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Anomalous structure and properties of poly(dA)·poly(dT). Computer simulation of the polynucleotide structure with the spine of hydration in the minor groove

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ABSTRACT

The results of the search for low-energy conformations of poly(dA)·poly(dT) and of the poly(dA)·poly(dT) "complex" with the spine of hydration similar to that found by Dickerson and co-workers (Kopka, M.L., Fratini, A.V., Drew, H.R. and Dickerson, R.E. (1983) J. Mol. Biol. 163, 129-146) in the minor groove of the CGCGAATTCGCG crystals are described. It is shown that the existence of such a spine in the minor groove of poly(dA)·poly(dT) is energetically favourable. Moreover, the spine of hydration makes the polynucleotide conformation similar to the poly(dA)·poly(dT) structure in fibers and to the conformation of the central part of CGCGAATTCGCG in crystals; it also acquires features characteristic of the structure of poly(dA)·poly(dT) and DNA oligo(dA)-tracts in solution. It is shown that the existence of the TpA step in conformations characteristic of the poly(dA)·poly(dT) complex with the spine of hydration is energetically unfavourable (in contrast to the ApT step) and therefore this step should result in destabilization of the spine of hydration in the DNA minor groove.

Thus, it appears that the spine of hydration as described by Dickerson and co-workers is unlikely to exist in the poly d(A-T)·poly d(A-T) structure. The data obtained permit us to interpret a large body of experimental facts concerning the unusual structure and properties of poly(dA)·poly(dT) and oligo(dA)-tracts in DNA both in fibers and in solution. The results provide evidence of the existence of the minor groove spine of hydration both in fibers and in solution on A/T tracts of DNA which do not contain the TpA step. The spine plays an active role in the formation of the anomalous conformation of these tracts.

INTRODUCTION

One of the important features of the crystal structure of the double-stranded CGCGAATTCGCG dodecamer is the presence in the minor groove of its central part of an ordered zig-zag structure formed by water molecules of the first and the second hydration shells (spine of hydration). The first shell is formed by water molecules hydrogen-bonded with thymine O2 and adenine N3 atoms, thus bridging adjacent bases pertaining to different chains. The second shell consists of water molecules which connect two adjacent water molecules of the first shell (1,2). Dickerson and co-workers noted (1,2) the important role of this spine of hydration in stabilization of the B form and

in the mechanism of the B-A transition. Later (3) we used energy estimates and the analysis of space-filling models to study the possibility of the spine formation on different sequences of B-like DNAs as well as its role in stabilization and modification of the DNA structure. It has been suggested that the unusual structure of poly(dA)•poly(dT) is related to the existence of this spine of hydration in the minor groove. The influence of the spine of hydration on the DNA structure was roughly modelled by reducing the N3(A)...O2(T) distances and the distances between oxygens of adjacent water molecules of the first shell to the values characteristic of the central part of the CGCGAATTCGCG dodecamer (~3.5 Å and 4.5 Å, respectively); however, it was not clear whether the spine of water molecules would in fact reduce these distances in the same way.

Here we present the results of energy calculations for the poly(dA)• •poly(dT) complex with the spine of hydration. The results suggest that the interaction of the spine of hydration with the polynucleotide duplex in the minor groove is energetically favourable; the spine of hydration contributes much to abnormality of the poly(dA)•poly(dT) structure. The data obtained permit us to interpret a large body of experimental facts concerning the unusual structure and properties of poly(dA)•poly(dT).

METHODS

The low-energy conformations were determined using calculations of the energy of nonbonded interactions by the method of atom-atom potential functions. The parameters of potential functions were taken from ref. (4) for nucleic acids and from ref. (5) for water-water and water-DNA interactions. Bond lengths and bond angles in DNA (except for the internal bond angles of deoxyribose and the C3'-O3'-P angle) were assumed to be constant and the same as in ref. (6-8).

The energies of poly(dA)•poly(dT) and of the poly(dA)•poly(dT) complex with the spine of hydration were optimized in the space of independent variables. The independent variables were as follows: (1) only one parameter characterizes the mutual position of the two bases in the pair: half of the dihedral angle between the bases formed at rotation of these bases around the line passing approximately through the C8 atom of purine and the C6 atom of pyrimidine, i.e. half of the propeller twist (TW); (2) the parameters characterizing the position of the base pair relative to the helix axis: roll (RL), tilt (TL), slide (SL) and displacement from the helix axis (D); in ref. (9) these parameters are denoted as θ_R, θ_T, s and d, respectively

(see footnote to Table 1); (3) the helical parameters, i.e. the distance between adjacent base pairs along the helix axis (d) and the turn of one pair relative to the adjacent one, the winding angle (τ); (4) two dihedral angles χ determining the glycosylic conformations for each antiparallel chain; (5) the eight angles determining conformations of sugar rings of both antiparallel chains (two dihedral and two bond angles for each sugar).

For the poly(dA)•poly(dT) complex with the spine of hydration twelve more degrees of freedom were added: (1) six parameters characterizing the position of a water molecule of the first hydration shell relative to the polynucleotide; the position of all other molecules of the first shell is imposed by helical symmetry (a turn by τ about and a shift by d along the helix axis); (2) six parameters characterizing the position of a water molecule of the second shell relative to the polynucleotide; the position of all other molecules is also determined by the helical symmetry.

The energy of the poly(dA)•poly(dT) complex with the spine of hydration (E) consists of the energy of the regular polynucleotide itself (E_p), the energy of the polynucleotide interaction with the spine of hydration (E_{pw}) and the energy within the spine of hydration (E_w):

$$E = E_p + E_{pw} + E_w$$

The energy is normalized per one nucleotide pair.

