

## DNA-bound lipids: computer modeling of DNA interaction with stearic acid and unsaturated fatty acids

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It was shown for the first time by computer experiments that fatty acids are strongly bound to DNA. This is consistent with the presence of free fatty acids in the specimens of DNA-bound lipids isolated from various cells. Binding of all fatty acids to the DNA minor groove is stronger than to the major groove, which is correlated with the presence of two pools of free fatty acids isolated from DNA specimens by biochemical methods. Since DNA polymerase is also bound to the DNA minor groove, fatty acids can play an important role in the regulation mechanism of DNA replication and signal transmission. The energy of interaction of fatty acids with DNA depends on both the number of double bonds and the geometric configuration of the fatty acid and the nucleotide composition of DNA. Dependence on the bond energy in the DNA–fatty acid complex on the nucleotide composition attests to the possibility of site-specific binding of lipids to DNA. On passing from a saturated fatty acid to unsaturated acids containing one, two, or three double *trans*-bonds, the bond energy of DNA with the fatty acid gradually decreases. The presence of one or three double *cis*-bonds results in weakening of the strength of the DNA–fatty acid complexes compared to those with the saturated acid. The strongest binding between DNA and fatty acid was found for the unsaturated acid with two double *cis*-bonds (linoleic). This can be explained by the fact that the bent (boomerang) shape of the molecule of this acid follows the curve of the DNA helix. The pattern of variation of the energy of DNA complexes with stearic, linoleic, oleic, and linolenic acids correlates with experimental data on the melting points of these complexes: the more stable the DNA–fatty acid complex, the lower the melting point of DNA.

**Key words:** oligodeoxyribonucleotides, DNA-bound lipids, fatty acids, linoleic acid, molecular mechanics, structure, stability of complexes.

Lipids are present in the nuclear membrane; they are important integral component of chromosomes, chromatin, and the nuclear matrix; they actively participate in signal transmission, regulation of DNA replication and transcription and apoptosis induction.<sup>1–8</sup> The presence of lipids in DNA specimens has been demonstrated by various physicochemical, physical, and biochemical methods, in particular, sedimentation in CsCl<sup>9</sup> and alkaline saccharose<sup>10</sup> gradients, small-angle X-ray diffraction,<sup>11,12</sup> microcalorimetry,<sup>13</sup> circular dichroism,<sup>14</sup> and ESR,<sup>15</sup> and in experiments with lipolytic and proteolytic enzymes.<sup>13</sup> Using the mild phenolic method,<sup>16</sup> we isolated natu-

ral supramolecular complexes, *i.e.*, DNA-bound lipids, from eukaryote and procaryote cells (thymus, rat liver, regenerating rat liver in the S-phase and the G<sub>2</sub>-phase, loach sperm, pigeon erythrocytes, Ehrlich ascitic carcinoma, Seidel ascitic hepatoma, sarcoma 37, *E. coli* B, and phage T2).<sup>13,17–21</sup> Experiments with hens showed that these DNA complexes possess high transforming activity.<sup>13</sup> They contain 94–97% high-molecular-weight DNA ( $3 \cdot 10^8$ – $3 \cdot 10^9$  Da), 1–3% lipids, and 1–3% acidic nonhistone proteins (NHP). Previously,<sup>13</sup> by special experiments with the addition of labeled and unlabeled lipids to a cell homogenate prior to DNA isolation, we have

demonstrated that DNA-bound lipids are natural DNA components rather than artifacts originating during the isolation procedure. The presence of DNA-bound lipids *in vivo* has also been demonstrated using isotope-labeled lipids or their precursors, *i.e.*, [ $^{14}\text{C}$ ]acetate,<sup>13</sup> [ $^{14}\text{C}$ ]cholesterol, and [ $^3\text{H}$ ]glycerol.<sup>22</sup> Other researchers utilized our method<sup>16</sup> to isolate DNA-bound lipids from *Salmonella thyphimurium*<sup>23</sup> and two strains of *Shigella sonnei*<sup>24</sup> and also used a different method to isolate such complexes from Ehrlich ascitic carcinoma.<sup>15,22</sup>

