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Torsional flexibility of $\bar{B}$-DNA as revealed by conformational analysis

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ABSTRACT

The thermal fluctuations of a regular double helix belonging to the $\bar{B}$-family were studied by means of atom-atomic potentials method. The winding angle fluctuation was found to be 2.4° for poly(dA):poly(dT) and 3.0° for poly(dG):poly(dC). The reasonable agreement of these estimations with those obtained experimentally reveals the essential role of the small-amplitude torsional vibrations of atoms in the mechanism of the double helix flexibility. The calculated equilibrium winding angle, τ_o, essentially depends on the degree of neutralization of phosphate groups, being about 35.5° for the full neutralization. The deoxyribose pucker is closely related to the τ angle: while τ proceeds from 30° to 45° the pseudorotation phase angle, P, increases from 126° to 164°. Fluctuations of the angles TL and TW, which specify inclination of the bases to the helix axis, were evaluated to be 5°-10°. Possible correlation between conformational changes in the adjacent nucleotides is discussed.

INTRODUCTION

Knowledge of the precise value of torsional stiffness of the DNA double helix and its relation to the bending stiffness has become very important in recent years because of intensive study of the covalently closed supercoiled DNA (1-3) and also since some detailed models of DNA arrangement in chromatin were proposed (4-7). Closely related to this subject is the dependence of the equilibrium winding angle, τ_o, on the environmental conditions (8-10).

The first quantitative data on the torsional rigidity of DNA were provided by conformational analysis of the regular double helix, i.e. the helix in which all nucleotides have the same geometry (11-15). Such calculations imitate the uniform smooth deformation of the duplex under change of ionic strength, temperature etc. within a certain family of forms, e.g. the $\bar{B}$-

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family (8). In other words, it is supposed that all the dihedral angles describing the helix geometry remain in the same local minima (14). As a result of these computations the dependence of the energy of helix, E, on winding angle, τ , is obtained. After that the r.m.s. fluctuation of τ, $\Delta\tau$, can be easily calculated as such a deviation from equilibrium value , which corresponds to the increase in energy by RT/2. (This definition is correct for the harmonic oscillator and we will use it in our pseudoquadratic case as well.) The torsional rigidity constant, g, is related to the r.m.s. fluctuation of τ by a simple formula: $g = RT/(\Delta\tau)^2$.

Recently the torsional stiffness of DNA in the standard environment was estimated on the basis of experimental data by several groups of authors (16-22). These findings make it possible to compare the results of conformational analysis with the data found independently. It is of a fundamental interest since this comparison would provide us with the information on the nature of the double helix flexibility; namely, whether it is induced by small amplitude torsional vibrations of atoms or by rotational isomerism (23). The latter means in the case of DNA abrupt transition into another family of forms (8,14) or kinking of the helix (24). This problem seems to be particularly intriguing now that the left helices (25,26) as well as some "noncanonical" right helices, e.g. Watson-Crick forms with the C4'-C5' angle in the "trans" region (27) are proved to exist under special conditions (earlier such forms were postulated theoretically (14)).

Indeed, if the magnitude of $\Delta\tau$ calculated for the $\bar{B}$-family forms is in good agreement with the value found experimentally, then it means that the $\bar{B}$-forms are indeed predominant in normal conditions and other forms can be neglected. If, on the contrary, the computated $\Delta\tau$ is much less than the experimental estimation, then one should suppose that $\bar{B} \rightarrow \bar{A}$, $\bar{B} \rightarrow \bar{Z}$, $\bar{B} \rightarrow \overline{WC}$ transitions, kinks etc. affect the DNA flexibility markedly even in the standard environment, and experimentally the equilibrium between these forms is observed. (We do not consider the local melting of the double helix since the fraction of the disrupted base pairs was estimated quite reliably to be 10^{-5} at the room

temperature (28).)

Now, before comparing the calculated and experimental values of $\underset{\sim}{\Delta}\tau$, let us first examine accurately how the experimental one is obtained and what it means. In most of the investigations published up to now (17-22) the dispersion of twisting for a long DNA fragment, $\underset{\sim}{\Delta}\underline{tw}$, is actually found. The length of a fragment under consideration attains several thousands base pairs in the case of supercoiled DNA (17,21) and in the studies which deal with the fluorescence anisotropy decay (18-22) the DNA segment consists of hundreds b.p. When calculating the fluctuation of winding angle, $\underset{\sim}{\Delta}\tau$, the neighbouring monomers are supposed to fluctuate independently. In other words, the DNA duplex is considered as a chain consisting of N discrete links (N + 1 base pairs), each link being characterized by its winding angle τ_i, so that $\underline{tw} = \sum \tau_i$. Energy of the duplex is assumed to be $E = g/2 \cdot \sum (\Delta\tau_i)^2$, where $\Delta\tau_i$ is deviation of the winding angle from the equilibrium value. In this case

