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Conformational flexibility of DNA: Polymorphism and handedness

(DNA structure/right-handed duplexes/left-handed duplexes/RL models)

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ABSTRACT **It is shown that left-handed duplexes are possible for A, B, and D forms of DNA. These duplexes are stereochemically satisfactory and are consistent with the observed x-ray intensity data. On scrutiny the refined right-handed models of B and D DNA by Arnott and coworkers are found to be stereochemically unacceptable. It was possible to formulate a stereochemical guideline for molecular model building based on theory and analysis of single-crystal structure data of dinucleoside monophosphate and higher oligomers. This led to both right- and left-handed DNA duplexes. The right-handed B and D DNA duplexes so obtained are stereochemically superior to earlier models and agree well with the observed x-ray intensity data. The observation that DNA can exist in either handedness for all the polymorphous forms of DNA at once explained A ⇌ B and B ⇌ D transitions. Hence it is confirmed that polymorphism of DNA is a reflection on the conformational flexibility inherent in DNA, the same cause that ultimately allows DNA in either handedness. The possibility of various types of right- and left-handed duplexes generated by using dinucleoside monophosphate and trinucleoside diphosphate as repeating units resulted in a variety of models, called RL models. All these models have alternating right and left helical segments and inverted stacking at the bend region as suggested by us earlier. It turns out that the B-Z DNA model of Wang *et al.* is only an example of RL models.**

Subtle changes of environmental conditions such as relative humidity and salt concentration induce conformational transitions in DNA fibers. Studies of various polymorphous forms of DNA help us understand the nature of conformational flexibility inherent in the DNA molecule. With this in view the present investigation on three polymorphous forms of DNA, A, B, and D, was taken up. These forms are well characterized in terms of their helical parameters n (nucleotides per turn) and h (distance between repeating units): for the A form $n = 11$, $h = 2.56$ Å; for the B form $n = 10$, $h = 3.40$ Å; and for the D form $n = 8$, $h = 3.03$ Å (1–3).

By using fiber diffraction data alone it is not possible to obtain precise stereochemical details of the repeating unit. Therefore, analysis of fiber diffraction data requires choice of a proper repeating unit that reflects essentially all the properties of polymeric DNA. In our studies, to begin with, base-paired dinucleoside monophosphate (Fig. 1) was chosen as the model fragment because it embodies all the essential attributes of polymeric DNA: (*i*) Watson–Crick base pairing, (*ii*) three major sources of flexibility—sugar pucker, rotation around phosphodiester linkages (P—O bonds) and glycosyl torsion, and (*iii*) stacking interaction, a vital stabilizing force in DNA. Having identified a typical repeating unit, we investigated the conformational flexibility of DNA along the following lines. First, single-crystal structure data of dinucleoside monophosphates and higher oligomers were analyzed. This analysis rendered information about the stereochemistry of the repeating unit and also suggested a few correlations that exist among the various torsional degrees of freedom. Second, by using these data double helical models for A, B, and D forms of DNA were generated. The acceptability of a given model was judged on the basis of the following four criteria: (*i*) Watson–Crick base pairing scheme throughout the structure, (*ii*) allowed stereochemistry of the backbone consistent with the single crystal structure data of nucleic acid components, (*iii*) energetically favorable stacking interactions, and (*iv*) agreement with the observed x-ray data. It was then examined whether both right- and left-handed duplexes are possible for the three forms of DNA. Finally, the possibility of combining such conformational variants in a given structure was investigated in an attempt to arrive at a stereochemical pathway of superfolding.