The fact that the DNA complex contains similar amounts of lipids and NHP (1–3%) does not rule out their functioning as lipoproteins. We showed<sup>13</sup> that, unlike proteolytic enzymes (trypsin, chymotrypsin, pronase E and P), only lipolytic enzymes (phospholipases A<sub>2</sub>, C, D and lipase) cause pronounced degradation of high-molecular-weight DNA complexes (molecular mass of up to  $40 \cdot 10^6$  Da). Note also that a highly rigorous method of isolation of DNA (*p*-aminosalicylic acid, phenol, ethanol, RNase, pronase) from the guinea-pig thymus resulted in a low-molecular-weight DNA specimen ( $5 \cdot 10^6$  Da), which contained only strongly bound phospholipids (PL), because the sedimentation profile of DNA in CsCl changed only after pre-treatment with phospholipase A rather than with pronase.<sup>9</sup>

DNA-bound lipids have a specific composition differing from the lipid composition of the nuclear membrane, chromatin, nuclear matrix, mitochondria, or microsomes. They are characterized by (1) the predominance of neutral lipids (NL) over PL; (2) enrichment of NL with cholesterol esters (CE), free fatty acids (FFA), and diglycerides (DG) and depletion in free cholesterol (FC) (35, 30, 25, and 10% of the total NL, respectively); (3) enrichment of PL with cardiolipin (CL) and depletion in phosphatidyl ethanolamine (P), phosphatidyl choline (PC), phosphatidyl serine (PS), and phosphatidyl inositol (PI) and the presence of only traces of sphingomyelin<sup>5</sup> (50, 25, 12, 10, and 3% of the total PL, respectively); (4) the presence of almost all chromatin CL in DNA,<sup>5,13</sup> which is in line with the presence of a common "inter-phosphate" motif between the CL and DNA.<sup>25</sup>

The DNA-bound lipids consist of two pools,<sup>13</sup> differing in the degree of binding to DNA and in the composition, in particular, a pool of weakly bound lipids, which is extracted from DNA into 35% ethanol (24 h at 37 °C without stirring) and a pool of strongly bound lipids, which can be extracted with a  $\text{CHCl}_3$ –MeOH mixture (2 : 1) only after pre-incubation of the DNA residue (after extraction with 35% ethanol) with DNase I (2 h, 37 °C). Each pool consists of four NL fractions (FFA, CE, FC, and DG) and five PL fractions (CL, P, PC, PS, and PI). It is significant that two independent methods used to isolate DNA from the Ehrlich ascitic carcinoma<sup>13,22</sup> gave similar contents of strongly bound lipids (8.6 and

9.1  $\mu\text{g}$  per mg of DNA) and similar percentages of FFA (27.0 and 23.7% of the total NL).

Fatty acids represent a new class of signal molecules, which participate in signal transmission,<sup>13</sup> regulation of gene expression,<sup>26</sup> and the activity of ion channels.<sup>27</sup> In recent publications,<sup>28–31</sup> the effects of the *cis*- and *trans*-isomers of fatty acids on the biochemical processes in the cell were shown to be different depending on the number of double bonds or stereoisomers of retinoic acid. For instance, only *cis*-unsaturated fatty acids (arachidonic acid), unlike *trans*-isomers or saturated acids, activate the adenosine receptor A1 binding sites of the rat brain.<sup>28</sup> It was found that *cis*-isomers of arachidonic (C20:4)\* and linoleic acids (C18:2) increase the intracellular concentration of the  $\text{Ca}^{2+}$  ion more efficiently than *cis*-oleic (C18:1) and myristic acids (C14:0).<sup>27</sup> It was shown in a review<sup>29</sup> that sphingolipids with a 4-*trans*-double bond and those without a double bond exert different effects on cell growth, differentiation, and apoptosis. The mouse liver and kidney cells were found to contain natural stereoisomers of retinoic acid (9-*cis*-, 11-*cis*-, and 13-*cis*-retinoic acids), which are produced from fully *trans*-retinoic acid by means of membrane-bound isomerase;<sup>30,31</sup> this provided the conclusion that isomerization of retinoids is a key mechanism of signal transmission.