$$\langle (\Delta \underline{tw})^2 \rangle = N \cdot \langle (\Delta\tau)^2 \rangle = N \cdot RT/g$$

since $\Delta\ \underline{tw} = \sum \Delta\tau_i$ and all $\Delta\tau_i$ vary independently (17). Fluctuation of the winding angle found according to the above equation: $\underset{\sim}{\Delta}\tau = \sqrt{\langle (\Delta\tau)^2 \rangle} = \sqrt{\langle (\Delta \underline{tw})^2 \rangle / N}$, proved to be 3.3°-5.9° (16-22).

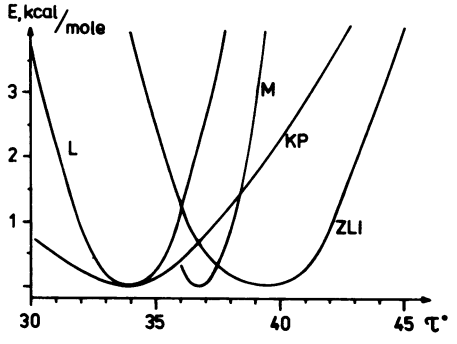
It is clear that for such a simple system the both ways to determine $\underset{\sim}{\Delta}\tau$ (consideration of the dependence of E on τ for the regular helices and estimation of the twisting fluctuation for a long DNA fragment) would lead to the same value. Application of this idealized model to the DNA duplex has several shortcomings, however. Firstly, winding angle τ is not a normal coordinate because its variation is strongly coupled with changes of other helical parameters: inclination of bases (TL), twist (TW) and so on (14). Secondly, in the double helix, having rather complicated structure, one should expect some correlation between fluctuations in the adjacent monomers; it might be caused for instance by unfavorable interaction of the neighbouring base pairs if they were tilted towards each other. In such a case the dependence of the twisting dispersion on N is not linear and dispersion of τ proves to be less than $\langle (\Delta \underline{tw})^2 \rangle / N^2$, this discrepancy being the more pronounced the stronger corre-

lation is (29). Therefore the values of $\Delta\tau$ obtained in the studies (17-22) are probably overestimated.

In this situation the relation between the two evaluations of the torsional stiffness which were mentioned above is far from being clear. The detailed analysis of this matter (29) has shown that for large N the magnitude $\langle(\Delta tw)^2\rangle/N^2$ equals $\langle(\Delta\tau)^2\rangle$ defined for regular polymers. Thus in order to reveal the mechanism of the double helix flexibility we can compare the winding angle fluctuation found for the regular helices with that obtained in general case.

Review of the Published Conformational Studies of the double helix flexibility within the B-family.

The energy profiles obtained in these studies are presented in Figure 1. They vary markedly both in width and position of minimum. According to calculations of Zhurkin et al. (11,14) equilibrium winding angle, τ_o, equals to 40°, and thermal fluc-



	L	KP	M	ZLI
τ°_{min}	34	34	37	40
$\Delta\tau^\circ$	1.2	2.4	0.7	1.6

Fig. 1 Dependence of energy of the DNA double helix on winding angle as obtained by different authors:
ZLI - Zhurkin et al.(14), poly(dA):poly(dT);
M - Miller, poly(dA):poly(dT), from Fig.8 of Ref.(15);
KP - Khutorsky and Poltev (13), poly(dA):poly(dU);
L - Levitt (5), random sequence.
The relative energy values are presented per mole of base pairs.

tuation of τ is 1.6° for poly(dA):poly(dT). The imperfection of this study was that the suger ring was assumed to be inflexible in a standard C2'-endo conformation. Besides that the potential functions used (30) had unrealistically short interatomic equilibrium distances.

Miller (15) has frozen not only the conformation of deoxyribose but mutual disposition of the base pairs as well: they were strictly perpendicular to the helix axis (TL=TW=0) and the interplane distance was constant (H=3.38 Å). In result the fluctuation of winding angle decreased: $\underset{\sim}{\Delta}\tau$=0.65° for poly(dA):poly(dT) and $\underset{\sim}{\Delta}\tau$=0.95° for poly(dG):poly(dC). According to his computations the equilibrium value of the τ angle is sequence-dependent and varies from 36° to 39°; for poly(dA):poly(dT) τ_o=37° (Fig. 1).