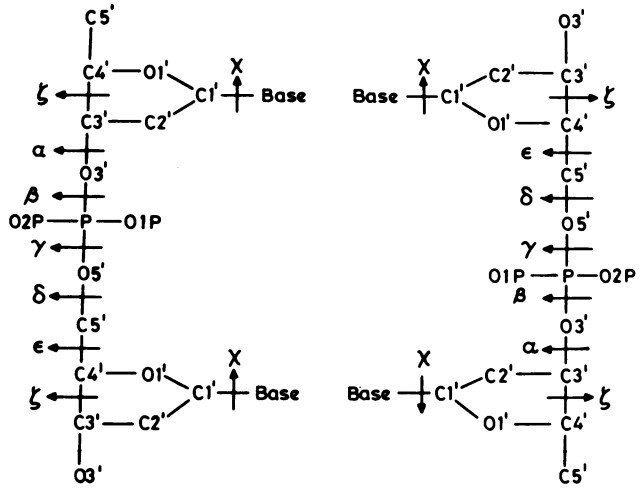

FIG. 1. Schematic representation of the base-paired dinucleoside monophosphate chosen as the repeating unit. Different torsion angles are indicated. The alphabetical nomenclature follows the convention adopted by Seeman *et al.* (4). Note that the exocyclic torsion angle ζ is same for the sugars at the 5′ and 3′ ends. Thus, the mononucleotide turns out to be the true repeat. But in view of the fact that a dinucleoside monophosphate embodies two essential features of polymeric DNA—viz., torsions around two P—O bonds and base stacking—the former was chosen as a repeating unit to formulate a stereochemical guideline for molecular model building.

Helical domains in the (β-γ) space

Theoretical studies and analysis of single-crystal structure data of dinucleoside monophosphates and higher oligomers revealed certain correlations among the major torsional degrees of freedom present in the structure (5, 6). The puckering of the sugar can be broadly classified into two regions, C3′-*endo* ($70° \leq \zeta \leq 100°$) and C2′-*endo* ($130° \leq \zeta \leq 160°$). For sugar pucker in the C3′-*endo* region, P—O torsions were found to fall in the g^-g^- domain in the (β-γ) space (see Fig. 1 for the notation of various torsion angles), while for sugar pucker in the C2′-*endo*

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region, the tg^- domain was found to be most favored. This correlation between the sugar pucker and P—O torsions (henceforth referred to as the preferred correlation) formed the basis of molecular model building for the three polymorphous forms of DNA. For dinucleoside monophosphates and higher oligomers, the most frequently observed conformation around C4′—C5′ bond is *gg* (i.e., $40° \leq \epsilon \leq 70°$). The other two torsion angles α and δ (see Fig. 1) take up the *trans* conformation with $200° \leq \alpha \leq 255°$ and $160° \leq \delta \leq 200°$, respectively (6).

Thus, using the observed ranges of values for all the backbone torsion angles, stereochemically allowed helical duplexes for different forms of DNA were generated by using a modified linked atom least squares method. The duplexes fall into two conformationally distinct domains as shown in Fig. 2: C3′-*endo* puckering with g^-g^- conformation around P—O bonds (henceforth referred to as C3′-*endo*, g^-g^- conformation) and C2′-*endo* puckering with tg^- conformation around the P—O bonds (C2′-*endo*, tg^- conformation). In each of the two domains (I and II in Fig. 2), both right- and left-handed duplexes were found to be possible. The backbone torsion angles of the right- and left-handed duplexes in a given domain are only slightly different. The significant difference lies in the value of χ: for the left-handed duplex it is about 50°–60° lower than that of the right-handed duplex in the same helical domain. For example, the right-handed duplexes with (C3′-*endo*, g^-g^-) conformation have χ in the range of 10° to 45°, whereas for the left-handed duplexes with the same conformation χ falls in the range of −50° to −15°. Similarly, the right-handed duplexes with (C2′-*endo*, tg^-) conformation have χ in the range of 55° to 75°, about 60° higher than the values obtained for the left-handed duplexes ($-5° \leq \chi \leq 15°$) in the same domain.

Discussion of different molecular models

Thus, model building studies demonstrated that, by using the preferred correlation, stereochemically allowed models of both right- and left-handed duplexes can be built for all the polymorphous forms of DNA. For a particular form of DNA, that model in a given domain was chosen as the most probable one which satisfactorily met all four of the criteria mentioned earlier. The conformational parameters of different models, so obtained, are given in Table 1. The values of torsion angles for different models fall within or near the range of values observed from the single-crystal structure data of nucleic acid components. It is to be noted that the values of α in the refined models of B DNA and D DNA (2, 3), are 155° and 145°, respectively (see Table 1), well below the lowest value of α observed so far in the single crystals of nucleic acid components (6). Such a low value of α, in fact, leads to short contacts be-

