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Molecular Dynamics of Oligonucleotide DNA Complexes with Phosphatidylserine

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Abstract: A formation of DNA-lipid complexes was studied by molec.docking and dynamics methods using the interaction of DNA with phosphatidylserine (PS). We have previously shown that some fatty acids and phosphatidylglycerol can specifically bind to oligonucleotide DNA $(dA)_{20}$•$(dT)_{20}$. It is shown that $(dA)_{20}$•$(dT)_{20}$ and phosphatidylserine formed a stable complex with a 6.3 kcal/mol binding energy and the PS molecule located in the minor groove of DNA. This complex contains 342 atom groups (interatomic distance ≤3.4 Å). The types of bonds in the PS-DNA(oligonucleotide) complex are suggested as hydrogen bonds, and hydrophobic and dipole-induced dipole interactions. In our previous study, a similar arrangement with close binding energy of 5.8 kcal/mol was shown for phosphatidylglycerol complex with the same oligonucleotide (complex contained 354 groups of atoms). The present study is of interest due to the importance of such complexes for applied biochemistry and biotechnology, genetics and molecular biology, cytology and pharmacology, nanotechnology, and self-assembly smart materials.

Keywords: interfacial interactions; interatomic distances; phosphatidylserine; oligonucleotides; DNA-lipid complexes; docking; molecular dynamics.

1. Introduction

DNA, proteins, and lipids are widely studied because they are the most important biologically active substances (BAS) for all living creatures [1,2]. The numerous interactions of DNA with proteins are of great interest in connection with the functions they perform in cells and are well established [2]. In contrast, DNA interactions with lipids are just getting to become an interesting field of modern research [3,4], while a few earlier general publications in this field have been known [5,6]. Some lipid fractions are tightly bound to different nucleic acids or oligonucleotides [4-8]. It is assumed that such lipids can form a new information level in genomic DNA. The authors accomplished "molec.docking and molec.dynamics simulation" (i.e., MD&MDS) calculations to assess the stability of oligonucleotide complexes with numerous fatty acids in minor or major grooves [4,8], as well as with some phospholipids [3].

MD&MDS allowed us to identify possible contacts and types of bonds between DNA and lipids, which is of interest in connection with the functions they perform in the cells of living organisms - structural and regulatory [9,10], as well as identification of novel inhibitors of SARS-CoV-2 [11].

It is well-known that phosphatidylserine (PS) is one of the major phospholipids in humans, animals, some bacteria, and fungi [1,12-14]. On the acidity scale, PS can be placed between phosphatidic acid and phosphatidylethanolamine [13]. PS is concentrated mainly in the inner layer of cell membranes. PS is preferentially associated with membrane proteins, especially during oxidative stress [15-17]. PS has facilitated some cell responses to biologically active substances [12-18]. The PS amount in the human cerebral cortex and cell nuclei membrane accounted for 13–15 % [18-20] and 4-9% of the total phospholipids, respectively [12].

The authors [19] are believed that PS receptors (PS-Rs [20]) increase the penetration of "enveloped viruses in cells" [19]. A study of the authors [19] indicates that the PS-Rs of numerous human cells interact with PS molecules [19]. The authors [21] are believed that phosphatidylserine is involved in the "inflammation of COVID-19" [21]. The research [21] devoted to the influence of PS on inflammation and coagulation processes will enhance therapeutic efforts in disease treatment [21]. For example, phosphatidylserine (in the mixture with C20:5 n-3 and C22:6 n-3 acids [22])) exhibits positive effects of "Alzheimer's disease" [22], as well as induces one of the major roles in the research concerning medical problems of the membrane lipid metabolism disorders [23].

The phosphatidylserine molecule is also of interest as a precursor of some "phospholipids" [12]. In this work, the structure of phosphatidylserine includes linoleic acid residues with two double bonds (C18:2), while in nature, there is normally a mixture of saturated and unsaturated fatty acids presented.

In the recent works [24-31], some MD&MDS approaches for BAS-complexes were proposed [24-31]. In the recent review [32], the authors provided a historical context and compiled suitable methods for DNA–BAC interactions. For example, the authors [33] employed some modern spectroscopic techniques, etc. in order to investigate the interactions of bishomoleptic and trisheteroleptic "ruthenium (II) polypyridyl complexes with calf thymus DNA (CT-DNA)" [33]. These data were proved by MD&MDS, and detailed graphs were presented by the authors [33]. The authors [33] calculated the binding energies of these complexes with "CT-DNA" [33], which were close to the experimental one ("-7.7 kcal·mol^{-1}" [33]). The authors [34] applied the methods of hybrid localized molecular orbitals (HALMO) energy decomposition analysis (EDA) to dissect the interactions within double-stranded DNA.