E_p was calculated in the same manner as for the regular homopolynucleotides (7,8). If the phosphate groups were completely neutralized, the interaction between non-adjacent nucleotide pairs was neglected, and only interactions between the bases were taken into account in the interaction of antiparallel chains. Changes of the phosphate group charges were simulated by changing the charge of the two phosphate oxygens as in ref. (10). If the phosphate groups of the polynucleotide were not completely neutralized, the energy of interaction of all phosphate groups of the six nearest nucleotide pairs was added to E_p.

E_p consists of the energy of van-der-Waals interactions, electrostatic interactions, the torsional energy of rotations around single bonds of the sugar-phosphate backbone and the deformation energy of variable bond angles. Parametrization is the same as in ref. (4,7,8).

As the total energy (E) of the complex of the regular homopolynucleotide with the spine of hydration is normalized per one base pair, the energy E_{pw} consists of the energy of interaction of the poly(dA)•poly(dT) polynucleotide with one water molecule of the first shell (E1) and the energy of interaction of poly(dA)•poly(dT) with one water molecule of the second shell (E2).

To calculate E1 and E2, poly(dA)•poly(dT) was replaced by the hexamer d(pApApApApApA)•d(pTpTpTpTpTpT). The figures under the hexamer designate the number of the nucleotide (see also Fig. 1B). The nucleotide includes the base, the adjacent sugar and the 5'-end phosphate. The term "sugar" will be used here to designate the deoxyribose residue with the atoms O3', H1', C5' and one of the H5' atoms. All the nucleotide groups have the same number as the whole nucleotide. The water molecule of the first hydration shell was located between the third adenine of one chain and the nineth thymine of the anti-parallel chain (the central base pairs of the hexamer) while the water molecule of the second hydration shell was near the A•T pair formed by the fourth adenine and the nineth thymine. In Fig. 1B these water molecules are drawn in bold lines. The substitution of the hexamer for poly(dA)•poly(dT) seems to be quite reasonable because the interaction of these water molecules of the first and the second hydration shells is weak even with the ends of the hexamer.

E_w consists of the interaction energy between the water molecules of the first hydration shell, the energy between the molecules of the second hydration shell and the energy of interaction of the molecules of the first with the second hydration shells. The energy was also normalized per one nucleotide pair. Interactions of non-adjacent molecules of the first and the second hydration shells were neglected.

Calculations were performed assuming that the dielectric constant is equal to 4 unless noted otherwise. This value was used to take into account the effective influence of the medium. All the results, except those described in subsection 3, were obtained for completely neutralized phosphate groups (see also the footnote to Table 1). Hydrogens of the thymine CH_3-group were oriented as in ref. (10). Conformations of flexible deoxyriboses of both anti-parallel chains were taken in the C2'-endo region according to the recent data of NMR, Raman spectroscopy (11,12) and X-ray studies of poly(dA)•poly(dT) fibers (13,14).

RESULTS AND DISCUSSION

To simulate the regular spine of hydration in the DNA minor groove, water molecules were placed on low-energy conformations of the B-family in the same way as in the spine of hydration in Dickerson's dodecamer. Then the obtained poly(dA)•poly(dT) complexes with the spine of hydration were optimized by energy. It appears that the location of such a spine of hydration in the minor groove of poly(dA)•poly(dT) is energetically favourable, the spine is firmly

positioned there. Below we report the results of calculations.

1. Low-Energy Conformations at Fixed Values of d=3.23 Å and τ=36.0°. Comparison with the poly(dA)•poly(dT) Structure in Fibers

Before discussing our results we would like to note the main conclusions obtained on the structure of the calcium and sodium salts of poly(dA)• •poly(dT) in fibers (13,14) as these data are important for the understanding and interpretation of our results.

(1) On the basis of a comprehensive Patterson analysis of the obtained X-ray pattern of the poly(dA)•poly(dT) calcium salt it has been concluded (13,14) that the antiparallel chains of the polynucleotide must be equivalent. These results permit one to reject, for the calcium salt of poly(dA)•poly(dT) the heteronomous model suggested for the sodium salt of this polynucleotide (15). The heteronomous model of Arnott et al. (15) is characterized by essentially different conformations of the two chains: poly(dA) is in the A-type conformation with the C3'-endo sugar while poly(dT) is in the B-type conformation with the C2'-endo sugar.

(2) Proceeding from the conformation obtained for the calcium salt of poly(dA)•poly(dT) and using the X-ray data of Arnott et al. (15) Alexeev et al. (13,14) found the structure of poly(dA)•poly(dT) sodium salt with a smaller R-factor than in the heteronomous model (15) and with the sugars of both chains in conformations close to C2'-endo. The structure of the sodium salt is very similar to that of the calcium salt and is characterized, in particular, by a negative tilt (-6°), a large positive propeller in the pair (2 TW = +20°) and a narrowed minor groove (9.2 Å) (see Table 1, conformation 4).

(3) The authors of papers (13,14) were particularly interested in the position of calcium and sodium counterions around poly(dA)•poly(dT) in fibers as an earlier study on the cesium salt of thymus DNA (16) showed that half of the ions are located deep in the narrow B-DNA groove making a good contact with the bases. They found that calcium is located near the phosphates rather than deep in the poly(dA)•poly(dT) minor groove. The X-ray pattern did not allow sodium localization, however, the modelling suggests that sodium cannot be placed deep in the narrow groove of the obtained poly(dA)•poly(dT) structure.