Khutorsky and Poltev (12,13) were the first to analyse the double helix with the flexible deoxyribose. Introduction of a new variable has naturally widened the energy profile, $\underset{\sim}{\Delta}\tau$ increased up to 2.4°. Another distinction of their data is the shift of τ_o to 34° from 37°-40° (Fig. 1). It should be mentioned,however, that Khutorsky and Poltev (12,13) did not take into account the electrostatic and torsional interactions; the latter being very important when flexible pentose ring is considered (see below).

The study of Levitt (5) stands apart in this row. Indeed, in the cited papers (11-15) five parameters specifying the disposition of bases were independent variables, and for the given set of basic parameters the geometry of sugarphosphate backbone was calculated. So the search of energetically optimal forms was performed in a 5-dimensional (or 9-dimensional in the case of flexible furanose) space. In the Levitt's case (5) the cartesian coordinates of atoms are assumed to be independent variables, so that when analysing the DNA duplex consisting of 20 b.p. he deals with 2500-dimensional space. At first it seems to be absolutely comprehensive approach, but really there is no hope to find the precise minimum of function of so many variables. Another shortcoming of this paper is neglect of the hydrogen atoms and electrostatic interactions.

As to the results obtained using the discussed procedure

one may expect that fluctuation of τ would further increase due to unfreezing all the constraints in the molecule. It did not happen, however. On the contrary, $\Delta\tau$ proved to be even less than that found with rigid sugar ring (14)(Fig. 1). From our point of view, this discrepancy is explained by that in the structures computated by Levitt inclination of the bases changes insufficiently (see below).

Note the studies of Tumanyan and coworkers (31,32) who dealt with flexible sugar ring also. They estimate the equilibrium winding angle as 36.2°-38.5° for poly(dA):poly(dT) depending on parametrization of potential functions. In their approach the dihedral angles are independent variables and therefore energy profile E (τ) was not investigated.

So we see that the published computations present rather contradictory picture and a new research with no disadvantages mentioned above is badly needed.

METHODS

Five base parameters of Arnott (33) (τ, H, D, TL, TW) and four angles (two dihedral and two valency angles) controlling the furanose pucker (34) served as independent variables in this study. The geometry of the sugar-phosphate backbone was found by the method described earlier (14).

Energy was computated as a sum of van der Waals, electrostatic and torsional terms (35,55) and deformational energy of the valency angles in deoxyribose. The parameters of van der Waals interactions were chosen so as to reproduce correctly the interplanar base-to-base distance (35). When estimating the electrostatic term the following three approaches were used: (1) $\varepsilon = \infty$;(2) $\varepsilon = 4$;(3) $\varepsilon = 1$ for adjacent bases and $\varepsilon = 4$ for all other interactions. Ethane-like torsional potentials for the C-C and C-O bonds, $U = U_{xy}/2 \cdot (1 + \cos 3\varphi)$, had the barrier heights $U_{CC} = 2.5$ and $U_{CO} = 0.6$ kcal/mole, respectively. Deformational energy of the valency angles was evaluated as $U = U_{xyz}/2 \cdot (\theta - \theta_o)^2$ where $U_{COC} = 100$, $U_{CCC} = U_{CCO} = 75$, $U_{HCX} = 60$ kcal/mole·rad^2 (here x is for atoms H, C, O). For all angles in the sugar ring the tetrahedral angle $\theta_o = 109.5°$ served as an equilibrium value. The bond lengths and the remain-

ing valency angles were chosen as in the study (36), the distance CH was chosen to be 1.08 Å, OH=NH=1.0 Å.

The calculations were performed for the infinite regular helix neglecting the end effects. All the interactions within the complementary pair of nucleotides were taken into account as well as the interactions with N neighbouring pairs (N=1,5). Due to the regularity of the helix it was sufficient to consider the neighbouring monomers from one side only (14). Poly(dA):poly(dT) and poly(dG):poly(dC) were investigated. The control calculations with the unfreezed exocyclic bonds in deoxyribose have shown that introduction of these degrees of freedom does not affect the torsional flexibility of DNA. A short account of this study has been published elsewhere (37).

RESULTS AND DISCUSSION

Flexibility of Deoxyribose and Equilibrium Winding Angle of DNA

First we studied how the energy profile $E(\tau)$ of the double helix depends on the conformation of the sugar ring. Poly(dA):poly(dT) was examined with rigid deoxyribose for the different values of the pseudorotational phase angle P (38). It was found that diminution in the P angle from 162° to 108° (which means shift of the sugar pucker from the standard C2'-endo conformation to the "unusual" O1'-endo) leads to a decrease in the value from 40° to 32° (Fig. 2).