The numerous fundamental studies of the theoretical complexes of oligonucleotides, native and G-quadruplex DNA with various BAC are promising for novel medical formulations, metal-included drugs, etc. [32-36]. In particular, the authors [35] performed an estimation of MD&MDS approaches [35] for BAS-complexes with biological activity [36].

The work is aimed to study the interaction between phosphatidylserine and $(A)_{20}$:$(T)_{20}$-oligonucleotide by MD&MDS approaches.

2. Materials and Methods

2.1. The ligand's and complexe's structure.

The PS with two linoleic acid residues was obtained from the HIC-Up database [24,25], and the structures were constructed using the "Avogadro-program". The structures of double-helix oligonucleotides containing twenty A-T pairs $(dA)_{20}$:$(dT)_{20}$ were generated using the NAB utility programs from the "AmberTools" software package ("AMBER 11, University of California, San Francisco, 2010" [26,27]). The atomic coordinates of the complexes are used in accordance with the generally accepted nomenclature [26,27]. To prepare initial structure of the complex, the PS molecule was placed in a DNA minor groove (in their crystallographic configurations) using the "VMD" program. The complex was performed using "AutoDock" with the vina.exe application [3,4].

2.2. MD&MDS

The molecular dynamics parameters of the system were calculated using the "NAMD" program [28]. The bond lengths have been gotten using the "SHAKE" algorithm, which allowed using a path calculation time step 2 fs. All results were obtained for temperature 300 K. Before calculations, all free binding energy and collecting parameters and the system as whole was brought to equilibrium for 200 ps. This time value was selected from our previous computer experiments [2-4] and remained equal for all our calculations to compare the data correctly. Moreover, the selected time 200 ps exceeded the equilibrium time, commonly used in most molecular dynamics computer experiments (see *http://ambermd.org/tutorials/basic/tutorial1/section5.htm* as an example). The resulting data were analyzed using the "VMD" software package and using additional scripts written in Python programs [37, 38]. When analyzing non-covalent contacts between fatty acyl residue and oligonucleotide atoms, we took part the atoms, the distance between which did not exceed 3.4 Å (a value of 3.4 Å corresponds to the maximum of the van der Waals attraction between carbon atoms) [4].

3. Results and Discussion

3.1. Results.

Previously, we studied the interaction of DNA with phospholipids (e.g. phosphatidylglycerol [3] and phosphatidylethanolamine [4]) by the MD&MDS methods. In our computer experiments, the result of docking of the PS molecule with the DNA oligomer $(dA)_{20}$•$(dT)_{20}$ (see the Methods section) was the PS displacement from the starting unstable position to the "DNA groove" [3,4], with the binding energy value equaled to 6.3 kcal/mol. In this area, 342 groups of elements are used (having less than 3.4 Å). with the binding energy equaled to 6.3 kcal/mol. In this area, 342 groups of elements are used (with distances less than 3.4 Å). It is important to highlight that 89 of such pairs are of interest because only these atoms show pronounced interactions due to the certain distances between them.

These are element pairs: O-H (61), N-H (13), O-C (5), N-C (1), O-N (4), O-O (3), and pairs with P (2 with P-H). In earlier works [2,4], the complex binding energy for the DNA with phosphatidylethanolamine was determined as 6.3 kcal/mol. [4], and for phosphatidylglycerol-

DNA complex - 5.8 kcal/mol [3]. The structure of the B-form of a DNA oligomer with a PS molecule in the minor groove of DNA, obtained as a result of docking, is shown in Figure 1.