Let us turn now to our results. Table 1 lists the parameters of both the low-energy poly(dA)•poly(dT) conformation (No.1) and the low-energy conformations of the complex of poly(dA)•poly(dT) with the spine of hydration (No.2) at fixed d=3.23 Å and τ=36.0° (these are the structural parameters of the poly(dA)•poly(dT) calcium and sodium salts (13-15)). A comparison of

Table 1. Conformational parameters of low-energy poly(dA)·poly(dT) structure and poly(dA)·poly(dT) complexes with the spine of hydration in the minor groove

	1	2	3	4
ε	183	179	181	174
ζ	-101	-103	-105	-102
α	-69	-61	-65	-50
β	178	181	180	188
γ	61	54	58	41
δ	128	132	133	137
χ	-120	-118	-120	-112
P	141	146	145	149
D	1.1	1.0	1.0	~0.8
TL	-0.1	-5.3	-6.0	-6
TW	7.5	7.5	6.1	10
N3(A)...O2(T)	3.7	3.3	3.4	3.32
L	10.8	9.4	9.5	9.23
d	3.23	3.23	3.34	3.23
τ	36.0	36.0	36.0	36.0

No.1, low-energy poly(dA)·poly(dT) conformation at fixed d=3.23 Å and τ=36.0°; No.2, low-energy conformation of the poly(dA)·poly(dT) complex with the spine of hydration at fixed d=3.23 Å and τ= 36.0°; No.3, low-energy conformation of the poly(dA)·poly(dT) complex optimized by all independent variables; No.4, structure of the calcium salt of poly(dA)·poly(dT) in fibers from ref.(14). Distances are in Å, angles in degrees. Designations of structural parameters: torsion angles are defined as $P^{\alpha}O5'^{\beta}C5'^{\gamma}C4'^{\delta}C3'^{\varepsilon}O3'^{\zeta}P$ and χ for glycosyl, O1'-C1'-N9-C4 for purine or O1'-C1'-N1-C2 for pyrimidine; P, angle of pseudorotation (17); D, shift of the base pair from the helix axis, corresponds to the parameter d in ref.(9); TL, rotation of the base pair about its short axis relative to the plane normal to the helix axis; TW, half of the propeller twist of the dihedral angle between bases in the pair; TL and TW are determined according to Arnott (18); L is the minor groove width, the distance between the nearest phosphorus atoms of the antiparallel chains; d is the distance between the adjacent base pairs along the helix axis; τ is the winding angle between the adjacent base pairs. The values of the backbone dihedral angles for structures Nos.1,2 and 3 are averaged for the two chains. The difference between the analogous angles pertaining to different chains of the structure was no more than 3-5°. The phosphates for structures Nos.1 and 2 were considered to be completely neutralized (see Methods). However, when the phosphates were charged, the characteristic differences between structures Nos.1 and 2 remained. For structure No.3 the phosphate charge was about -0.6.

It is seen from the table that noticeable changes of the structure are possible at slight changes of the dihedral angles of the sugar-phosphate backbone. This is what our calculations on incorporation of non-Watson-Crick pairs into the DNA double helix have shown (19,20). In structure No.2 TW is 2.5° smaller than in model No.4, but it is easy to obtain a better agreement and to increase it by ~2.5°: at fixed d=3.23 Å and τ=36.0° the energy of the poly(dA)·poly(dT) complex with the spine of hydration will increase by no more than ~0.5 kcal/mol. On the other hand, it should be noted that in the model of the poly(dA)·poly(dT) structure in fibers (13,14) deoxyribose with one independent parameter (P) was used while we used deoxyribose with four independent parameters. There are indications that the use of a more rigid deoxyribose results in an increase of TW in the structure.