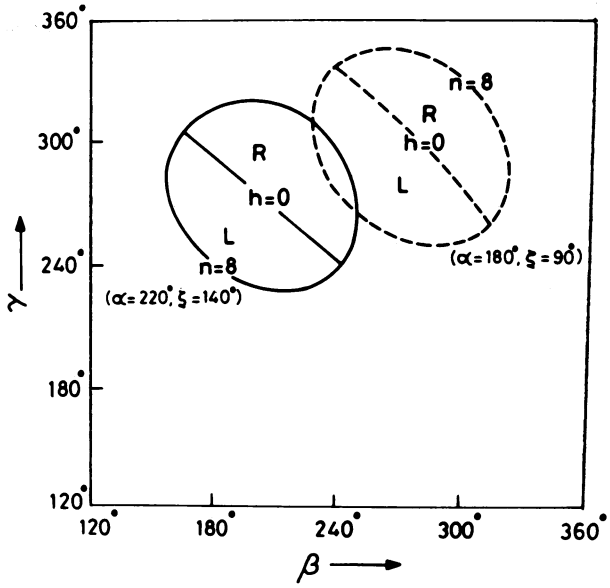

FIG. 2. A typical set of helical domains in the (β-γ) space, for C2′-*endo* (solid line) and C3′-*endo* (broken line) sugar puckering and *gg* conformation around the C4′—C5′ bond. The phosphodiester conformations refer to the phosphate group attached at the 3′ end of the sugar. The values of the torsion angles α and ζ are given for each case. The $n = 8$ contour shown encloses the helical domains corresponding to $n \geq 9$. The $n = 0$ contour divides each domain into right (R) and left (L) helical sections.

Table 1. Conformational parameters and agreement indices of different B and D DNA duplexes

Form and handedness of DNA duplex	Molecular conformation	Sugar pucker (ζ)	P—O torsions (β, γ)	Glycosyl torsion (χ)	Other torsion angles α	δ	ϵ	Agreement indices R, R''
Right-handed B	(C3′-*endo*, g^-g^-)	C3′-*endo* (97°)	g^-g^- (292°, 269°)	*anti* (34°)	184°	179°	75°	0.36, 0.39
	(C2′-*endo*, tg^-)	C2′-*endo* (149°)	tg^- (202°, 302°)	*anti* (74°)	228°	144°	41°	0.35, 0.38
	Alternate puckering*							
	C3′-*endo* fragment	C3′-*endo* (89°)	g^-g^- (292°, 295°)	*anti* (24°)	183°	181°	66°	0.33, 0.36
	C2′-*endo* fragment	C2′-*endo* (156°)	tg^- (210°, 286°)	*anti* (58°)	205°	147°	49°	
	(C2′-*endo*, g^-g^-)†	C2′-*endo* (156°)	g^-g^- (264°, 314°)	*anti* (80°)	155°	214°	36°	0.31, 0.37
Left-handed B	(C_2′-*endo*, tg^-)	C2′-*endo* (137°)	tg^- (204°, 270°)	Low *anti* (−3°)	241°	135°	36°	0.37, 0.40
Right-handed D	(C_2′-*endo*, tg^-)	C2′-*endo* (154°)	tg^- (209°, 302°)	*anti* (72°)	208°	147°	61°	0.37, 0.40
	(C2′-*endo*, g^-g^-)‡	C2′-*endo* (156°)	g^-g^- (260°, 298°)	*anti* (82°)	141°	208°	69°	0.33, 0.39
Left-handed D	(C2′-*endo*, tg^-)	C2′-*endo* (152°)	tg^- (215°, 263°)	Low *anti* (−4°)	225°	156°	32°	0.36, 0.40

The models were arrived at by using a modified linked atom least squares procedure (7) that incorporates the flexibility of the furanose ring. Standard bond lengths and bond angles were maintained. The (C3′-*endo*, g^-g^-) conformation refers to g^-g^- conformations for the phosphate group attached at the 3′ end of the sugar with C3′-*endo* puckering; similarly for (C2′-*endo*, tg^-) conformation. Agreement indices R and R'' were computed for different models by following the procedure of Arnott and Hukins (2). Atomic coordinates of the models can be obtained from the authors.

* C3′-*endo* fragment refers to (C3′-*endo*, g^-g^-–C2′-*endo*) sequence and C2′-*endo* fragment to (C2′-*endo*, tg^-–C3′-*endo*) sequence in Fig. 5. The model proposed by Klug *et al.* (8) has a large radius for the phosphorus atom (9.6 Å) and hence cannot be packed in the unit cell of B DNA. But all our B DNA models have a low radius for the phosphorus atom (9.0 Å) and thus are favorably packed.

