B* pattern at 36 °C and 75% r.h.; **c** the B* pattern obtained with a fibre of poly(dA)·2poly(dT)

poly(dA) · 2poly(dT) triple helix, since the authentic triple helix in solution has a melting temperature near 40 °C, compared with 72 °C for the poly(dA) · poly(dT) duplex (Sun and Helene 1993; Kan et al. 1997).

Discussion

For the helix-helix transitions of DNA, the different helical structures can be ordered according to the increasing values of the parameter p, the rise per residue (not the helix pitch). It then gives the following sequence of DNA structures: A, C, D, B, Z (S)… (Leslie et al. 1980; Saenger 1984). For DNA in fibre, the type of transformation actually observed when the r.h. is decreased corresponds generally to changes from a given double helix to another one with a smaller p value. This is the case when the r.h. is decreased from 98% to 20% for the B → A and B → C transitions (Premilat et al. 1990) and also for $S_{II} \rightarrow S_I$. Conversely, when the r.h. is increased, one can observe that p becomes larger or smaller depending on the helix-

helix transition. So, p becomes larger with increasing r.h. for the transitions A $\rightarrow$ B, C $\rightarrow$ B and $S_I \rightarrow S_{II}$. However, for poly(dA-dT) · poly(dA-dT), which can be in the D form (Davies and Baldwin 1963), it was observed (Abouelkassimi et al. 1991) that when the r.h. rises from 20% to 98%, p becomes smaller for D $\rightarrow$ A, then p increases for A $\rightarrow$ B. When the r.h. is lowered from 98% to 20%, p decreases for B $\rightarrow$ A but one cannot realise the transition A $\rightarrow$ D which would imply an increase of p with decreasing r.h.

Poly(dG-dC) · poly(dG-dC) double helices

For the alternating S-DNA conformation here proposed, the rise per unit (two base pairs) is indeed higher (p = 7.2 Å) than the sum of the rises for the A- and B-DNA structures. The following is observed with poly(dG-dC) · poly(dG-dC): when the r.h. is raised, p increases for $S_I \rightarrow S_{II}$ and p decreases for $S_{II} \rightarrow$ B. When the r.h. is abruptly changed from 98% to 20%, p becomes smaller for B $\rightarrow$ C, A is not obtained again and one finds S_I with the transition C $\rightarrow S_I$ when the r.h. is raised (Albiser and Premilat 1992). However, when the r.h. is lowered very slowly from 98% to 20%, p becomes larger for the B $\rightarrow$ S transition. So, one can note that the S- to B-DNA helix-helix transition of poly(dG-dC) · poly(dG-dC) in fibre is perfectly reversible.

It should be noted that short oligonucleotides in solution can very well present, depending on the physicochemical conditions, the reversible B $\leftrightarrow$ Z transition. However, when these molecules are organised in a crystal lattice, such transformations, implying a change of the helix handedness, become practically impossible and give all the more reason for long DNA chains closely packed in fibres. The S-DNA form here proposed is a right-handed double helix and the A-S-B transitions avoid therefore the mechanical difficulties inherent to the reversible change from a right- to a left-handed helix. This interpretation of the experimental results is based on a simpler hypothesis (Occam's law of parsimony in logic) than the A-Z-B transitions, and the molecular models presented for the S-DNA form allowed us to calculate patterns of diffracted intensities in accordance with the experimental data (Fig. 2). Besides, the present curves of the Fourier transform of S-DNA can be compared to those proposed by Rich and co-workers for Z_I-DNA (Wang et al. 1981). One can thus remark that the two calculated patterns of diffracted intensities are similar and in good agreement with experiment, at least for layer lines 0 to 8. However, the calculated intensities for the Z-DNA model are too weak on layer lines 9 and 11 whereas the present S-DNA models give curves which agree quite well with the experimental measurements. Hence, this comparison makes it possible to say that the presently proposed S-DNA structures are indeed better molecular models than the left-handed Z form for poly(dG-dC) · poly(dG-dC) in fibre. Moreover, one can see that the changes in torsion angles (Table 1) actually define the here-proposed S structure as a stable conformational intermediate in the A to B helix-helix transition of long molecular chains of poly(dG-dC) · poly(dG-dC). It should be noted that the present model is a regular alternating A-B conformation and it does not present any bending or kink as determined in crystals of oligonucleotide duplexes or protein-DNA (Wahl et al. 1996; Olson et al. 1998; Malinina et al. 1999).

Poly(dA) · poly(dT) double helices

We have a singular situation with poly(dA) · poly(dT) because p remains constant whatever the direction of variation of the r.h., even for the B′ $\rightarrow$ B* transition observed when the r.h. is lowered and the temperature is higher than 30 °C. However, concerning poly (dA) · 2poly(dT), one can see that the X-ray diffraction pattern obtained from fibres of this polymer mixture is in fact the one corresponding to a double helix of poly(dA) · poly(dT), the B* structure (Premilat and Albiser 1997). Moreover, we performed calculations of the diffracted intensities with different molecular models of the triplex using the geometrical parameters of the B* form. Results thus obtained are in bad agreement with experimental data, whereas these last are in accordance with calculations made using the B* double helix model. One can add that the effect of temperature, which stabilises the B* form, is also in accordance with the present interpretation of the experimental observations.

Furthermore, the reversible B′ $\rightarrow$ B* transition of poly(dA) · poly(dT) is well observed by fibre X-ray diffraction (Premilat and Albiser 1997) and one can hardly imagine a process allowing in that case a transition from a double to a triple helix structure while the molecules remain organised in a semicrystalline lattice. So, we suggest that the X-ray pattern ascribed to the triple helix structure of poly(dA) · 2poly(dT) (Arnott and Selsing 1974) is actually that of the B* double helix of poly (dA) · poly(dT) which appears when the r.h. is low and the temperature higher than 30 °C. It seems that when the very hydrophilic poly(dT) (Lamiri et al. 1998) is added to poly(dA) · poly(dT) double strands, it has the same effect on the duplex as the one induced by dimethyl sulfoxide (Herrera and Chaires 1989) and it therefore imposes the B* structure on the polynucleotide at ambient temperature (Fig. 5c).

The triple helix of poly(dA) · 2poly(dT) can possibly be present locally on a long double helix of poly (dA) · poly(dT) and thus be observed by spectroscopic methods applied on solutions or films of mixtures of poly(dA) · poly(dT) and poly(dT). Nevertheless, molecular units must have a unique conformation in order to be possibly organised in a crystal lattice. However, the interaction sites between a poly(dT) chain and a poly (dA) · poly(dT) double helix are so numerous that one should expect them to give a high multiplicity of different triplex conformations which give characteristic spectroscopic and thermal properties. They contribute to the amorphous part of the fibre and can hardly be

crystallographically ordered in a lattice like the poly (dA) · poly(dT) double helix.

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