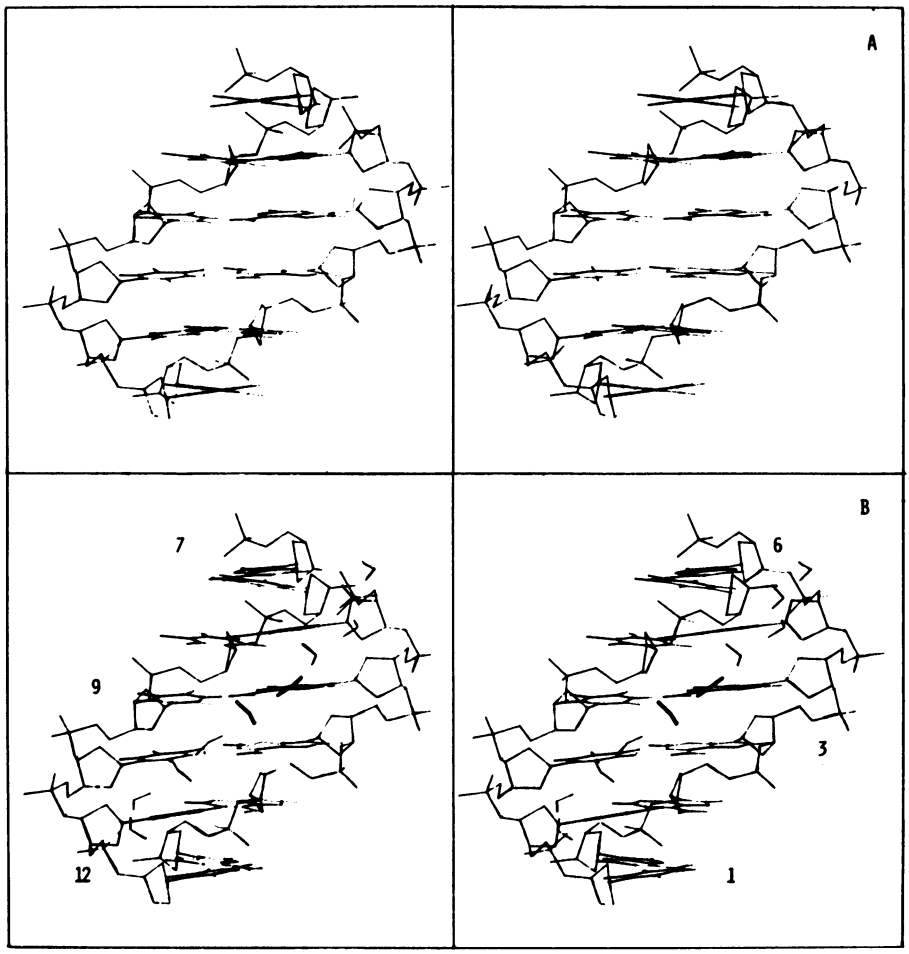

Figure 1. Stereo views of low-energy conformations at fixed values of d=3.23 Å and τ=36.0°. A, poly(dA)·poly(dT) (No.1 from Table 1); B, poly(dA)·poly(dT) with the spine of hydration in the minor groove (No.2 from Table 1). View from the minor groove. Nucleotides are numbered from 1 to 12 from the 3'- to the 5'-end. The bold line designates the molecules of the first and the second hydration shells whose energy of interaction with the hexamer was taken into account in the calculations (E_{pw}). It is seen that the conformation in (B) has a more narrow minor groove than that in (A) and a negative tilt of the base pair (TL).

the two conformations shows that the spine of hydration compresses the minor groove to ~9.4 Å, decreases the distances between adjacent N3 atoms of adenines and O2 atoms of thymines to ~3.3 Å and makes the tilt negative, ~-5° (Fig. 1). The structural parameters for the calcium salt of poly(dA)·poly(dT)

are given in Table 1 under No.4. The parameters for the sodium salt are similar to those for the calcium salt and therefore are not presented. A comparison of the structural parameters of low-energy conformations (Nos. 1,2) with those for the calcium salt of poly(dA)•poly(dT) shows that the conformation of the poly(dA)•poly(dT) complex with the spine of hydration (No.2), in contrast to the conformation without the spine, is similar to structure No.4 proposed in (14). As seen from Table 1, the dihedral angles of the sugar-phosphate backbone (conformation No.2) differ less than those of conformation No.1, from the dihedral angles of the structure of the poly(dA)•poly(dT) calcium salt (No.4).

Thus, our results provide evidence that in the minor groove of poly(dA)•poly(dT) fibers there is a spine of hydration similar to that found in the CGCGAATTCGCG crystal; this spine is greatly responsible for the unusual poly(dA)•poly(dT) structure in fibers, which is characterized by a narrow (~9 Å) minor groove, a small distance between N3 of adenine and O2 of the adjacent thymine (~3.2 Å) and the negative tilt value (~-6°). Furthermore, as we have noted in (3), the existence of this spine of hydration in the minor groove appears to account for the fact (21) that among all the known sequences in fibers only poly(dA)•poly(dT), poly(dI)•poly(dC) and poly d(A-I)••poly d(T-C) are not converted into other forms and remain always in the B' form (which somewhat differs from the orthodox B-form). As follows from our work (3), the spine of hydration can be formed only on the mentioned sequences and should be disrupted on all the others (see also subsection 4 of the present study). The fact that in poly(dA)•poly(dT) fibers the ions are not located deep in the minor groove also supports our conclusion on the presence of water molecules in it.

Let us consider the individual energy contributions to the poly(dA)••poly(dT) complex with the spine of hydration (No.2 in Table 1).

For the structure of complex No.2, the energy of the polynucleotide itself, E_p, is about 1 kcal/mol of nucleotide pairs higher than for structure No.1, this increase being due to a worse base stacking. Thus, the presence of the spine of hydration in the structure at fixed d=3.23 Å and τ=36.0° slightly increases the energy of the polynucleotide, this increase being even smaller when only τ is fixed (see below).

Hydrogen bonds between the water molecules of the first and the second hydration shells make the main contribution to E_w, the energy of the spine. Each water molecule of the second hydration shell forms two almost linear hydrogen bonds with oxygens of the water molecules of the first hydration

Table 2. Components of the interaction energy of poly(dA)·poly(dT) with the spine of hydration (E_{pw})

E1A					E1T					E2A		E2T	
E_{2S}	E_{2b}	E_{3SP}	E_{3b}	E_{4b}	E_{7SP}	E_{8S}	E_{8b}	E_{9SP}	E_{9b}	E_{23S}	E_{34b}	E_{78S}	E_{89b}
-0.7	-0.2	-0.2	-3.5	-0.5	-0.2	-2.0	-0.3	-0.1	-2.3	-1.3	-1.0	-1.6	-0.9
-5.1					-4.9					-2.3		-2.5	

E_{pw} for conformation No.2 from Table 1:

$E_{pw} = E1 + E2$

$E1 = E1A + E1T$ $\qquad$ $E2 = E2A + E2T$

$E1A = E_{2S} + E_{2b} + E_{3SP} + E_{3b} + E_{4b}$ $\qquad$ $E2A = E_{23S} + E_{34b}$

$E1T = E_{7SP} + E_{8S} + E_{8b} + E_{9SP} + E_{9b}$ $\qquad$ $E2T = E_{78S} + E_{89b}$

E1A and E1T are the interaction energies of the water molecule of the first hydration shell (shown in bold line in Fig. 1B) with the hexamer chain containing adenines and thymines, respectively; E2A and E2T are the interaction energies of the water molecule of the second hydration shell (shown in bold line in Fig. 1B) with the hexamer chain containing adenines and thymines, respectively; E_{NS} is the interaction energy of the Nth sugar of the double-stranded hexamer with the water molecule of the first hydration shell; E_{NSP} is the interaction energy of the Nth residue of the sugar-phosphate backbone with the water molecule of the first hydration shell; E_{Nb} is the interaction energy of the Nth base with the water molecule of the first hydration shell; E_{23S} and E_{78S} are the interaction energies of the water molecule of the second hydration shell with the sugar-phosphate of the second nucleotide and with the sugar of the third nucleotide (E_{23S}) and with the seventh sugar-phosphate and the eigth sugar, respectively (E_{78S}); E_{34b} and E_{89b} are the interaction energies of the water molecule of the second hydration shell with the third and the fourth (E_{34b}) and with the eighth and the ninth (E_{89b}) bases, respectively. The total E_{pw} = -15.0 kcal/mol while the sum of the components listed in the table is -14.8 kcal/mol. This difference is due to omission of the components whose contribution to the energy is less than 0.1 kcal/mol.

shell according to the scheme described by Dickerson and co-workers (1,2). The distances O1H...O2 and O1...O2 between the atoms of these molecules are equal to 1.9 Å and 2.8 Å, respectively, being within the limits characteristic of hydrogen bonds. The value of E_w for conformation No.2 is about -3 kcal/mol of nucleotide pairs.