† (C2′-*endo*, g^-g^-) refers to the "best B DNA model" of Arnott and Hukins (2).

‡ The right-handed D DNA model by Arnott *et al.* (3) has (C2′-*endo*, g^-g^-) conformation.

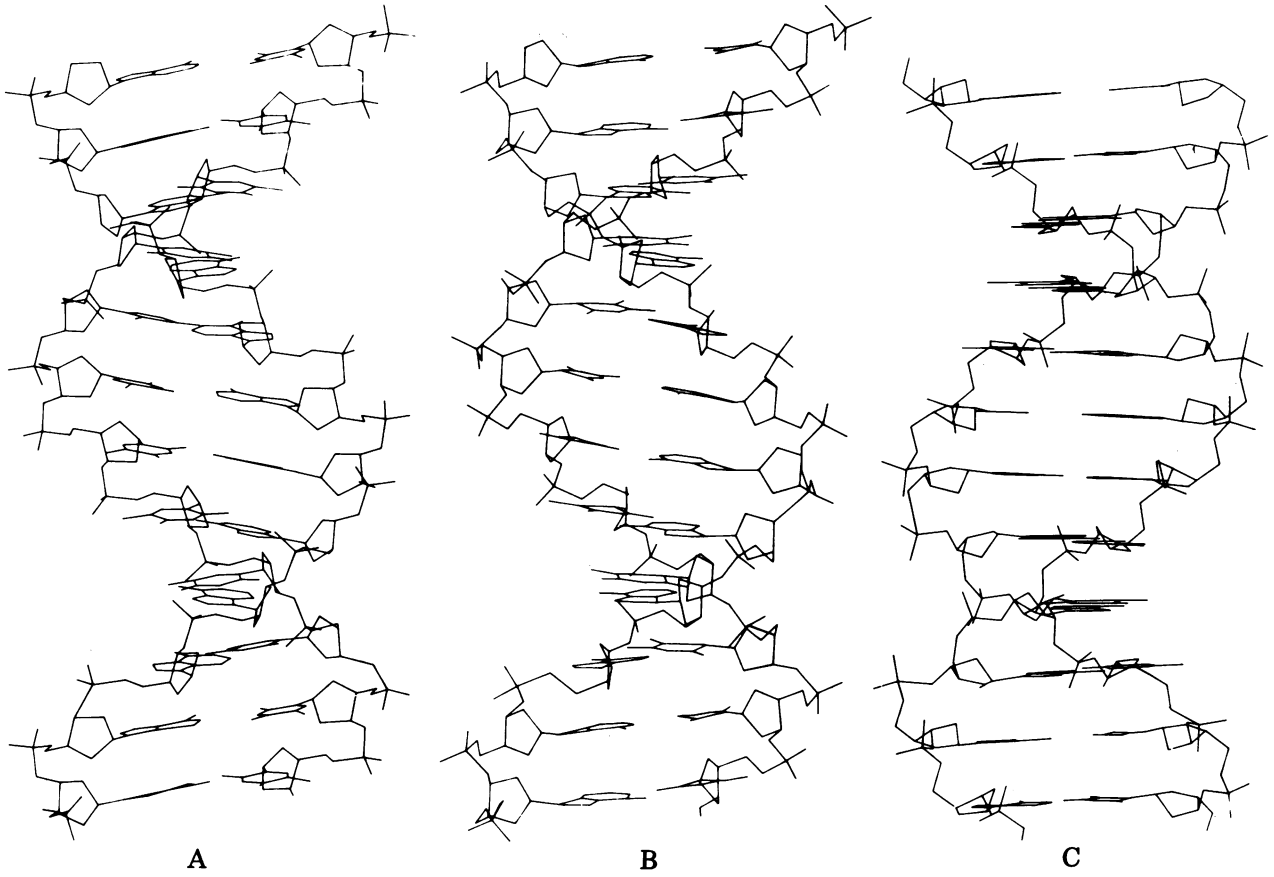

FIG. 3. Line diagrams of three B DNA models obtained by using dinucleoside monophosphate as the repeating unit. (*A*) Right-handed duplex with (C3′-*endo*, g^-g^-) conformation, (*B*) right-handed duplex with (C2′-*endo*, tg^-) conformation, and (*C*) left-handed duplex with (C2′-*endo*, tg^-) conformation.