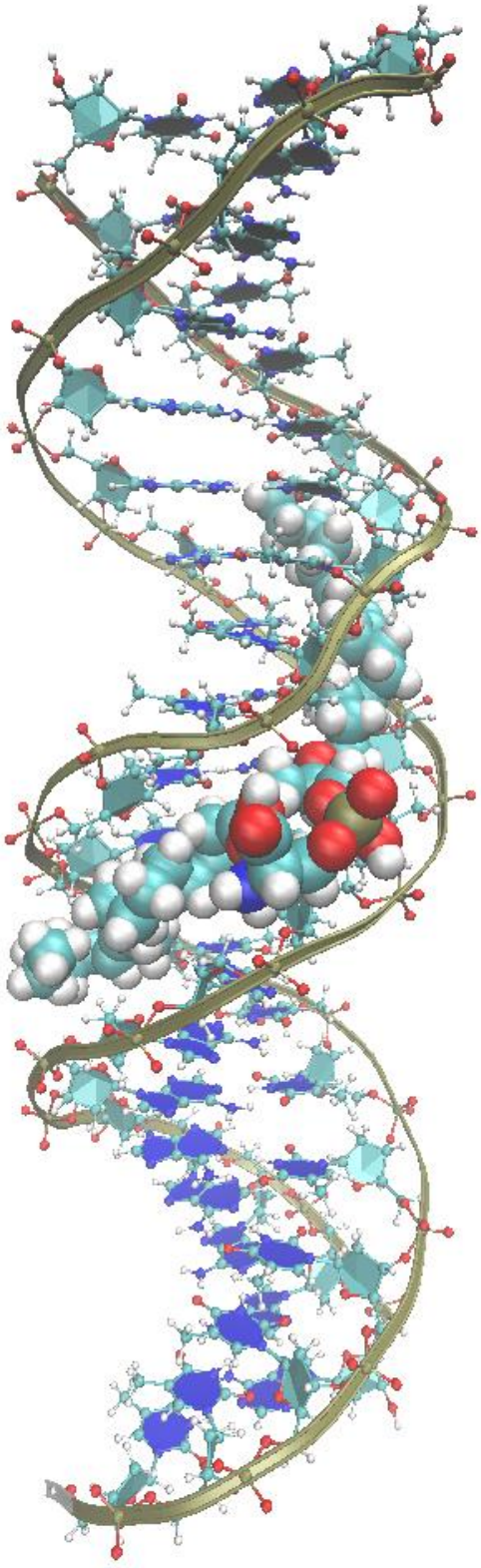

Figure 1. MD&MDS of phosphatidylserin-DNA complexes. The structure of the oligomer$(dA)_{20}$•$(dT)_{20}$-phosphatidylserine complex (PS - in the minor groove) obtained by the AutoDock in the "vina.exe" application.

We calculated the track of atoms in the complex of DNA oligomer and PS with two residues of linoleic acid by molecular dynamics methods as described earlier for complexes of DNA with linoleic acid [3, 4]. There are changes in the root mean of the structure's square deviation ("RMSD") vs. time (Figure 2).

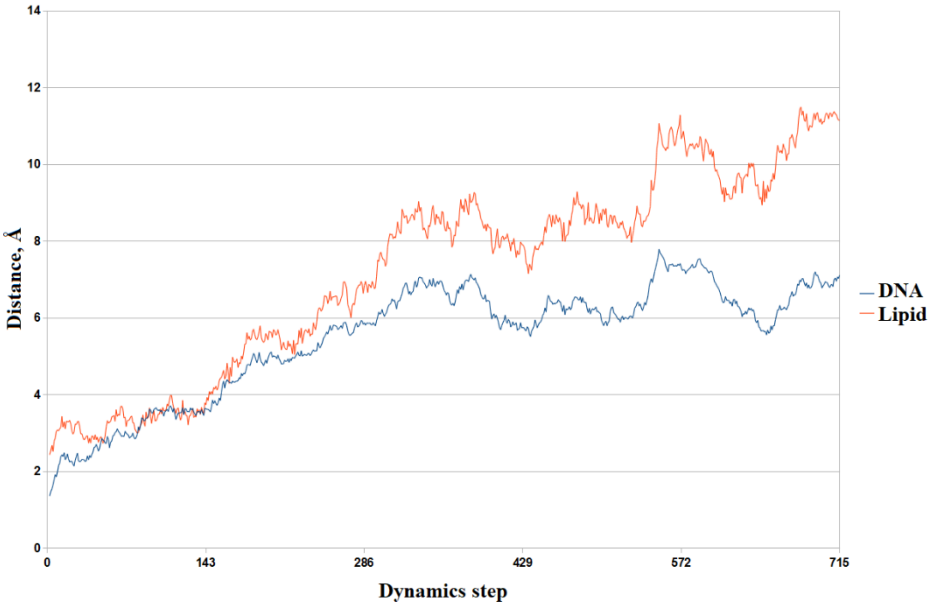

Figure 2. MD&MDS of phosphatidylserin-DNA complexes. Change of the distance (Y, RMSD [4]) in the oligomer$(dA)_{20}$•$(dT)_{20}$-PS complex at each saved step of the dynamics (X), according to the methods section.