E_{pw} (see METHODS) consists of the energy of the hexamer interaction with the water molecule of the first hydration shell (E1) and with the water molecule of the second hydration shell (E2) (bold lines in Fig. 1). As seen in Table 2, the main interactions of the water molecule of the first hydration shell with the hexamer are as follows:

(1) strong interaction with the third adenine, E_{3b}=-3.5 kcal/mol. Table 3

Table 3. Some typical nearest interatomic distances and angles between poly(dA)·poly(dT) and the water molecule of the first hydration shell in conformation No.2 (table 1)

	O	H1	H2	α_1	α_2
N3(A)	2.9		1.9		175
O1'(A)	3.4		3.0		103
O2(T)	2.7	2.0		128	
O1'(T)	2.7	2.0		128	

O1'(A), O1'(T) are oxygens of adenosine and thymidine sugar, respectively; O, H1, H2 are water oxygen and hydrogen atoms, respectively; α_1 and α_2 are the angles between O-H1 and O-H2 water bonds and the line connecting the H atoms of water and the nearest proton-acceptor sites of the polynucleotide. The distances are in Å, the angles are in degrees. Close association of sugar O1' atoms with water molecules of the spine has been also noted in ref. (1,2).

shows that this interaction falls within the limits of the hydrogen bond between N3 of adenine and H of water;

(2) a rather good interaction with the sugar of the second nucleotide (E_{2S}=-0.7 kcal/mol); the distance between O of water and O1' of deoxyribose is 3.4 Å;

(3) strong interaction with the nineth thymine (E_{9b}=-2.3 kcal/mol) falls within the limits of the hydrogen bond between O2 of thymine and H of water (Table 3);

(4) a rather strong interaction between the sugar of the eighth nucleotide and the water molecule of the first hydration shell (E_{8S}=-2.0 kcal/mol, the energy of the water molecule interaction with the O1' atom being -1.3 kcal/mol); the distance between O of water and O1' of deoxyribose is 2.7 Å.

Some typical nearest interatomic distances and angles between the polynucleotide and the water molecule of the first hydration shell are given in Table 3.

As for the interactions of the water molecule of the second hydration shell with the hexamer, Table 2 shows that this molecule effectively interacts not only with sugar-phosphates 2 and 7 of nucleotides and sugars 3 and 8 of nucleotides, but also with bases 3, 4, 8 and 9.

It should be noted that the data are given for the dielectric constant ε=4; at ε=1 the values of the main components of E_{pw} listed in Table 2 decrease by 60-70% on the average. The minor groove becomes 0.2-0.3 Å narrower, the tilt becomes more negative (~-7°) and the distance between O of water of the first hydration shell and O1'(A) of deoxyribose decreases by 0.2-0.3 Å.

Thus, the formation of a spine of hydration similar to that described by Dickerson and co-workers (1,2) is in fact a very favourable event.

2. Low-Energy Conformations at Fixed $\tau=36.0^{\circ}$

Calculations show that the low-energy conformations of the poly(dA)·poly(dT) complex with the spine of hydration at fixed $\tau=36.0^{\circ}$ involve not only structures with a rather large TW, but also with a small TW. Owing to the presence of a spine of hydration in the minor groove the low-energy complexes of poly(dA)·poly(dT) display a stronger dependence between TL and TW than for poly(dA)·poly(dT). Thus, for example, the low-energy complexes can have the structures with $TW\sim3^{\circ}$, $TL\sim-12^{\circ}$, $d\sim3.49$ Å, $D\sim0.7$ Å and the minor groove width ~9.1 Å as well as the structures with a large $TW\sim10^{\circ}$, $TL\sim-4^{\circ}$, $d\sim3.26$ Å, $D\sim1.0$ Å and the minor groove width ~9.4 Å, the energy of these complexes differing by only some tenths of kcal/mol. A comparison of the parameters of these two types of structures shows that both the structure with a large propeller ($2 \times TW \sim 20^{\circ}$) and a moderate negative tilt TL ($\sim-4^{\circ}$), and that with a small propeller ($2 \times TW \sim 6^{\circ}$) and a large negative TL ($\sim-12^{\circ}$) can have a narrow minor groove. The optimal conformation of the poly(dA)·poly(dT) complex with the spine of hydration in this extended region of low-energy conformations is similar to structure No.3 from Table 1.

The energy E_p of the polynucleotide itself in optimal complexes of poly(dA)·poly(dT) with the spine of hydration is only some tenths of kcal/mol higher than the energy of optimal structures of poly(dA)·poly(dT) at fixed $\tau=36.0^{\circ}$. The energy E_w is almost the same as for structure No.2 in Table 1. Individual contributions to E_{pw} and the interatomic distances between the water molecules and the polynucleotide are almost the same as for complex No.2 in Table 1.