tween C2′ . . . O1P and H2′ . . . O1P (see Fig. 1). This is because of the fact that both the models (for B and D DNA) have unorthodox (C2′-*endo*, g^-g^-) conformation instead of the preferred (C2′-*endo*, tg^-) conformation and the steric problem mentioned above is an inevitable consequence of such a conformation. Therefore, it turns out that the preferred correlation as predicted by theory and as observed in the single crystals of dinucleoside monophosphates and higher oligomers has a sound stereochemical basis. It is seen from Table 1 that the models for B and D forms of DNA of the present study conform to the preferred correlation and hence they are stereochemically superior to those given by Arnott and coworkers (2, 3). Further, the agreement indices obtained for our models of B and D DNA are close to those given by Arnott and coworkers (2, 3). The line diagrams of the three models of B DNA obtained by using dinucleoside monophosphate as the repeating unit are shown in Fig. 3. Two of them are right-handed duplexes: one with (C3′-*endo*, g^-g^-) conformation and the other with (C2′-*endo*, tg^-) conformation. The third one is a left-handed duplex with (C2′-*endo*, tg^-) conformation. In all the three cases, the bases are favorably stacked. Left-handed B DNA duplexes were stereochemically possible in the (C3′-*endo*, g^-g^-) domain. But these models always had small separation (5–7 Å) between the neighboring phosphate chains, which is well below the optimum value (10–14 Å) required to match the x-ray intensity data of B DNA. Hence, these models are not discussed here. Line diagrams of two D DNA models are shown in Fig. 4. Both belong to (C2′-*endo*, tg^-) domain: one is right-handed and the other is left-handed. It is seen for the right-handed duplex that bases in a 5′-pdApdT sequence are better stacked than those in a 5′-pdTpdA sequence; the reverse is the case for the left-handed duplex. Therefore, both the D DNA models are equally favorable as far as the stacking interaction is concerned.

Only recently have we have had access to the x-ray intensity data of A DNA and the refinement of the stereochemical

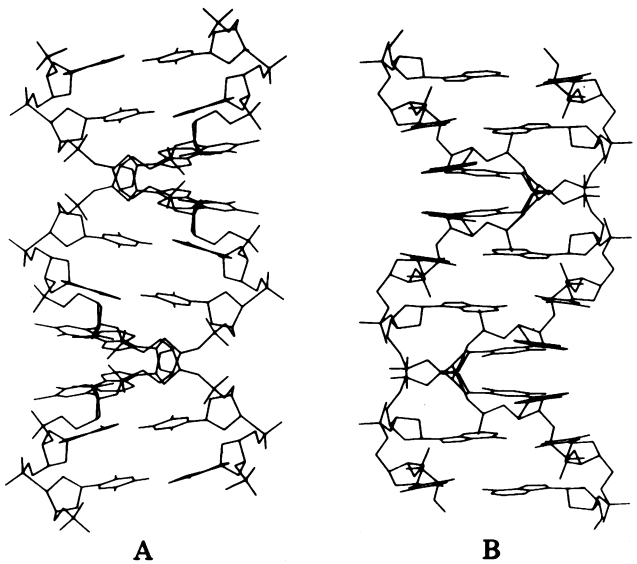

FIG. 4. Line diagrams of two D DNA duplexes. (*A*) Right-handed duplex with (C2′-*endo*, tg^-) conformation and (*B*) left-handed duplex with (C2′-*endo*, tg^-) conformation.