The fact of binding of lipids to DNA in the absence of proteins is also indicated by the results we obtained by the titration (at a lipid : DNA base pair molar ratio ranging from 1 : 20 to 1 : 1) of synthetic double-stranded polynucleotides with a homo- or alternating sequence (polyA·polyT, poly(AT), polyG·polyC, poly(GC)) by oleic acid using a number of physicochemical methods, in particular, spectrophotometry, CD spectroscopy, plasmon resonance (biosensor), atomic force microscopy (nanoscope), and dialysis of DNA–lipid complexes.<sup>32</sup> These methods revealed strong interaction between the fatty acid and DNA even for (1 : 10)–(1 : 5) ratios. It was shown by CD spectroscopy that binding of poly(AT) and polyA·polyT occurs according to a recognition pattern: the CD spectrum changes when the lipid : DNA base pair ratio is equal to 1 : 10 (the amplitude of the absorption band at 260 nm decreases) and subsequently, the CD spectrum does not change up to 1 : 1 ratio. However, in the case of the poly(GC) and polyG·polyC polynucleotides, oleic acid interacts with DNA according to the saturation pattern: the CD spectrum changes during the titration of DNA with oleic acid up to 1 : 1 ratio; the band at 260 nm disappears and a new band appears at 280 nm. This fact obviously points to a strong interaction of the ligand (oleic acid) with poly(GC) DNA in the absence of proteins.

\* This designation shows the number of C atoms in the fatty acid and the number of double bonds in it.

Despite the abundance of biochemical data concerning the composition and biological significance of chromatin- or DNA-bound lipids, there is little adequate information concerning the structures and strengths of such complexes. In 1994, the existence of DNA–phospholipid recognition was hypothesized, *i.e.*, the interaction of nucleic acids and phospholipids was suggested to be specific, depending on the sequence of nucleic acid bases and the nature of the polar group of phospholipid or the metal ion.<sup>33</sup> In 1995, we started computer experiments aimed at estimating the energies of interaction of double-stranded oligonucleotides (all 64 triplets of the pApApNpNpNpApA genetic code, where N is nucleoside, A is adenosine, p is phosphate) with phospholipids (stability of complexes) in the absence or in the presence of metal(II) ions, depending on the nucleotide sequence in the oligomer or the nature of the phospholipid.<sup>33–35</sup> In particular, we found that the formation energy (stability) of phosphatidyl choline complexes with DNA oligomers tends to increase for GC-rich triplets containing metal ions ( $\text{Mg}^{2+}$ ) compared to similar complexes devoid of metal ions.<sup>36,37</sup> The formation energies of complexes differ by 1.7–2.6 kcal mol<sup>−1</sup> in the former case, which can be regarded as recognition at the molecular level. The

formation energies of the complexes with sphingomyelin are 4–16 kcal mol<sup>−1</sup> higher than the corresponding values for the phosphatidyl choline complexes,<sup>37</sup> which is correlated with the enhanced content of sphingomyelin in the nuclear membrane to which chromatin is attached.

Then we studied the structure and the formation energies of the DNA–elaidic (*trans*-oleic) acid complexes by molecular mechanics.<sup>38</sup> It was found that this acid is attached more firmly to the DNA minor groove than to the major groove, which may be the reason for the presence of two pools of fatty acids extracted from DNA specimens by biochemical methods.<sup>13</sup> In addition, the computer experiments showed that the energy of this interaction for elaidic acid depends on the nucleotide composition of DNA. The fact that DNA binding to this acid is accompanied by weakening of hydrogen bonds in the complex may also be indicative of the participation of fatty acids in the regulation of transcription.

The purpose of this study is to perform the computer simulation of this interaction for a wider range of fatty acids and to calculate the bond energies for the DNA complexes with lipids by molecular mechanics. For computer experiments, we took saturated and unsaturated fatty acids containing 18 carbon atoms (Fig. 1): stearic (1)