The E_{pw} contribution to the total energy of low-energy complexes of poly(dA)·poly(dT) with the spine of hydration at fixed $\tau=36^{\circ}$ and $TW\sim10^{\circ}$ is almost 1 kcal/mol smaller by the absolute values than for the complexes with $TW\sim3^{\circ}$ (the minor groove width for the first complex is ~9.4 Å and for the second one ~9.1 Å). The complex with $TW\sim10^{\circ}$ and the same E_{pw} values as for the low-energy complex with TW~3 will not be energetically preferable owing to an increase of E_p.

The spine of hydration in the minor groove of poly(dA)·poly(dT) not only changes the polynucleotide conformation, but also contributes considerably to stabilization of the obtained structure. Thus, if the optimal structure of poly(dA)·poly(dT) at fixed $\tau=36.0^{\circ}$ is altered so that TL=TW=0, thus widening the groove to ~12 Å and increasing the N3(A)...O2(T) distance to ~ 4 Å, the

energy would increase by no more than 1.0-1.5 kcal/mol (less than 1 kcal/mol if the CH_3 group of thymine is replaced by one united atom). If the same alteration is done for the low-energy complex of poly(dA)·poly(dT) with the spine of hydration at fixed $\tau=36.0^{\circ}$ (TL=TW=0, the minor groove widening to ~ 12 Å and the N3(A)...O2(T) distance increasing to ~ 4 Å), the energy will rise by 4 kcal/mol. 80% of this value is due to a worse interaction of the spine with the polynucleotide (E_{pw}) and 20% to an increased energy of the nucleotide (E_p).

All the considered structures of the poly(dA)·poly(dT) complex with the spine of hydration are characterized by a negative TL and a narrowed minor groove (~9 Å). We shall analyze the reasons of this by examining some conformations of the poly(dA)·poly(dT) complex with the spine of hydration. E_w in these complexes is practically the same. One of the structures is the low-energy conformation obtained at fixed $\tau=36.0^{\circ}$, TL=TW=0, the minor groove width is ~12 Å and the N3(A)...O2(T) distance is ~4 Å. E_{pw} in this conformation is ~3 kcal/mol higher than in the optimal complex at $\tau=36.0$, of which ~1.5 kcal/mol is the increase of the interaction energy of the water molecule of the first hydration shell with the polynucleotide and the other 1.5 kcal/mol is caused by an increase of the interaction energy of the water molecule of the second hydration shell with the sugar-phosphate backbone (these water molecules are represented in bold lines in Fig. 1B). The former value consists of the increase by ~2 kcal/mol of the interaction energy of the water molecule of the first hydration shell with the 2nd and the 8th sugars and the decrease by ~0.5 kcal/mol of the interaction energy of this water molecule with the 9th thymine. (The energy of interaction of the 8th sugar with the water molecule of the first hydration shell increases by ~1.5 kcal/mol, of which ~1.1 kcal/mol is due to a worse interaction with O1' of the eighth sugar). In its turn, from 1.5 kcal/mol of the increase of the interaction energy of the water molecule of the second hydration shell with the sugar-phosphate backbone, 0.6 kcal/mol is due to a worse van-der-Waals interaction of this water molecule with the 3rd and the 8th sugars and ~0.9 kcal/mol is due to a worse interaction with the 2nd and the 7th sugar-phosphates.

It should be noted that at TL=TW=0 and $\tau=36.0^{\circ}$ in the complex, the energy of interaction and the hydrogen bonds of the first hydration shell with the adjacent adenine and thymine bases are not worse (for thymine even better) than in the energetically optimal complex. The spine of hydration results not only in a negative TL and the minor groove compression, but also in additional stabilization of the positive propeller TW as compared with poly(dA)·poly(dT). Thus, E_{pw} increases by no more than 1.5 kcal/mol in the low-energy complex with

TL=0 and $\tau=36.0^{\circ}$ as compared with E_{pw} of the energetically optimal complex of poly(dA)·poly(dT) with the spine of hydration at $\tau=36.0^{\circ}$. Hence it follows that from 3 kcal/mol of the E_{pw} increase in the complex with TL=TW=0 as compared with E_{pw} of the optimal complex at $\tau=36.0^{\circ}$ (see above) no less than 50% is due to a decrease of the propeller TW to zero.

Thus, the decrease of the total energy of the complex at transition of the polynucleotide conformation into the anomalous one is mainly due to a more favourable interaction of the spine of hydration with the sugar-phosphate backbone of both chains of poly(dA)·poly(dT) in this anomalous conformation. It is characterized, in particular, by a narrow minor groove (~9 Å), a negative TL, a small N3(A)...O2(T) distance (~3.3 Å) and a positive TW. The spine not only changes the poly(dA)·poly(dT) structure, but also considerably stabilizes the resulting anomalous structure.

3. Low-Energy Conformations Optimized by All the Variables. Comparison With the Solution Data

As follows from ref. (10) and from our data, the calculated equilibrium winding angle τ for poly(dA)·poly(dT) is equal to 36° at completely neutralized phosphates. If the phosphates are charged, the helix unwinds and at the phosphate charge of ~-0.6 the angle τ is ~33° (see Fig. 2A). These results are in agreement with the experimental data showing that a decrease of ionic strength is accompanied by an unwinding of the double helix (22-25). On the other hand, our calculations of the low-energy complexes of poly(dA)·poly(dT) with the spine of hydration indicate that the spine increases the winding angle of the double helix. If the phosphate charge is ~-0.6, the low-energy poly(dA)·poly(dT) structure is characterized by τ equal to ~33°, the minor groove width of ~12 Å and the N3(A)...O2(T) distance of ~4 Å (see Fig. 2A) while the structure with the same phosphate charge, but with the spine of hydration, has the minor groove narrowed to 9.5 Å, the N3(A)...O2(T) distance decreased to 3.3 Å and the winding angle τ increased to 36°. The parameters of the low-energy complex of poly(dA)·poly(dT) with the spine of hydration are given in Table 1 (No.3). The results were obtained with the dielectric constant $\varepsilon=4$; at a smaller ε and the same phosphate charge the winding angle τ will be more than 36°.