The dynamics results showed the relative stabilization of the phospholipid-DNA complex. At the dynamic's starting point, significant changes in the molecule's vibration were found. The complex fluctuated insignificantly (within the limits of 1 Å) in the central part. At the dynamic's final stage, the complex fluctuated within 2 Å. There were no sharp changes in such vibrations except for the change in the RMSD of the lipid in the middle of the dynamic's second half (~2.5 Å).

Moreover, we analyzed the starting and last saved steps of MD&MDS for the number of pairs of atoms with a distance between ≤3.4 Å. At the starting saved step of the dynamics (Figure 3), a total of 213 pairs of atoms were detected (with a distance of less than 3.4 Å), and 51 of them are of interest because the atoms (between which these distances are observed) are capable of interacting.

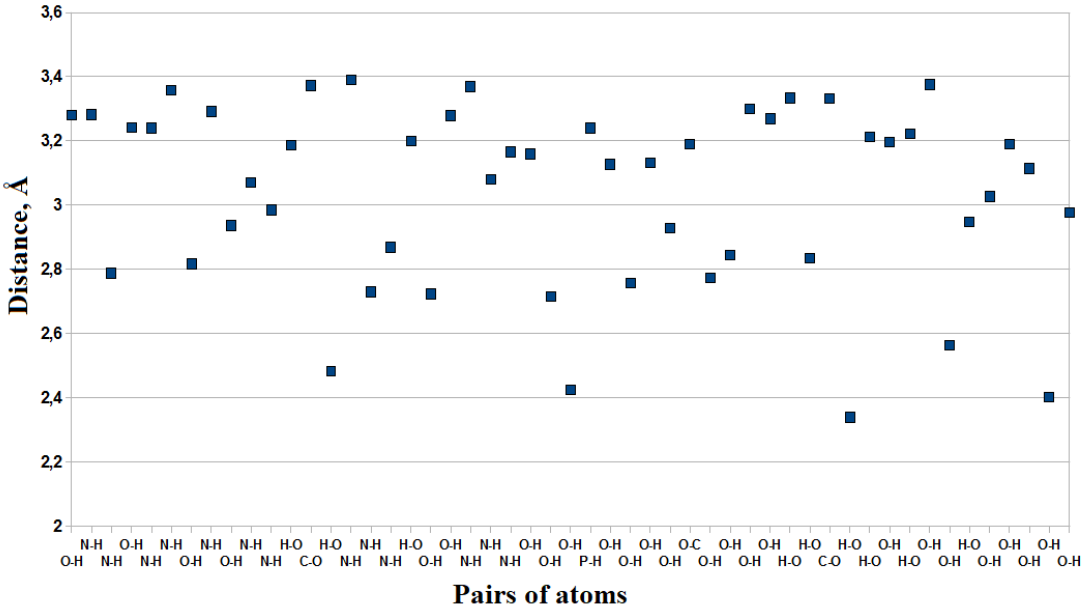

Figure 3. MD&MDS of phosphatidylserin-DNA complexes. Distance's change (Y) between the atom pairs (X) in the starting step, according to the methods section.

These are pairs of atoms O-H (34), N-H (13), O-C (3), as well as the pair of P-H (1). Compared to the number of atom pairs during MD&MDS, there are 129 pairs fewer. This is because, in the course of the dynamics, the structures constantly fluctuate.

At the last saved step of dynamics (Figure 4), the distance between them is less than 3.4 Å, and a total of 196 pairs of atoms were found, of which 51 are of interest due to the fact that the atoms (between which these distances are observed) are capable of interacting.

