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Molecular Dynamics and Free Energy of Binding of Oleic Acid to DNA in Aqueous Solutions

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Interaction of DNA with low-molecular-weight ligands is of great theoretical and practical interest for molecular biology and pharmacology [1]. DNA is a target for peptide antibiotics, which bind in the minor groove, and for various antitumor drugs, which interact with it both covalently (through the formation of chemical bonds) and noncovalently (due to electrostatic, van der Waals, and hydrophobic forces) [1, 2]. The presence of fatty acid residues in the fraction of lipids tightly bound to DNA has been shown for bacteria, particularly for the gram-negative bacterium *Pseudomonas aurantiaca* [3]. The feasibility of interaction between DNA and natural fatty acids and a possible regulatory role of the latter in gene expression was assumed and confirmed in a series of studies of the stability of complexes formed by DNA oligomers with lipids, C18 fatty acids, and cholesterol, performed using the molecular mechanics approach [4–6]. Most of the results are based on the modeling of DNA–lipid complexes in vacuum and on the analysis of steady states of the complexes and isolated reagents [5, 6]. In a recent study [7], in which DNA–lipid complexes were investigated by molecular docking, the effects of the solvent were taken into account implicitly and the conformational flexibility of the DNA molecule was not taken into account at all [7].

Thus, the study of structure and conformational dynamics of DNA–fatty acid complexes is of undoubted interest. After the formation of a complex between DNA and lipids [8] (in particular, oleic acid [9]) was confirmed, no data on the spatial structure and molecular dynamics of such complexes have been published. Linoleic acid was chosen for the computer-aided experiments on the DNA binding because up to 70% of molecules of the natural phospholipid cardiolipin in mitochondria contain four linoleic acid residues.

In this study, the complexes of a DNA oligonucleotide consisting of 25 A–T base pairs $(dA)_{25} \cdot (dT)_{25}$ and linoleic acid (*trans*-9,12 octadecadienoic acid) in neutral and ionized forms were studied by the molecular dynamics method with the use of an "explicit solvent model." It is shown that these complexes have a high conformational mobility. The free energy of formation of complexes between DNA and linoleic acid (8 and 13 kcal/mol for the anion and acid, respectively), were determined on the basis of molecular dynamics trajectories using the adaptive biasing force approach. It is shown that the proton of the acid is involved in hydrogen bonding with the phosphoryl group of DNA (the bond length in the equilibrium structure was 1.68 Å). Two main conformations of the complex of linoleic acid with DNA were identified. In the course of conformational dynamics, the formation and destruction of interactions between the polar and nonpolar residues of linoleic acid, on the one hand, and DNA, on the other, were observed.

MATERIALS AND METHODS

The Structure of the Ligands and Complexes

The structural parameters of linoleic acid were obtained from the database HIC-Up [10]. The structure of the double-stranded oligonucleotide consisting of 25 A–T pairs $(dA)_{25} \cdot (dT)_{25}$ was generated using the NAB utility of the AmberTools software package (AMBER 11, University of California, San Francisco, 2010). The coordinates of the atoms of complexes are given in accordance with the generally accepted nomenclature. To obtain the initial structures of complexes, the fatty acid was placed in the minor groove of DNA (in crystallographic configurations) using the VMD program. The complex was dissolved in a rectangular periodicity cell using the TIP3P model of water molecules so that the distance between the peri-

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Representative distances of interatomic noncovalent interactions for the optimized complexes and in the course of molecular dynamic simulation

Molecular dynamics stage	Distance between linoleic acid sites and DNA					
	EIC41:C18-dT34:H5'1	EIC41:C10-dT34:H1'	EIC41:H31-dT37:O1P	dA10:H1'-EIC41:C17	dT35:H5'-EIC41:C13	dT35:H5'-EIC41:C13
	Linoleic acid, neutral form			Linoleic acid anion		
After optimization, Å	2.96	2.88	1.68	3.57	2.67	2.83
At the end of molecular dynamics, Å	3.77	5.62	4.89	3.14	5.76	3.10
Average value during the dynamics, Å	5.669	9.22	4.87	4.34	5.19	2.91

odic images of the complex was at least 15 Å. To ensure the electrical neutrality of the system, the required amount of sodium ions was added. For the resulting structure, the energy minimization was performed using the conjugated gradient method in the NAMD program [11]. When generating the DNA structure, we used the AMBER99 force field parameters [12]. Ligands were characterized using General Amber Force Field (GAFF) parameters with AM1-BCC charges.