The main regularities found for fixed values of d and /or τ (subsections 1 and 2) are also valid when all the independent parameters are allowed to vary. The individual energy contributions to E_{pw} and the interatomic water-polynucleotide distances are similar to those found earlier.

Assuming that the qualitative conclusions obtained for the polynucleotide

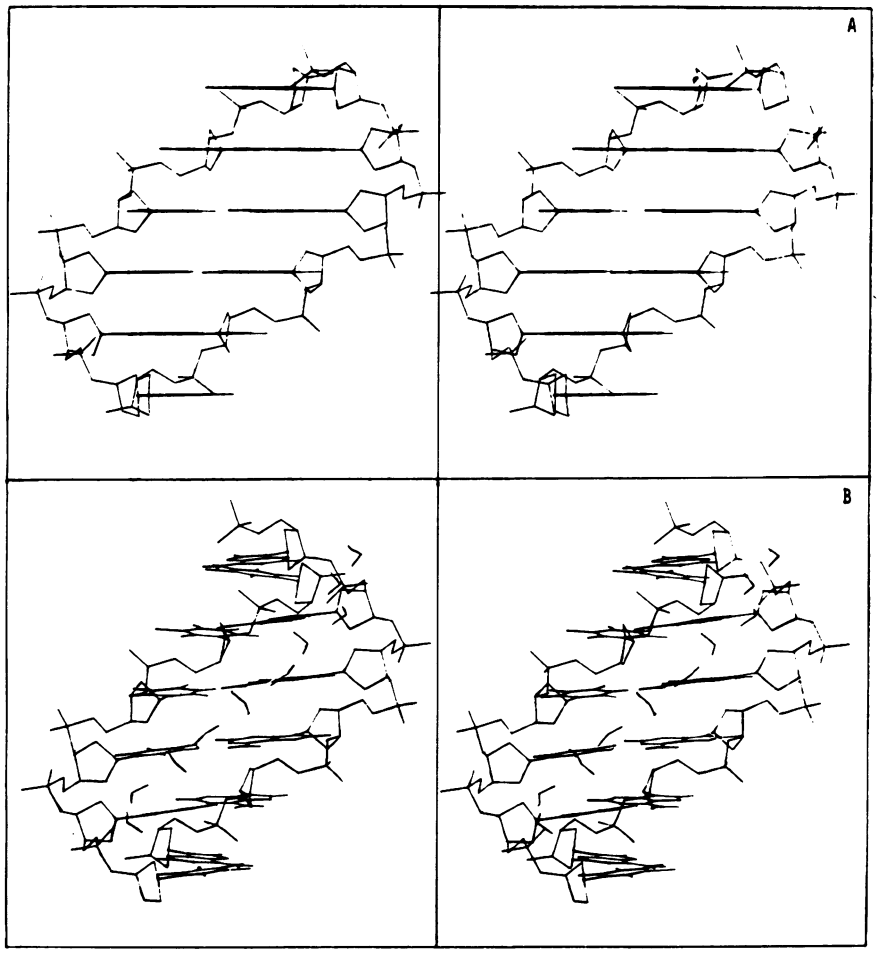

Figure 2. Stereo views of low-energy conformations. A, low-energy structure of poly(dA).poly(dT) without the spine and with the phosphate charges of ~-0.6. B, low-energy structure of the poly(dA)·poly(dT) complex with the spine of hydration in the minor groove and with the same phosphate charges of ~-0.6 (No.3 from Table 1). The conformation in (A) as compared to that in (B) is characterized, in particular, by a wider minor groove (~12 Å), a larger N3(A)...O2(T) distance (~4 Å) and a smaller winding angle (τ~33°).

should be also valid for oligo (dA)-tracts, we shall interpret some experimental data on DNA solutions.

(1) Among the sequences with the known winding angle τ only for poly(dA)·poly(dT) τ is equal to 36° while for other sequences it is equal to 33.5-34.5° (26-28). We have noted (3) that among all these sequences only poly(dA)·

•poly(dT) can have a spine of hydration (the data on destabilization of the spine on the TpA step and stability on the ApT step are also given below). The calculations presented here suggest that the increase of τ to 36° in this polynucleotide seems to be due to the existence of the spine of hydration in the minor groove.

(2) The results reported in ref. (29,30) indicate that on fragments of the DNA duplex consisting of A/T runs without the TpA step, DNAase I cuts the sugar-phosphate backbone much more poorly than on fragments with other runs. Only on these runs the probability of cuts considerably increases upon heating. The authors (29,30) propose that this is because only on A/T runs (which do not contain the TpA step) the narrow minor groove becomes wider upon heating. We share this view and propose the following explanation: at room temperature the minor groove is compressed to ~9 Å owing to the existence of a spine of hydration on A/T runs (without the TpA step); upon heating the spine is disrupted and, according to our calculations, the minor groove becomes larger (see Fig. 2). Moreover, it follows from our data that this should be accompanied by unwinding of these runs from 36° to $33\text{-}34^{\circ}$.

(3) DNA bending has been found for sequences containing A/T runs (31-34). The bending is thought to occur at the junction between a specific structure formed by the adenine tract and the usual B-form characteristic of other DNA regions (31-33). As we have shown, anomaly of the adenine tract is largely due to the existence of the minor groove spine of hydration which can be disrupted upon heating, the unusual structure of the adenine tract being converted to the usual one. As a result, bending should decrease upon heating. This is exactly what the experiment shows (31-34).