Because of the principal influence of the deoxyribose conformation on geometry of the whole helix let us consider the problem in more detail. As it follows from the distribution of the X-ray structures of mono- and dimers in the phase angle P (Fig. 3) the most favorable energetically are the C3'-endo and C2'-endo conformations of the sugar ($P \approx 18°$ and 162°) whereas O1'-endo conformation (P=90°) corresponds to energetical barrier. This is in qualitative agreement with all calculations published before. The only point in which they differ is the height of this barrier. So, according to Lugovskoy & Dashevsky (34), Sato (39) and Olson & Sussman (42) it is about 2-2.5 kcal/mole, Il'icheva et al. (40) estimated it as 1 kcal/mole, but Levitt & Warshel (41) found it to be only 0.6 kcal/mole.

According to our calculations the barrier at P=90° equals

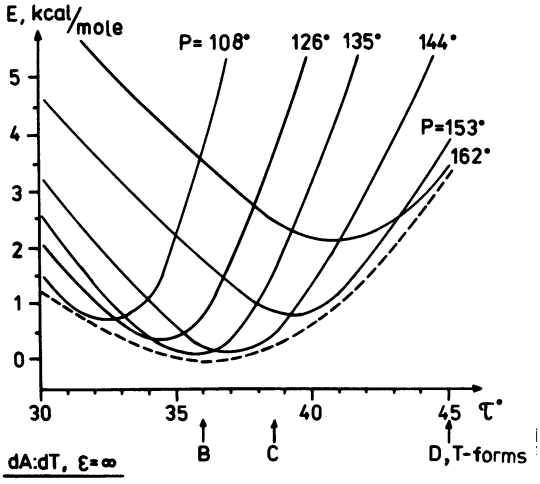


Fig. 2 Energy of poly(dA):poly(dT) as a function of the helical winding angle for different values of the pseudorotation phase angle, P.
The calculations were performed with $\varepsilon = \infty$, N=1; the angle was scanned with a 2° step; energy of the deoxyribose was included in the total energy. The broken line shows the function E(τ) for the unfrozen angle P.

2.2 and 2.6 kcal/mole for deoxy- and riboadenosine respectively (Fig. 3). This tendency is in good agreement with X-ray (43) and NMR (44) observations. We varied all components of the potential energy within the reasonable limits and found that parametrization of van der Waals and electrostatic interaction as well as choice of bending constants for the valency angles control the amplitude of pseudorotation, τ_m (38), but practically do not influence the magnitude of the barrier - its change is not more than 0.3 kcal/mole. But the interconversion barrier does depend crucially on the torsional potentials: the larger the difference between U_{cc} and U_{co} (see Methods) the higher the barrier at P=90°. This inference derives from the fact that in the O1'-endo puckering of the pentose the C2'-C3' dihedral angle is in unfavorable cis-conformation while in C2'-endo (C3'-endo) puckering it is O1'-C4' (O1'-C1') bond which is overstrained (see the right diagram of Fig. 3).

Now it is clear why Levitt & Warshel (41) found the furanose to be extremely flexible: they assume U_{cc} and U_{co} to be

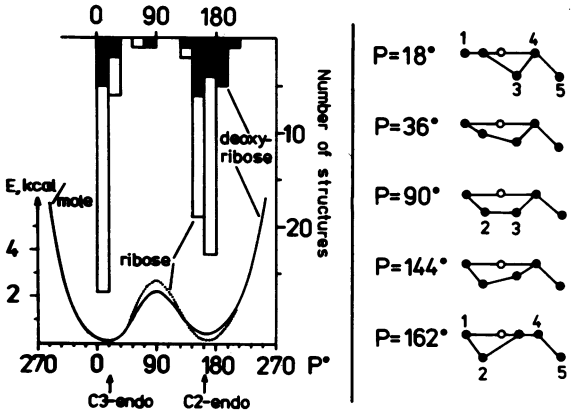


Fig. 3 Histogram of the phase angle P in the crystal mono- and dimeric structures as well as dependence of energy of ribo- and deoxyadenosine on P (ε =4). Some "twist" and "envelope" furanose conformations are presented in projection to the plane C1'-O'-C4' (the right half of the Figure).

practically the same (2.3 and 2.1 kcal/mole respectively). Note that this choice of U_{cc} and U_{co} contradicts the wellknown experimental fact that the rotational barrier in ethane is about 3 kcal/mole but in methanole it equals 1 kcal/mole only.

According to NMR data the barrier for C3'-endo-C2'-endo transition in adenosine is 4.7±0.5 kcal/mole (45). It should be mentioned, however, that the NMR estimation is an effective one to which other transitions, e.g. syn-anti, also contribute. When comparing the NMR results with those obtained theoretically one should have in mind that interaction with solvent, which is not considered here, would also influence the discussed conversion. Therefore our present estimation of the barrier at P=90° seems to be quite realistic though perhaps somewhat underestimated.