Molecular Dynamics

The molecular dynamics trajectory was calculated using the NAMD software package. Bond lengths were fixed using the SHAKE algorithm, which allowed the calculation of trajectories with a 2-fs step. All trajectories were calculated for 300 K. Before calculating the free energy of binding and the collection of parameters, the system was brought to equilibrium within 200 ps. The resulting trajectories were analyzed using the VMD software package as well as additional scripts written in Python. When analyzing the noncovalent contacts between the atoms of fatty acids and the oligonucleotide, the distance between them was not greater than 3.4 Å (this value corresponds to the maximum van der Waals attraction between the carbon atoms).

Binding Free Energy

The binding free energy (A) was determined using the adaptive biasing force approach [13] with the use of the NAMD software [14]. This method is based on calculating the average force F_x along the reaction coordinate ξ, whose action is then abolished by the action of a biasing force equal in magnitude and opposite in direction, which allows the system to overcome energy barriers. The dynamics of the system with respect to ξ corresponds to a random wandering with a zero mean active force. The F_x values accumulate in small intervals (ranges) ξ, which makes it possible to estimate the derivative of the free energy $dA(\xi)/d\xi$. The distance between the centers of linoleic acid atoms and the oligonucleotide atoms interacting with them was used as a reaction coordinate. The binding free energy was calculated in the course of calculation of a 5-ns molecular dynamics trajectory.

RESULTS AND DISCUSSION

The Structure of the Complexes of the Oligonucleotide and Linoleic Acid

After optimization of the complex of linoleic acid (EIC LA) and DNA (Fig. 1a), the number of interatomic interactions (van der Waals contacts) between the two structures was 106. The EIC was located parallel to the phosphate groups of DNA. To analyze the relative positions of the molecules, we selected three atoms in the EIC and three atoms in DNA that were the closest to the selected atoms of EIC. The atoms of the first pair (EIC41 : H31–dT37 : O1P) were located at a distance of 1.68 Å from each other (table). In this case, the hydrogen bond between the hydrogen atom of the carboxyl group of EIC and the oxygen atom of the phosphate group of DNA is formed. The distance between the atoms in the next pair (EIC41 : C10–dT34 : H1') was 2.88 Å. This pair consists of the carbon atoms of EIC that form the double bond in the EIC itself and the hydrogen atom in the deoxyribose of pyrimidine. The distance between the penultimate carbon atom in the EIC and the hydrogen atom in the deoxyribose of pyrimidine (dT34 : H5'1) was 2.96 Å.

The number of interatomic distances between the atoms of DNA and linoleic acid in the anionic form that were shorter than 3.4 Å was 30% less than in the neutral form and amounted to 74 (Fig. 1b). To describe the complex formed by the anionic form of EIC and DNA, we chose the same groups of atoms as in describing the previous complex. Due to the lack of hydrogen, the carboxyl group acquires a negative charge, which leads to distancing the fatty acid "head" from the negatively charged oxygen of the phosphate group of DNA; as a result, the distance in dT37 : O1P–EIC41 : O2 was 3.82 Å. It should be noted that