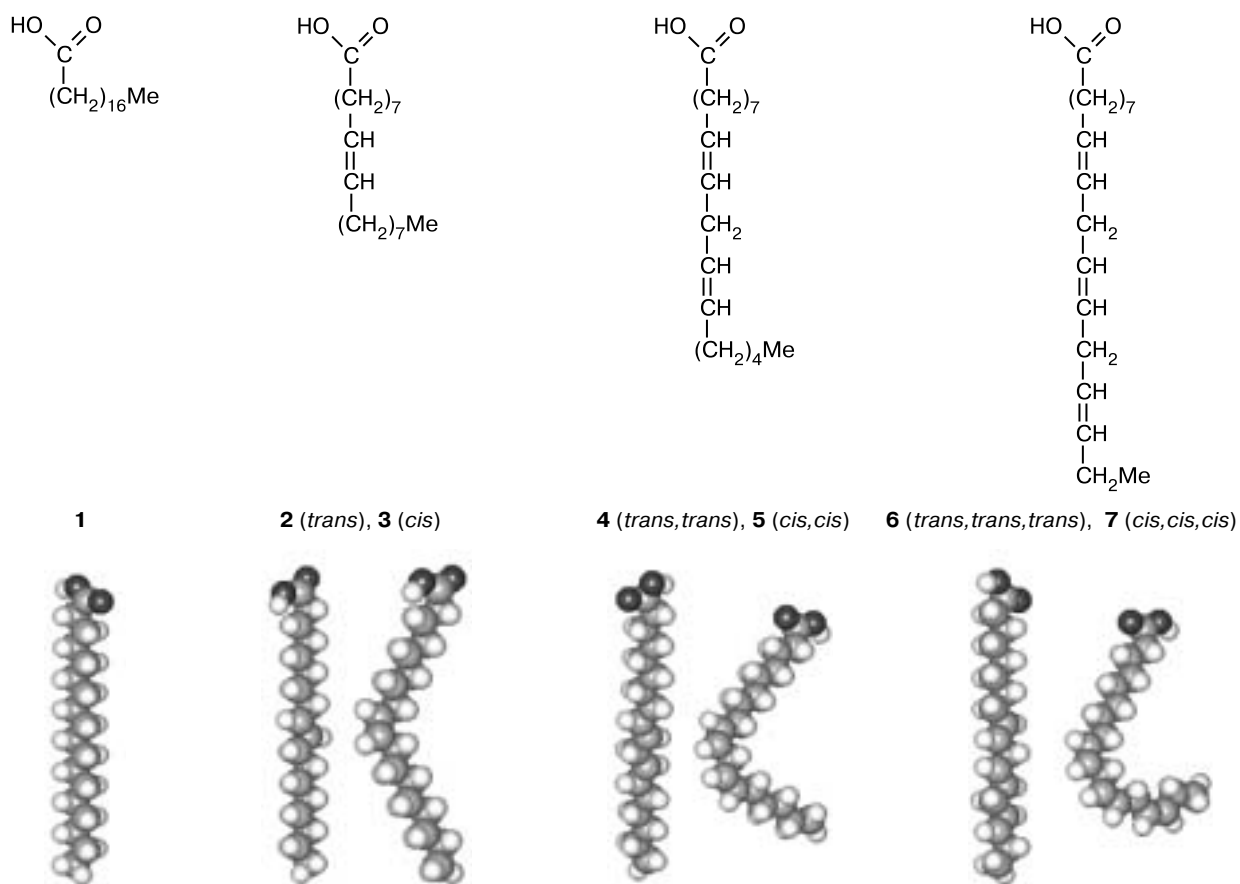


Fig. 1. Fatty acids 1–7 and the corresponding staggered configurations optimized by MM+ calculations.

(C18:0), oleic (**3**) (one *cis*-double bond, C18:1), linoleic (**5**) (two *cis*-double bonds, C18:2), and linolenic (**7**) (three *cis*-double bonds, C18:3) acids and the corresponding *trans*-isomers **2**, **4**, **6**, respectively.