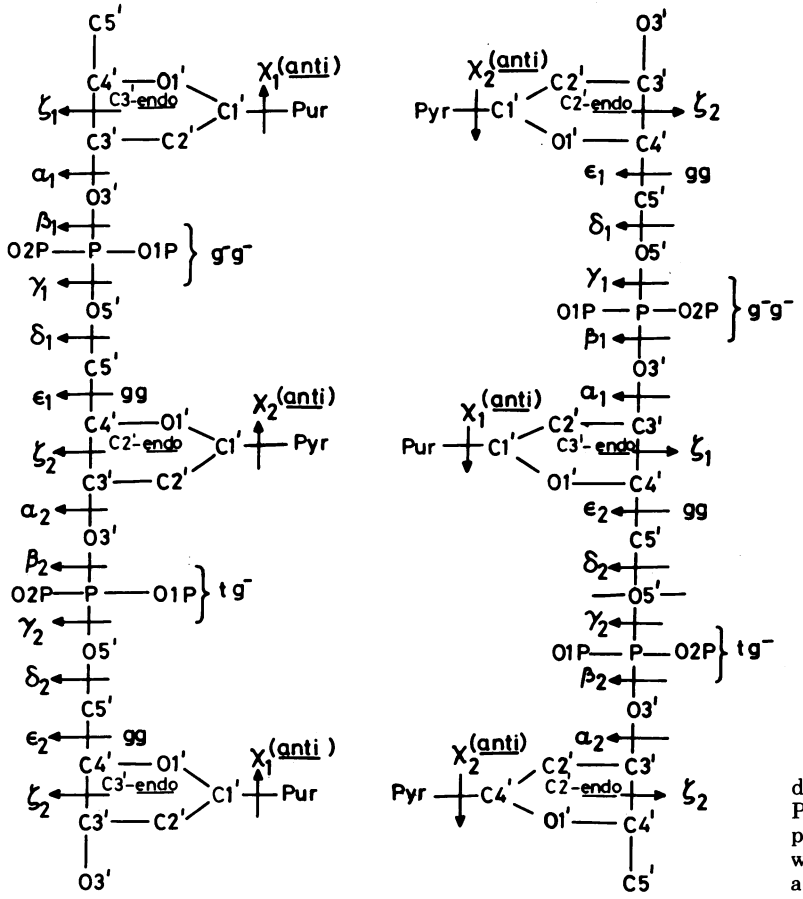

FIG. 5. Schematic representation of trinucleoside diphosphate. Different torsion angles are indicated. Purines (Pur) are attached to sugars with C3′-*endo* puckering and pyrmidines (Pyr) are attached to sugars with C2′-*endo* puckering. Note that the true repeat is a dinucleotide.

models with respect to x-ray intensity data is not yet complete. Nevertheless, it is clear that both right- and left-handed duplexes are stereochemically possible for A DNA. In view of the fact that the right-handed A DNA model in the (C3′-*endo*, g^-g^-) domain (1) is stereochemically satisfactory, only left-handed models, one with (C3′-*endo*, g^-g^-) conformation and the other with (C2′-*endo*, tg^-) conformation, were investigated. The values of the conformational parameters of the two models are as listed in Table 2.

From Table 1, one finds an interesting possibility for B DNA when trinucleoside diphosphate (see Fig. 5) is chosen as the repeating unit and a right-handed duplex with $n = 5$ and $h =$ 6.8 Å is generated. The resulting structure is obtained by joining two dinucleoside monophosphate fragments: one with (C3′-*endo*, g^-g^-) conformation and other with (C2′-*endo*, tg^-) conformation. Recently such a structure has been proposed by Klug *et al.* (8) for poly(dA-dT)·poly(dT-dA) in B form. A line diagram of the model is shown in Fig. 6. Structural details of

Table 2. Conformational parameters of two left-handed A DNA duplexes

Molecular conformation	Sugar pucker (ζ)	P—O torsions (β, γ)	Glycosyl torsion (χ)	Other torsion angles		
				α	δ	ϵ
(C3′-*endo*, g^-g^-)	C3′-*endo* (85°)	g^-g^- (290°, 290°)	Low *anti* (−25°)	176°	170°	40°
(C2′-*endo*, tg^-)	C2′-*endo* (150°)	tg^- (208°, 308°)	Low *anti* (13°)	201°	146°	35°

The parameters were obtained by using dinucleoside monophosphate as the repeating unit.

various models generated by using trinucleoside diphosphate as the repeating unit are reported elsewhere (5).

Possibility of left-handed duplexes

It, therefore, turns out that left-handed duplexes are possible for all three forms of DNA. This is in agreement with the fact that, in order to explain the smooth A $\rightleftharpoons$ B transition and also the B $\rightleftharpoons$ D transition in the case of poly(dA-dT)·poly(dT-dA), a left-handed helix should be possible for all three forms of DNA. It may be recalled that Fuller *et al.* (1) admitted that left-handed B DNA was stereochemically possible. But they encountered stereochemical problems while building left-handed A DNA. Thus, in order to explain a smooth A $\rightleftharpoons$ B transition, left-handed models for both A and B forms were discarded. The systematic analysis that was carried out in the present study not only enabled us to obtain stereochemically allowed models for left-handed A DNA but also revealed exactly where the previous workers went wrong in constructing a left-handed A DNA duplex. In their model, they were working in the high *anti* region ($\chi \geq 90°$) and that was precisely the reason they had severe short contacts between sugar ring carbons and neighboring pyrimidine ring carbon atoms. However, all our left-handed helical models have low χ values (discussed earlier) and thus no stereochemical problem is encountered. It is also to be noted that Mitsui *et al.* (9) could obtain a left-handed model for D DNA, but it was discarded by Arnott *et al.* (3) because the model had a rather unusual O1′-*endo* sugar puckering. However, we could obtain a left-handed model for D DNA with the frequently observed C2′-*endo* puckering.