Thus we have seen that the equilibrium winding angle, τ_o, is a compromise between two opposite tendencies: diminishing the phase angle P leads to a decrease in τ_o and makes the interactions in sugar-phosphate moiety more favorable, while the energy of the sugar ring itself increases. Therefore the resulting value of τ_o depends on the height of the pseudorotational barrier: the higher the barrier, the larger τ_o value. This cor-

relation explains the data presented in Fig. 1: calculations with the rigid furanose give τ_o =37-40° (14,15), whereas the extreme flexibility of sugar decreases τ_o to 33.5-34° (5,13)(the deoxyribose was presumably overflexible in the studies of Khutorsky and Poltev (12,13), since they did not take into account the torsional interactions). In the present study the rigidity of deoxyribose is increased in comparison with that in the studies (5,13) and it results in the rise of τ_o up to 36° (Fig.2). As it follows from the data presented in Fig. 2, unfreezing the sugar ring leads to widening the energetic profile and thereby to an increase in $\underset{\sim}{\Delta}\tau$ (*cf.* the parabola for P=162° and the envelope curve shown by the broken line).

It is worth mentioning that the τ_o values under discussion are not to be compared (5) with the experimentally found magnitude τ =34.6° (10) for the DNA in solution of 0.2 M ionic strength since the calculated values were obtained with no consideration of repulsion of the negatively charged phosphate groups.

Dependence of the Equilibrium Winding Angle on the Degree of Neutralization of Phosphates

In order to evaluate the repulsion of opposite chains we calculated interaction of a pair with 5 neighbours from each side (N=5). Thus we took into account the phosphates of both major and minor grooves, as it follows from the consideration of different forms belonging to the $\overline{B}$-family (14). The shielding effect of counterions was modelled as follows: if neutralization was equal to x·100% then the positive charge of x/2 was added to both negatively charged phosphate oxygens.

It is found that decrease in the neutralization from 100% to 50% leads to unwinding of the double helix by 1.5-2.0°, while in the absence of counterions the duplex tends to unwind far beyond τ =30° and probably becomes unstable (Fig. 4). Note that the increase in the dielectric constant for the stacking interactions from 1 to 4 practically does not influence this trend: the τ_o value for a 100% neutralization shifts from 35° to 35.8° whereas it remains constant for a 50% neutralization, τ_o=33.5°.

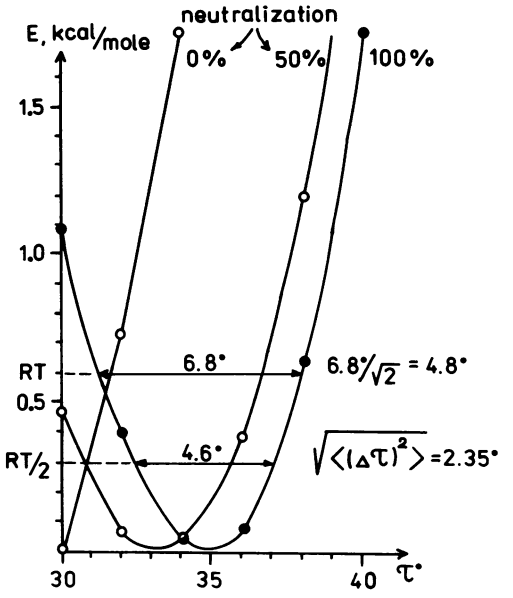


Fig. 4 Energy of poly(dA):poly(dT) as a function of winding angle and the degree of neutralization of phosphate groups. The computations were made for N=5; $\mathcal{E}$ =1 for the stacking interactions and $\mathcal{E}$ =4 otherwise.

The data presented above are in qualitative agreement with the known experimental fact, i.e. with unwinding of the double helix under decrease in ionic strength (8-10). This effect can be explained with the use of simple geometrical considerations. Indeed, reduction of the ionic strength results in deshielding of the phosphates by counterions and increase in the electrostatic repulsion, which in its turn leads to widening the narrow groove where the density of charges is the largest. The width of the narrow groove is reversely proportional to the helical winding angle in the forms of the $\overline{B}$-family (8,14), thus it becomes clear why the decrease in the counterions concentration controls the τ_o angle.