### Calculation Procedure

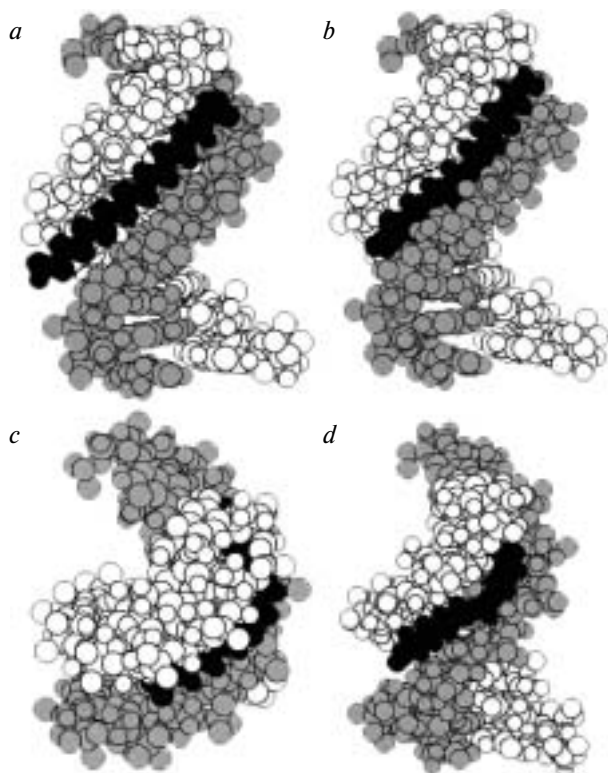
As previously,<sup>36–38</sup> all molecular mechanics calculations were carried out using the HYPERCHEM program package, MM+ and AMBER force fields.<sup>39</sup> The energies of bonding of fatty acids to DNA ( $E_{\text{bond}}$ ) were calculated by the MM+ method, as its system of parameters seems to be more versatile and applicable to a wider range of compounds. The lengths of hydrogen bonds in the (AT)<sub>10</sub>—**2** complexes were determined using the AMBER force field, whose parameters were adapted to calculations of the structures of nucleic acids. The B-form was chosen for DNA and sodium ions were added to ensure the electric neutrality of the system. The  $E_{\text{bond}}$  values were determined as the difference between the sum of the total energies ( $E_{\text{tot}}$ ) of isolated molecules and DNA—fatty acid complexes. Taking into account the geometry of the DNA B-form in terms of the double strand model, the ligands were arranged in the minor or major groove and the energies of bonding of the ligands upon complexation with the minor and major grooves of DNA, respectively, were estimated. In all cases, the fully staggered conformation

was chosen for the saturated fragments of the hydrocarbon chains of the ligands (see Fig. 1). The equilibrium geometries of the (AT)<sub>10</sub> complexes with fatty acids **1**, **3**, **5**, and **7** optimized using the results of molecular mechanics calculations are presented in Fig. 2.

### Results and Discussion

Table 1 presents the averaged data on the number of FFA molecules attached to DNA per 1000 base pairs, derived from experimental data on the content of FFA (μg) per 10 mg of DNA.<sup>13</sup> In all objects, both eukaryotes and procaryotes (except for the S-phase of the regenerating rat liver), the density of FFA is higher in the weakly bound NL pool than in the strongly bound pool. This is especially pronounced for metabolically inactive genomes (by a factor of 16 for loach sperm and by a factor of 3.3 for pigeon erythrocytes). The greatest number of FFA molecules (29 per 1000 base pairs) was detected in the rat liver in the G<sub>2</sub>-phase. The DNA of malignant cells is enriched in FFA compared to the rat liver DNA in the stage of active synthesis. Note also that the DNA of the T2 phage contains only the strongly bound FFA pool.

In our previous publication,<sup>38</sup> which presents a detailed study of the complexation of DNA with elaidic acid (**2**), it was shown that calculation of the interaction of DNA with fatty acids containing not more than 20 carbon atoms in a hydrophobic shell can be restricted to considering a DNA oligomer composed of 10 nucleotide pairs. Therefore, in this work, we chose oligodeoxyribonucleotides with an alternative sequence of pyrimi-



**Fig. 2.** Equilibrium geometries of the complexes formed by oligo-(AT)<sub>10</sub> with the stearic (*a*), oleic (*b*), linoleic (*c*), and linolenic (*d*) acids in the minor groove optimized using the results of molecular mechanics calculations (the two DNA strands are shown in gray and white and the fatty acid is shown in black).