Having obtained both right- and left-handed models for A,

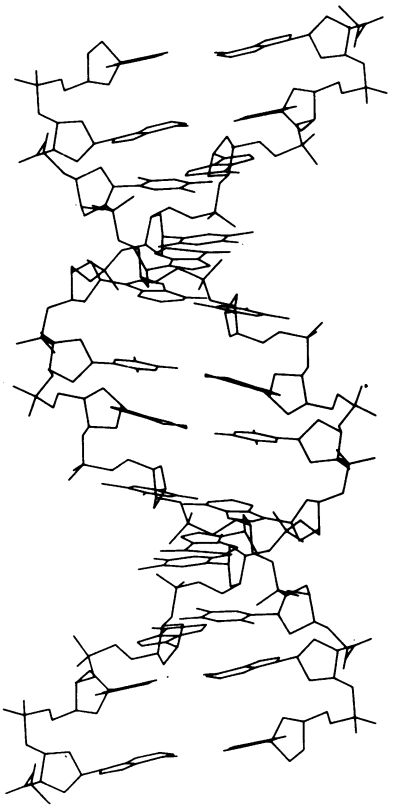

FIG. 6. Line diagram of the right-handed B DNA duplex with alternating sugar puckering.

B, and D forms of DNA and explained A ⇌ B and B ⇌ D transitions, we examined the principal differences between the left- and right-handed duplexes. Because right- and left-handed double helices have nearly the same backbone torsion angles, the essential difference comes from the mode of stacking. In one case, the bases are right stacked (in the right-handed duplex), whereas in the other they are left stacked (in the left-handed duplex). Interaction energies were computed for both right and left stacking (10) arrangements for different doublets (two neighboring base pairs). It was found that, for some sequences of doublets, right stacking was energetically more favorable than the left stacking and vice versa for some other sequences. However, considering all the doublets, both the stacking arrangements were found to be equally favorable on an average. Therefore, the left-handed duplexes are as likely to occur as their right-handed counterparts.

RL model of DNA

Thus, conformational flexibility of the repeating unit of the polynucleotide chain gives rise to both right- and left-handed duplexes. It was earlier shown (11, 12) that it was possible to combine alternately right- and left-handed double helical segments in one repeat (e.g., a repeat of 10 base pairs in the case of B DNA). The model so obtained was not a regular helix. This will henceforth be called an RL model of DNA. In such a structure, bends are formed at the places where right- and left-handed helical segments are joined. The bend regions satisfy the allowed stereochemistry and retain the base pairing scheme of Watson and Crick. However, an unusual kind of stacking arrangement results in the bend region, called inverted stacking, which was shown to be energetically stable for different doublets (10). Subsequently, the effect of specific base sequence on the RL model was examined. For this purpose, a polynucleotide duplex with alternating purine-pyrimidine sequence was chosen. For such a system, chemically and conformationally, a trinucleoside diphosphate is the repeating unit wherein two dinucleoside monophosphate fragments have different molecular conformations. This led to a variety of right- and left-handed duplexes. The structures obtained by joining such right- and left-handed duplexes retain inverted stacking at the bend region, an essential feature of the RL model proposed earlier (11, 12). In fact, any right-handed duplex (generated from dinucleoside monophosphate or trinucleoside diphosphate as the repeating unit) can be joined to any other left-handed duplex (generated from dinucleoside monophosphate or trinucleoside diphosphate as the repeating unit) to form such RL models. The combination of B DNA (right-handed duplex obtained from dinucleoside monophosphate as the repeating unit) with Z DNA (left-handed duplex generated from trinucleoside diphosphate as the repeating unit) is nothing but one such example of RL models (13).

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