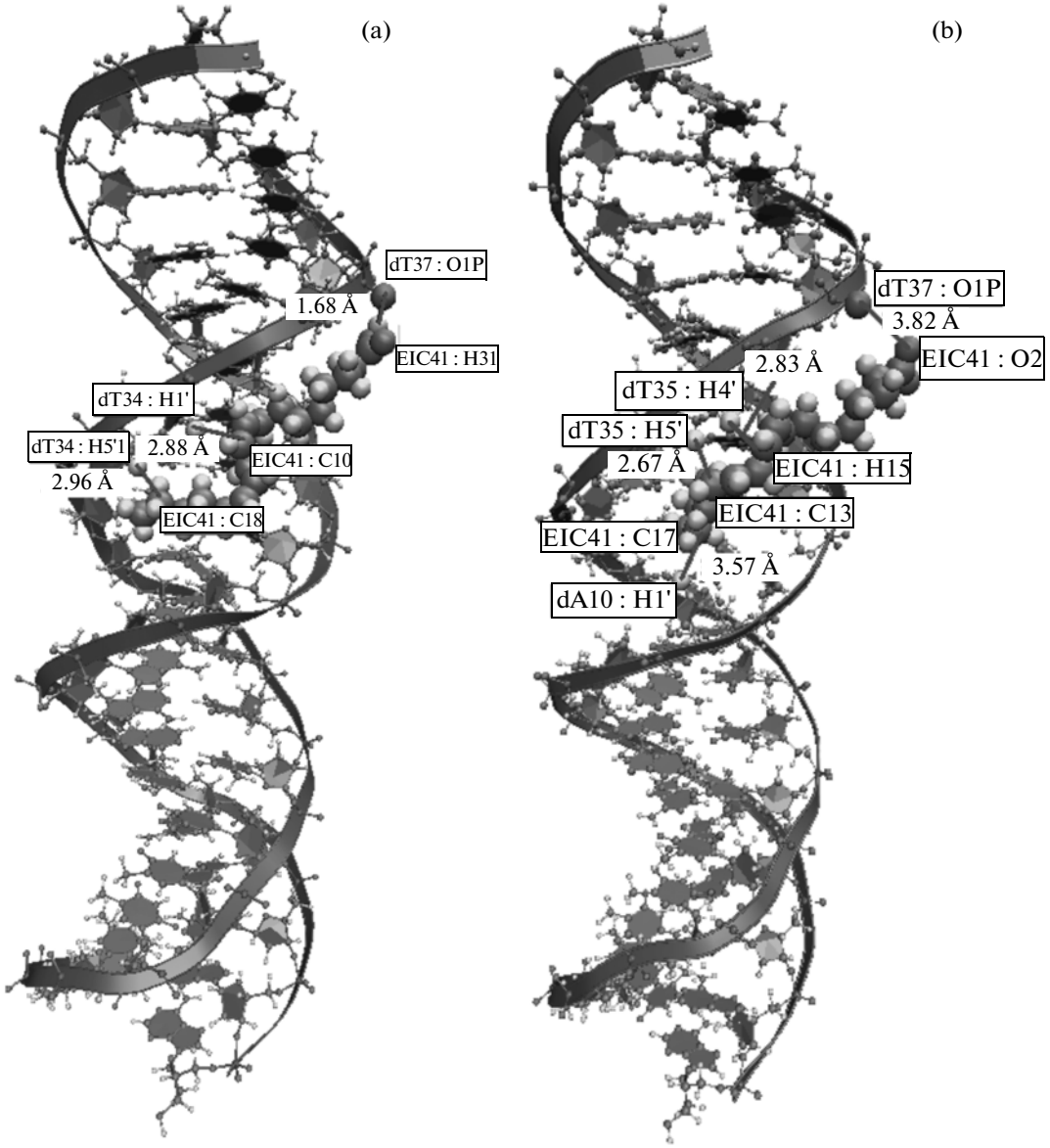

Fig. 1. The structure of complexes of fatty acids and the oligonucleotide $(dA)_{25} \cdot (dT)_{25}$ (computer experiment with the use of the NAMD software package): (a) linoleic acid (neutral form) and (b) linoleic acid anion. The orientation of the DNA strands was changed to provide a better viewing angle of the complex. Atoms of oxygen are shown in black; carbon, in gray; and hydrogen, in white.

carbon atoms involved in the double bond in the anion interact with the hydrogen atoms of the phosphate groups rather than with the hydrogen atoms of deoxyribose. The penultimate carbon of the fatty acid is located at a distance of 3.57 Å from the deoxyribose hydrogen. Thus, the center of the fatty acid molecule is located closer to DNA compared to its polar part and the hydrophobic "tail."

Molecular Dynamics

The molecular dynamics simulation of the complex formed by the neutral linoleic acid showed a high conformational mobility of the ligand (Fig. 2). Linoleic acid is retained in the minor groove of DNA through the hydrocarbon "tail," whereas the COOH group and the central moiety of the molecule (carbon atoms starting from C9) periodically lose contact with the atoms of DNA (Fig. 3). This is clearly seen from the dynamics of formation and break of the hydrogen bond between H31 of linoleic acid and oxygen of one of the phosphate groups of DNA. Despite this, the linoleic acid molecule remained bound for 2 ns. For the linoleic acid anion, the optimized structure initially had a smaller number of interatomic noncovalent interactions than the structure of the neutral com-