**Table 1.** Density of free fatty acids (FFA) in the genome DNA (the number of DNA-bound FFA molecules per 1000 base pairs)\*

Object	FFA density	
	I	II
Rat thymus	5.0	3.1
Rat liver		
G <sub>0</sub> -phase	5.3	4.5
S-phase	3.6	5.0
G <sub>2</sub> -phase	29.0	15.0
Loach sperm	10.0	0.56
Pigeon erythrocytes	3.3	0.96
Seidel ascitic hepatoma	9.1	6.3
Sarcoma 37	7.7	3.8
Ehrlich ascitic carcinoma	5.9	4.0
<i>E. coli</i> B	4.8	2.3
Phage T2	0	1.3

*Note.* The average lipid density values were calculated using the following molecular masses: for FFA, 260 Da; for DNA, 10<sup>8</sup> Da; I is the weakly bound lipid pool, II is the strongly bound lipid pool.

\* Error ±5%.

**Table 2.** Total energies ( $E_{\text{tot}}$ ) for DNA oligomers and fatty acid molecules

Compound	$E_{\text{tot}}$ /kcal mol <sup>-1</sup>
(AT) <sub>10</sub>	662.5
(GC) <sub>10</sub>	363.2
Stearic acid ( <b>1</b> )	30.0
<i>trans</i> -Oleic acid ( <b>2</b> )	10.6
<i>cis</i> -Oleic acid ( <b>3</b> )	10.9
<i>trans,trans</i> -Linoleic acid ( <b>4</b> )	9.3
<i>cis,cis</i> -Linoleic acid ( <b>5</b> )	15.6
<i>trans,trans,trans</i> -Linolenic acid ( <b>6</b> )	8.9
<i>cis,cis,cis</i> -Linolenic acid ( <b>7</b> )	12.2

**Table 3.** Total energies ( $E_{\text{tot}}$ ) and bond energies ( $E_{\text{bond}}$ ) of DNA complexes with fatty acids located in either the minor or the major groove of DNA (Min and Maj, respectively)

Composition of the complex	$E_{\text{tot}}$		$E_{\text{bond}}$	
	kcal mol <sup>-1</sup>			
	Min	Maj	Min	Maj
(AT) <sub>10</sub> – <b>1</b> (C18:0)	651.0	663.3	41.5	29.2
(GC) <sub>10</sub> – <b>1</b> (C18:0)	375.7	376.5	17.6	16.8
(AT) <sub>10</sub> – <b>2</b> ( <i>trans</i> -C18:1)	643.9	656.3	29.2	16.8
(GC) <sub>10</sub> – <b>2</b> ( <i>trans</i> -C18:1)	350.6	361.3	23.2	12.5
(AT) <sub>10</sub> – <b>3</b> ( <i>cis</i> -C18:1)	651.6	662.6	21.7	10.9
(GC) <sub>10</sub> – <b>3</b> ( <i>cis</i> -C18:1)	353.2	362.7	19.9	10.4
(AT) <sub>10</sub> – <b>4</b> ( <i>trans</i> -C18:2)	646.4	660.5	25.5	11.3
(GC) <sub>10</sub> – <b>4</b> ( <i>trans</i> -C18:2)	348.8	363.0	23.7	9.5
(AT) <sub>10</sub> – <b>5</b> ( <i>cis</i> -C18:2)	628.7	645.4	48.2	31.5
(GC) <sub>10</sub> – <b>5</b> ( <i>cis</i> -C18:2)	338.2	354.9	46.9	30.2
(AT) <sub>10</sub> – <b>6</b> ( <i>trans</i> -C18:3)	647.8	660.6	23.6	10.8
(GC) <sub>10</sub> – <b>6</b> ( <i>trans</i> -C18:3)	340.0	356.8	32.2	6.4
(AT) <sub>10</sub> – <b>7</b> ( <i>cis</i> -C18:3)	662.5	668.7	12.6	6.4
(GC) <sub>10</sub> – <b>7</b> ( <i>cis</i> -C18:3)	353.1	362.4	22.3	12.9

dine ((AT)<sub>10</sub>) or purine ((GC)<sub>10</sub>) nucleotides as DNA oligomers. The total energies of these oligonucleotides and FFA are summarized in Table 2, and the bonding energies for the complexes formed by fatty acids with DNA are listed in Table 3.