Quantitative comparison of our results presented in Fig. 4 with those obtained experimentally (8,9) is hardly possible since the shielding effect of ions was modelled here very roughly. Besides, it is still unclear what is the density of counterions in the close vicinity of phosphates and what is the

effective dielectric constant for intercharge distances 10-20 Å. It is worth mentioning, however, that equilibrium winding angle calculated here in the case of complete neutralization of phosphates (τ_o=36° for $\varepsilon=\infty$, Fig. 2, and τ_o=35-35.8° for ε=1 or 4, Fig. 4) is in a fair accord with the experimental value of 36±0.3° for poly(dA):poly(dT) at the standard ionic strength (45,46). In the case of poly(dG):poly(dC), N=5, the optimal winding angle is found to be nearly the same, but when only two neighbouring pairs are considered (N=1, ε=4) it decreases τ_o down to 34° in agreement with the data of Peck & Wang (45).

Thermal Fluctuation of the Helical Winding Angle proved to be $\underset{\sim}{\Delta}\tau$=2.4° for poly(dA):poly(dT) and $\underset{\sim}{\Delta}\tau$=3.0° for poly(dG):poly(dC) (Fig. 4). The geometrical procedure used here to define $\underset{\sim}{\Delta}\tau$ gives fairly high precision, since the dependence $E(\tau)$ is nearly quadratic (see Introduction). The latter follows from that twofold increase in ΔE corresponds to the growth of $\Delta\tau$ by a factor of $\sqrt{2}$ (Fig. 4). It is of interest that the presented magnitudes of $\underset{\sim}{\Delta}\tau$ are insensitive to the choice of dielectric constant and extent of phosphate shielding, i.e. to the parameters which define the optimal structure of the helix. In only one artificial case did $\underset{\sim}{\Delta}\tau$ increase up to 3.4° for poly(dA):poly(dT), namely, in the case of neglection of the coplanar A-T interactions with ε=1. That dG:dC polymer is more flexible than dA:dT seemingly correlates with destabilizing effect of electrostatic component in the case of G:C pairs and with definite conformational restrictions caused by thymine methyl group (see below).

It is known from the previous calculations (14) that all helical parameters are closely related. Therefore, in order to obtain the torsional stiffness of the double helix as a whole, one should vary all parameters (H, D, TL, TW) when scanning the τ angle; just what has been made above. On the contrary, fixation of these parameters allows one to learn what is the "local" flexibility of the duplex, i.e. the diversity of the winding angle between the two pairs when all the remaining helix is rigid. We carried out such computations for poly(dA):poly(dT) unfreezing only τ, H and D (their variation does not affect geometry of the neighbours) with the angles TL and TW as well

as sugar puckering being the same as in the minimum energy conformation. In this approach fluctuation of the τ angle proved to be only 0.6°, that is 4 times less than in the case of free change of all parameters. Thus the "local" torsional stiffness of DNA exceeds the "macroscopic" one by more than order of magnitude.

The magnitude of $\underset{\sim}{\Delta}\tau$, obtained here with the constant TL and TW, practically coincides with the results of Miller (15) for poly(dA):poly(dT) (see Introduction, Fig. 1). Fluctuation of τ, calculated by us without any restriction on parameters, is in fair agreement with the data of Khutorsky and Poltev (13) for poly(dA):poly(dU), though the optimal conformations differ markedly. This circumstance emphasizes independence of $\underset{\sim}{\Delta}\tau$ value on the particular parametrization of atom-atomic potentials or the computational scheme; the crucial point is to choose properly the independent parameters. In the light of these findings it is conceivable that underestimation of the τ angle fluctuation obtained by Levitt (5), $\underset{\sim}{\Delta}\tau$=1.2°, is explained by a constrained variation of the bases tilt in the B-family forms: when τ proceeds from 30° to 40° the TL and TW angles are changed not more than by 2°, while according to our calculations they alter by 6-12° (in the X-ray models of DNA in fibers the inclination of bases varies within the same limits (33,36)). In its turn, constancy of TL and TW in the Levitt's forms is probably linked with the choice of cartesian coordinates of atoms as independent variables (see Introduction).

Barkley and Zimm (18) have estimated fluctuation of the helical winding angle of DNA, $\underset{\sim}{\Delta}\tau$, to be 5.1°, on the basis of the known persistence length of DNA and using the Poisson ratio for the elastic isotropic rod. Here we present another evaluation of the $\underset{\sim}{\Delta}\tau$ magnitude, also based on the value of persistence length of DNA, P_∞, but using the discrete nature of the double helix and anisotropic mechanism of its bending flexibility (47,6). According to this model, further confirmed by the X-ray structure of the B-DNA dodecamer in crystal (48), the double helix bends into the both grooves much more easily than in perpendicular directions. The average bending of DNA helical axis, calculated after this model for P_∞=600 Å, equals 6°. As

was shown in Ref.(6) the local bending of the DNA axis into a groove by angle β changes a mutual disposition of the neighbouring base pairs in the same way as if the winding angle were increased by some magnitude $\Delta\tau$. The dependence $\Delta\tau(\beta)$ is pseudoquadratic, so that small values of β produce negligible $\Delta\tau$; $\beta=6°$ corresponds to $\Delta\tau=0.5°$. Note that this value of $\Delta\tau$ is very close to $\underset{\sim}{\Delta}\tau=0.6°$ calculated above for the fixed TL and TW.