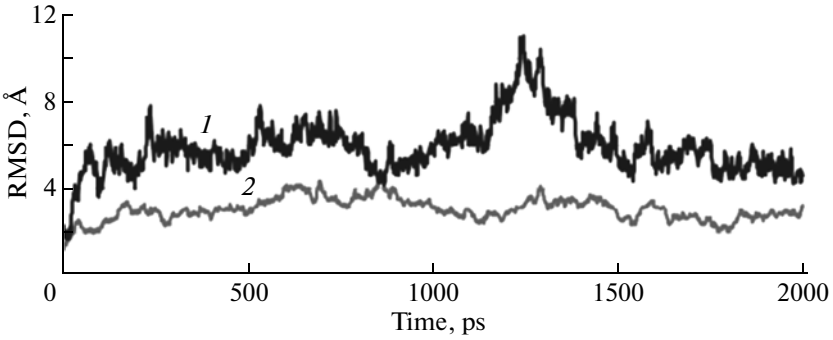

Fig. 2. Changes in the standard deviation of the coordinates of the structures (RMSD) in the course of molecular dynamics simulation with time: (*1*) linoleic acid and (*2*) DNA.

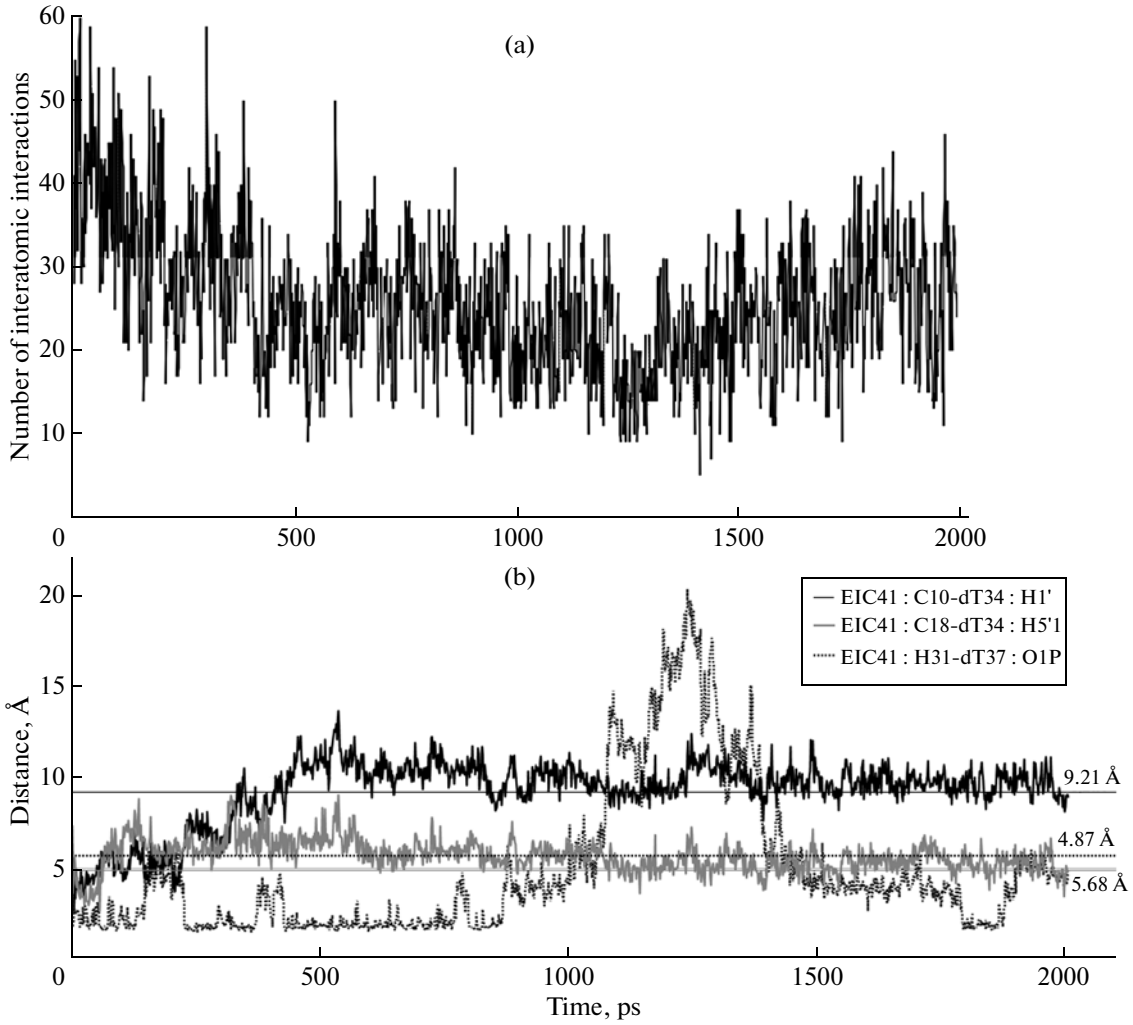

Fig. 3. Dynamics of interatomic noncovalent interactions between DNA and linoleic acid (neutral): (a) changes in the total number of interactions between DNA and linoleic acid over time and (b) changes in the distance between the representative atoms of DNA and linoleic acid over time.

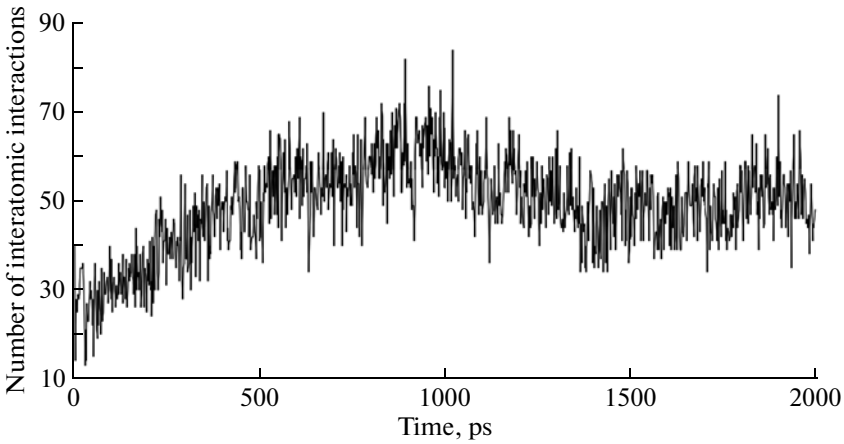

Fig. 4. Changes in the total number of interatomic noncovalent interactions of DNA and linoleic acid anion over time.

plex, which was due to the repulsion of the negatively charged oxygen of the carboxyl group of EIC and the oxygen of the phosphate groups of DNA. However, in the course of molecular dynamics simulation, the number of interatomic interactions increased, which was apparently due to the fact that the system leaved the local minimum and moved to a more favorable conformation (Fig. 4). A similar situation was observed for the molecular dynamics trajectory of linoleic acid.

The Free Energy of Binding of the Oligonucleotide and Linoleic Acid

The dependence of the free energy of binding of linoleic acid to DNA as a function of the reaction coordinate $A(\xi)$ yields the binding energy of 8 kcal/mol for the linoleic acid anion and 13 kcal/mol for the neutral molecule (data not shown). The difference in the binding energy values of the anion and the acid was probably due to the electrostatic repulsion between the phosphate groups of the sugar-phosphate backbone of DNA and the COO-group of fatty acids, whereas in the protonated form of fatty acid, hydrogen of the COOH group can form a hydrogen bond with the oxygen of the phosphate group of DNA. This value coincides with the value of the binding energy of linoleic acid and DNA decamer (13.3 kcal/mol), obtained by molecular docking [7]. The energy of binding of DNA to the neutral form of linoleic acid, calculated earlier for vacuum (48 kcal/mol) is too overestimated [5]. The high value of the energy of binding of linoleic acid to DNA is comparable to or even greater than the energy of binding of specific ligands, antibiotics, and anticancer drugs [15].

The results of this study confirm the possibility of existence of complexes formed by duplex DNA and fatty acids, in particular linoleic acid. They relate to the structure and dynamics of such complexes in an aqueous medium and are in agreement with the results of previous studies [7]. The complex formed by the neutral linoleic acid and DNA is more stable than the complex formed by the anion by 5 kcal/mol. Apparently, the negative charge of the DNA backbone is not an obstacle to the existence of such complexes. The neutral form of linoleic acid forms hydrogen bonds with the phosphate groups of DNA, which may affect the stability of the duplex.

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