In conclusion we would like to note that to remain in the anomalous conformation, the DNA fragment of A/T runs without the TpA step should be at least 4 b.p. long. This follows from the studies on DNA cuts by DNAase I (29), determination of the winding angle in supercoiled circular DNAs (27) as well as from the data on DNA bending (32). The DNA fragment should also be at least 4 b.p. long for the groove to be formed. This seems to be the minimal length on which the spine can be formed.

4. Energy Estimates Suggesting that Dickerson's Spine of Hydration Is Unlikely to Exist in the Minor Groove of Poly(dA-dT)•Poly(dA-dT)

In our earlier study (3) on the spine simulation by reducing some distances in the optimal structures to the values characteristic of the middle part of the CGCGAATTCGCG structure (1,2) we concluded that the spine should be disrupted on the TpA step when the propeller (2 x TW) in the base pairs is $\gtrsim 10^{\circ}$.

Here we studied the possibility of the existence of the steps TpA and ApT in conformations characteristic of the poly(dA)·poly(dT) complex with the spine of hydration in the case of a large (2 x TW ~ 20°) and a small (2 x TW ~ 6°) propeller. In our estimates we assumed that the adenylic and thymidylic sugar moieties have identical conformations in the C2'-endo region; this seems to be quite reasonable as shown by the data of Raman spectroscopy of poly d(A-T)·poly d(A-T) (12,35). The energy was optimized in the space of the same independent variables as for the regular homopolynucleotide (see METHODS).

The low-energy conformation of the double-stranded TpA with the fixed values of TW~3° and TL~-12° (the minor groove being ~9.4 Å wide) which is characteristic of the poly(dA)·poly(dT) structure with the spine of hydration at a small TW is about 4 kcal/mol less favourable than the conformation with a variable TL and a fixed TW. In the latter conformation TL tends to positive values, and the minor groove becomes wider. The TpA step in a conformation characteristic of the poly(dA)·poly(dT) structure with the spine of hydration at a small TW is unfavourable mainly because of a worse stacking of bases. As for the ApT step in this conformation, it is unfavourable by only some tenths of kcal/mol of nucleotide pairs, which should be quite acceptable for the existence of the spine of hydration (as we have shown, the increase of the polynucleotide energy E_p in the poly(dA)·poly(dT) complex with the spine of hydration is no more than 1 kcal/mol). The situation is about the same in conformations with TW~10°.

Thus, whatever the TW value, the existence of the ApT step is quite acceptable in conformations characteristic of poly(dA)·poly(dT) structures with the spine of hydration whereas the TpA step is unfavourable. The TpA step leads, in particular, to widening of the minor groove and, consequently, to destabilization of the spine (see subsections 1 and 2). So, in the poly d(A-T)·poly d(A-T) minor groove there seems to be no spine of hydration similar to that found by Dickerson and co-workers (1,2) in CGCGAATTCGCG. The spine can be formed on A/T runs which do not contain the TpA step.

The results described above permit a qualitative interpretation of the experimental data on poly(dA)·poly(dT) and poly d(A-T)·poly d(A-T) binding with ligands and intercalators which interact with the DNA in the minor groove (36-38). The experiments show that upon binding of the drug with the homopolymer the entropy change is considerably larger and the enthalpy change considerably smaller than upon binding with poly d(A-T)·poly d(A-T). The difference in the free energy change upon binding with the homo- and heteropolymer is only 1-2 kcal/mol (36-38). Our work permits a qualitative interpretation

of these results. Poly(dA)·poly(dT) has a regular spine of hydration in the minor groove, whereas poly d(A-T)·poly d(A-T) has none. Therefore binding of the drug with the homopolymer requires the removal of a more ordered hydration shell than in the case of binding with poly d(A-T)·poly d(A-T). As a result, the entropy changes much more upon binding of the ligand with poly(dA)·poly(dT) than with poly d(A-T)·poly d(A-T). On the other hand, the enthalpy change is smaller upon binding with poly(dA)·poly(dT) than with poly d(A-T)·poly d(A-T) because a part of the energy liberated upon binding of the polynucleotide with the ligand is spent to disrupt the bonds between the DNA and the spine of hydration and within the spine.

We would also like to note that our results suggest a correlation between the known (38) order of sequence-preferred binding of netropsin to the tetrameric duplex region: AAAA≈AATT>A-T-A-T, on the one hand, and the possibility of the spine existence, the corresponding minor groove width in these sequences and the energetical disadvantage of the minor groove compression (owing to the TpA step), on the other hand.

In conclusion we would like to note that the obtained results should not change qualitatively when A is replaced by I (inosine) and T by U.

CONCLUSIONS

Modelling of the poly(dA)·poly(dT) interaction with the two hydration shells in the minor groove shows that the existence of the spine resembling Dickerson's spine of hydration is energetically favourable. The spine affects the polynucleotide conformation so that it becomes strikingly similar to the conformation of poly(dA)·poly(dT) in fibers, that of the central part of the dodecamer CGCGAATTCGCG in crystals and acquires features of the A-tracts in DNA in solution. This conformation is, in particular, characterized by a narrowed minor groove (~9 Å), a small distance between N3(A) of one pair and O2(T) of the adjacent one (~3.3 Å), a negative tilt and a positive TW.

The major contribution to the formation and stabilization of such a conformation is made by the interaction of the first and the second hydration shells of the spine with the sugar-phosphate backbone of both polynucleotide chains. This interaction becomes weaker upon widening of the minor groove and a decrease of TL and TW (by the absolute value). The spine not only changes the poly(dA)·poly(dT) structure, but also considerably stabilizes the resulting anomalous structure.

Estimates show that the TpA step destabilizes such a spine of hydration and, therefore, it seems that the spine is absent in poly d(A-T)·poly d(A-T).

Several experimental facts concerning DNA fibers and solutions can be explained by the existence of the spine of hydration and its influence on the structure.

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