We will start the discussion with *trans*-oleic acid (**2**). According to MM+ calculations,  $E_{\text{tot}}(\mathbf{2}) = 10.6$  kcal mol<sup>-1</sup>. If the (AT)<sub>10</sub> DNA fragment and acid **2** do not interact, the total energy of the system would be 662.5 + 10.6 = 673.1 kcal mol<sup>-1</sup>, *i.e.*, it is found as the sum of the energies of isolated molecules. Placing acid **2** into the minor groove of (AT)<sub>10</sub> entails a rather great energy benefit, equal to 673.1 – 643.9 = 29.2 kcal mol<sup>-1</sup>. For comparison, the energy of the CO...HN hydrogen bond is ≤4 kcal mol<sup>-1</sup>.

One can see that the energy benefit caused by placing acid **2** into the DNA major groove is almost twice smaller

( $E_{\text{bond}} = 16.8$  kcal mol<sup>-1</sup>) than that in the case of its placing in the minor groove. Thus, acid **2** is attached rather strongly to (AT)<sub>10</sub> and should be located, first of all, in the minor groove.

The data collected in Table 3 attest to the predominant arrangement of all the studied fatty acids in the minor groove of DNA rather than in the major groove, which indicates that the gutter size in the DNA minor groove is better suited for the size of nonbranched hydrocarbon residues of these acids. The major groove is too spacious for them, resulting in weaker interaction between atoms of the fatty acid and the groove. It follows from the data of Table 3 that the  $E_{\text{bond}}$  value for the (GC)<sub>10</sub> complex with acid **2** is lower by 6 kcal mol<sup>-1</sup> in the minor groove and lower by 4.3 kcal mol<sup>-1</sup> in the major groove than those for the (AT)<sub>10</sub> complex with acid **2**, *i.e.*, the energy of interaction of acid **2** with the DNA molecule depends on the nucleotide composition of DNA.

Now we will consider stearic acid (**1**) containing no double bonds. It can be seen from Table 3 that placement of this acid into the minor groove of (AT)<sub>10</sub> produces an energy benefit equal to 41.5 kcal mol<sup>-1</sup>, which is 12.3 kcal mol<sup>-1</sup> greater than the corresponding bond energy in the complex with acid **2**. Binding to the large DNA groove is also stronger for stearic acid (**1**) than for elaidic acid **2**: when acid **1** is placed into the major groove of the (AT)<sub>10</sub> DNA,  $E_{\text{bond}} = 29.2$  kcal mol<sup>-1</sup>, which is 12.4 kcal mol<sup>-1</sup> greater than that for acid **2**. The stronger interaction of stearic acid with DNA can be explained by the fact that the hydrocarbon tail of the saturated acid is conformationally more flexible (does not contain rigid segments, that is, double bonds); hence, it better fits into DNA grooves by bending more readily along the grooves and adjoining more tightly the groove walls.

The energy of bonding of stearic acid to DNA, like that for acid **2**, depends on the nucleotide composition of DNA. For example, it can be seen from Table 3 that  $E_{\text{bond}}$  for the complex formed by (GC)<sub>10</sub> with stearic acid (**1**) is lower by 23.9 kcal mol<sup>-1</sup> for the minor groove and lower by 12.4 kcal mol<sup>-1</sup> in the major groove than the bonding energy for the (AT)<sub>10</sub> complex with **1**.

Now we will compare the strength of DNA complexes with acids **2** and **3** (*trans*- and *cis*-oleic acids). Transition from *trans*-isomer **2** to *cis*-isomer **3** is accompanied by weakening of the interaction of fatty acid with DNA: the bond energy with the minor groove of the (AT)<sub>10</sub> DNA decreases from 29.2 to 21.7 and that with the major groove, from 16.8 to 10.9 kcal mol<sup>-1</sup>; in the case of (GC)<sub>10</sub>, the bond energy in the minor groove changes from 23.2 to 19.9, while that in the major groove, from 12.5 to 10.4 kcal mol<sup>-1</sup>. This difference between the efficiency of binding of stereoisomers of oleic acid might be due to the fact that the hydrocarbon chain in the *cis*-isomer has a bent rigid segment, which prevents arrangement of the molecule along DNA grooves.