Thus we have got the estimation of $\underset{\sim}{\Delta}\tau$ which is 10 times less than that obtained in (18). The main difference between these approaches is that Barkley and Zimm deal with the "macroscopic" flexibility of DNA when all nucleotides are supposed to change its geometry while in our study only one pair of the complementary nucleotides is supposed to be flexible, and thereby the so called "local" rigidity of DNA is evaluated. This noticeable decrease in the value of $\underset{\sim}{\Delta}\tau$ found after fixation of some of the parameters of bases explains the known stiffening of the double helix in the vicinity of ethidium bromide intercalated in DNA (54).

Optimal Conformations of the Double Helix for $\tau=36°$ are presented in Table 1. Those are the forms very close to the total minima in the case of fully neutralized phosphates (see Fig. 4). It is seen that after unfreezing deoxyribose the dihedral angles in the sugar-phosphate backbone became essentially the same as in the monomers - this inference has already been mentioned by the others (5,31). In particular, the glycosidic angle χ has proceeded from 130-140° (in the models with C2'-endo (33) or C3'-exo (36) sugar puckering) to 120-125°, which is especially favorable for pyrimidine nucleotides. As a result of this rearrangement of the backbone, the orientation of the phosphate groups calculated here is in a much better agreement with the infra-red linear dichroism data (49) than in the C3'-exo structure (36).

It is of interest that the equilibrium values of dihedral angles in poly(dA):poly(dT) and poly(dG):poly(dC) are practically the same - they differ by 1-2° only; the angles are not altered by the choice of dielectric constant as well (Table 1). In contrast, the base parameters are different for these polymers; they also depend on the particular value of ε . Note the

Table 1. Optimal conformations for τ =36°

		Parameters of bases				Dihedral angles						
ε	N	H	D	TL	TW	χ	ξ	θ	ψ	φ	ω	P
						Poly(dA):poly(dT)						
∞	1	3.16	1.34	-2.6	-8.1	127.0	61.8	176.1	291.4	251.8	186.2	141.2
4	5	3.22	1.28	-0.2	-7.0	126.6	61.2	177.1	292.5	251.0	185.2	142.9
1,4	5	3.32	1.24	4.7	-4.5	123.0	62.4	177.2	292.2	249.6	185.2	143.6
						Poly(dG):poly(dC)						
∞	1	3.24	1.43	-2.8	-4.0	126.3	62.1	177.5	288.7	249.6	187.4	145.7
4	1	3.38	1.25	2.5	-1.9	125.1	61.7	178.2	290.9	246.9	186.6	148.5
1,4	5	3.61	0.95	11.6	0.4	119.3	63.4	178.1	292.6	247.2	185.5	146.3

The structures presented were calculated at complete neutralization of the phosphates. ε and N are defined in Methods, the pseudorotation phase angle P is described in Ref.(38). The basic parameters are defined in Ref.(14), note that conventions for the signs of tilt of the bases (TL) and propeller in a pair (TW) are opposite to those in Arnott's paper (33). The dihedral angles are measured as follows (33): χ(C8(Pu) or C6(Py)-N-C1'-C2'), ξ (C3'-C4'-C5'-O5'), θ (C4'-C5'-O5'-P), ψ(C5'-O5'-P-O3'), φ (O5'-P-O3'-C3'), ω (P-O3'-C3'-C4').

change in the H parameter (H=3.2Å for poly(dA):poly(dT) and H=3.4 Å for poly(dG):poly(dC) when ε =4), which is in accord with the X-ray data: H=3.29 Å in B'-DNA for poly(dA):poly(dT) and H=3.38 Å in B-DNA (36) with the random sequence.

The detailed analysis has shown that the different orientations of the A:T and G:C pairs can be explained by three reasons: (1) The guanine amino group prevents large propeller (TW) due to its hindrance with the cytosine O2 from the neighbouring pair. (2) On the contrary, the noticeable propeller is favorable for the A:T pair since it reduces the too tight contact of the thymine methyl with the C2'H_2-group of the neighbouring sugar ring and results in stabilizing hydrophobic interaction. (3) The electrostatic component in case of G:C pairs leads to inclination and moving the base pairs apart despite van der Waals attraction. As a consequence, dG:dC polymer is characterized by a smaller propeller in the pairs but a larger tilt of the bases, TL, and shift of bases along helix, H.