The presence of two double bonds (*i.e.*, two rigid segments) in the linear molecule of *trans,trans*-linoleic acid (**4**) may be expected to weaken the interaction with DNA relative to that of *trans*-acid **2**. Indeed, the numerical values demonstrate that the bond energies for acid **4** located in the minor and major grooves of (AT)<sub>10</sub> are lower than those for acid **2** by 3.7 and 5.5 kcal mol<sup>-1</sup>, respectively. In the case of complexes with (GC)<sub>10</sub>, the bond energies of acids **4** and **2** in the minor groove virtually coincide, being equal to 23.7 and 23.2 kcal mol<sup>-1</sup>, respectively. The replacement of *trans*-acid **2** by *trans,trans*-acid **4** in the major groove of (GC)<sub>10</sub> entails a loss in the bond energy (3 kcal mol<sup>-1</sup>). The *trans,trans,trans*-acid **6** contains one rigid double bond more than *trans,trans*-acid **4**, which should entail further weakening of the interaction of the acid with DNA. Direct computation confirmed the decrease in the interaction energy of DNA with acid **6** compared to that for acid **4**, except for the situation where the acid is in the minor groove of (GC)<sub>10</sub>.

According to the results presented in Table 3, of all the fatty acids we studied, *cis,cis*-linoleic acid (**5**) forms the strongest complexes with DNA. When acid **5** is arranged in the minor and major grooves of DNA, the bond energies are 47–48 and 30–32 kcal mol<sup>-1</sup>, respectively. This might be due to the fact that the conformation of this acid bent due to the two *cis*-bonds reproduces geometrically the curve of the double helix, which ensures the strong interaction with DNA. The presence of three *cis*-double bonds in the linolenic acid (**7**) entails a very pronounced bending of the molecule; therefore, transition from acid **5** to acid **7** violates the geometric compliance of the fatty acid with the shape of the DNA groove and results in a lower  $E_{\text{bond}}$  value.

Finally, note that fatty acids weaken the hydrogen bonds in DNA. As shown by calculations, the lengths of hydrogen bonds in the vicinity of acid **2** in the (AT)<sub>10</sub>–**2** complex are, on average, 2.12 Å, whereas in the initial (AT)<sub>10</sub> molecule, these lengths are 2.06 Å. The length of a step of the DNA helix virtually does not change upon the attachment of acid **2** (33.56 Å for DNA and 33.68 Å for the complex).

A thermal denaturation study of DNA complexes with fatty acids (C16:0, C18:0, C18:1, C18:2, C18:3) *in vitro* in the absence of proteins has shown<sup>40</sup> that stabilization or destabilization of hydrogen bonds in DNA depends on the length and the degree of unsaturation of the acids. In the researchers' opinion, the acids can be located in both minor and major grooves of DNA.

It should be noted that the strengths of DNA bonds with fatty acids predicted by calculations are confirmed by experimental data on the melting points of DNA complexes with these acids.<sup>40</sup> Typically, the interaction with stearic acid decreases the melting point of DNA by 6 °C and the reaction with linoleic acid, by 2 °C. Oleic and

linolenic acids have a weaker effect and even increase the melting point of DNA by 1–2 °C.

Thus, we found that all fatty acids bind more strongly to the minor groove of DNA than to the major groove. This is in line with the presence of two FFA pools extracted from DNA by biochemical procedures. Since DNA polymerase also interacts with the DNA minor groove,<sup>41</sup> fatty acids may play an important role in the mechanism of regulation of DNA replication and signal transmission. The energy of interaction of fatty acids with DNA depends both on the number of double bonds and the geometric configuration of the fatty acid and on the nucleotide composition of DNA. The dependence of the bond energy in the DNA–fatty acid complex on the nucleotide composition implies the possibility of site-specific binding of lipids to DNA. On passing from a saturated fatty acid to unsaturated fatty acids containing one, two, or three double *trans*-bonds, the bonding energy of DNA with the fatty acid gradually decreases. The presence of one or three double *cis*-bonds results in a decrease in the strength of the DNA–fatty acid complexes with respect to that of the saturated acid. The strongest binding between DNA and fatty acid was found for the unsaturated acid with two double *cis*-bonds, *i.e.*, linoleic acid. This effect can be attributed to the fact that the bent geometry of this acid (the boomerang shape) follows the curvature of the DNA helix.

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