The procedure used here to calculate $\underset{\sim}{\Delta}\tau$ was applied also to the TL and TW angles. It was found that unlike $\underset{\sim}{\Delta}\tau$, $\underset{\sim}{\Delta}$TL and $\underset{\sim}{\Delta}$TW do depend on parametrization of electrostatics. These fluctuations for poly(dA):poly(dT) are depicted below for $\varepsilon=\infty$ and ε =4: $\underset{\sim}{\Delta}$TL=6° and 11°, $\underset{\sim}{\Delta}$TW=4° and 9°. Thus fluctuation of the inclination angles, TL and TW, exceeds $\underset{\sim}{\Delta}\tau$ 2-3fold. These findings make it conceivable that the data of Hogan et al. (50, 51) on the comparatively large inclination of the bases, γ ($\gamma \approx \sqrt{TL^2 + TW^2} \approx 17°$) should be interpreted not on the basis of static (50,5), but dynamic model of DNA which the same authors have considered previously (51). In this connection it is worth mentioning that the average inclination of bases relative to the "macroscopic" axis of the real DNA molecule might be larger than in the regular helix studied here due to bending of the "regular" axis itself by 5-6° (47).

CONCLUSION

The computations presented above show that the two polymers, dA:dT and dG:dC, have somewhat different optimal conformations and thermal fluctuations of the helical winding angle:

$\Delta\tau$=2.4° for poly(dA):poly(dT) and $\Delta\tau$ =3.0° for poly(dG):poly(dC). It means that the corresponding torsional rigidities differ 1.5-fold. This variance, though not very significant, may play its role in the interaction of DNA with regulatory proteins or in DNA wrapping around the nucleosome cores. The calculated values of $\Delta\tau$ are in reasonable agreement with the other estimations of fluctuation of the τ angle based on the experimental data: $\Delta\tau$ =3.3-5.9° (16-22).

Our results were obtained on the assumption that the double helix is a member of the $\bar{B}$-family of forms, i.e. all its dihedral angles lie in the same local minima as in the canonical B-form. Of note is that variation of the atom-atomic potentials within rather broad limits affects the optimal conformation of the duplex, but not its torsional stiffness. In contrast, the details of procedure do change the resulting value of $\Delta\tau$, e.g. unfreezing the sugar puckering and all the parameters of bases markedly increases $\Delta\tau$. Besides, one may expect that consideration of irregular helices would further enlarge flexibility of DNA, thereby diminishing disagreement between theoretical and experimental data. The following reasons make it probable:
1. Bending and winding of the double helix are not independent (6), therefore the bends in the duplex might increase $\Delta\tau$.
2. There are some indications that C2'-endo → C3'-endo interconversion probably takes place in separate sugar rings (52) without actual $\bar{B} \rightarrow \bar{A}$ transition in the contiguous fragment of DNA (53). This possibility is not considered here.
3. Optimal configurations of poly(dA):poly(dT) and poly(dG):poly(dC) differ to some extent, so the DNA duplex with the random sequence might be more "loose" than a homopolymer and thus more flexible.

So we arrive at a conclusion that the basic mechanism of torsional elasticity of the double helix descends from the small amplitude torsional vibrations of the atoms - at least, they provide the DNA helix with the fluctuations which are comparable with the experimental data in physiological conditions. The role of rotational isomerism (23) in case of the double-stranded DNA is rather insignificant if any. It is interesting that our previous calculations of the bending stiffness of DNA

(6) have also shown that small variations of the dihedral angles in the sugar-phosphate backbone which keep the angles within the same local minima are consistent with the known persistence length of DNA. Though these computations do not evaluate the probability of creation of the noncanonical structures (left-handed helices, kinks etc.) it follows from them that the hypothesis on the existence of such structures is unnecessary to explain the experimental data on the flexibility of DNA in standard conditions.

The last point which is worthy of mentioning is that the values of $\underset{\sim}{\Delta}\tau$ estimated here do not measure the fluctuation of the winding angle between the neighbouring base pairs $-\underset{\sim}{\Delta}\tau$ serves only as a convenient measure of torsional stiffness of the whole duplex (see Introduction). Real fluctuation of the angle between the neighbours might be less, this difference being the more the larger is correlation of the conformational changes in adjacent nucleotides. This question is still open and we hope to answer it in the course of Monte-Carlo simulation of the DNA double helix.

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