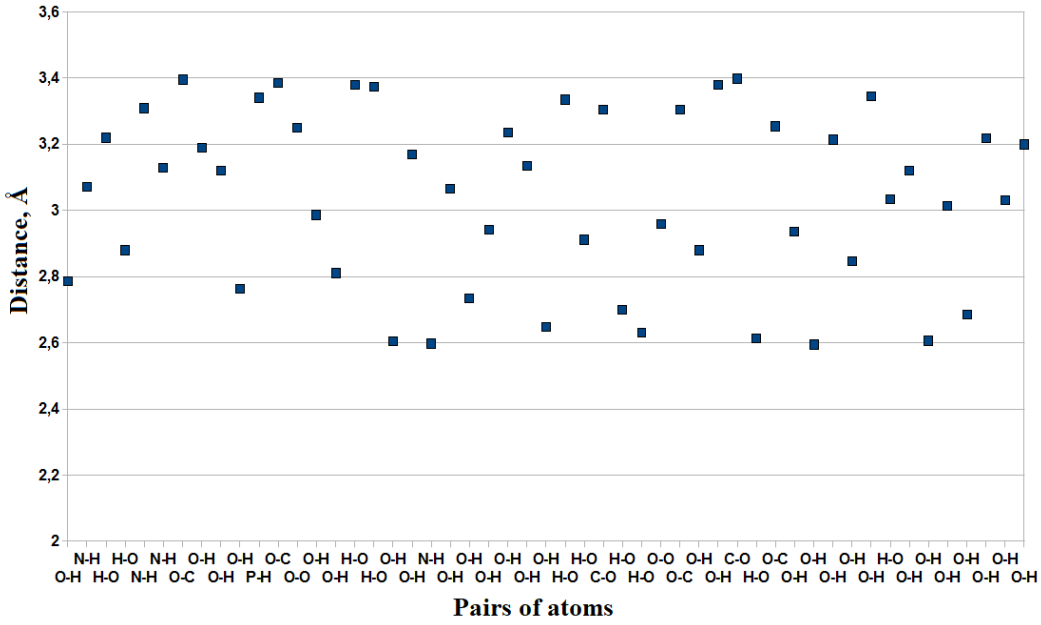

Figure 4. MD&MDS of phosphatidylserin-DNA complexes. Distance's change (Y) between the atom pairs (X) in the last step, according to the methods section.

These are pairs of atoms O-H (38), N-H (4), O-C (6), O-O (2), and the pair of P-H (1) in addition. The number of interatomic interactions was also calculated, i.e., MD&MDS for the number of atom pairs with the distance between ≤3.4 Å at each step of the dynamics (Figure 5).

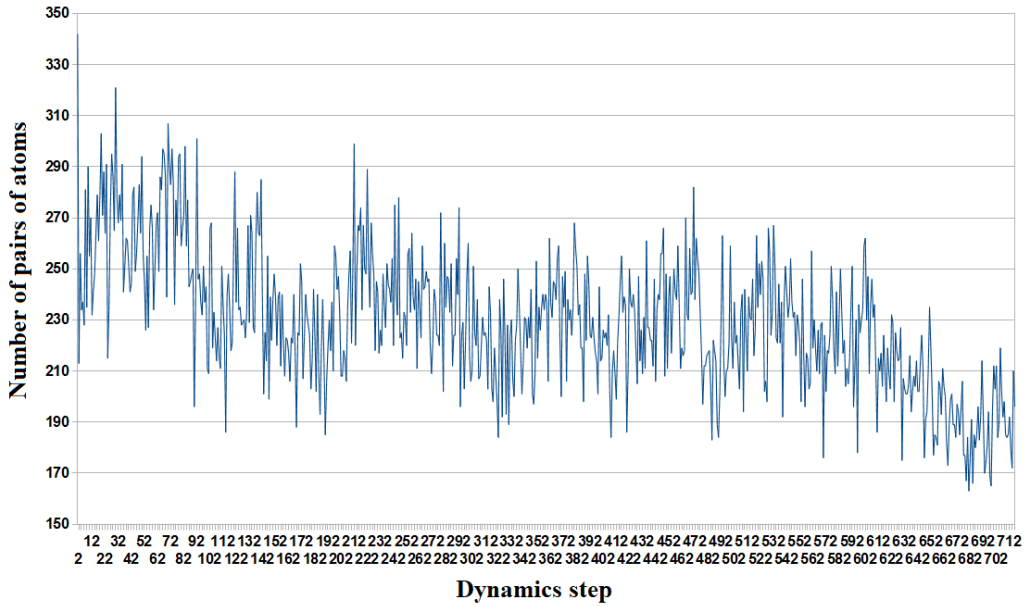

Figure 5. MD&MDS of phosphatidylserin-DNA complexes. The number of the atom pairs with the distance between ≤3.4 Å at each step of the dynamics.

The minimum number of pairs of atoms (equal to 163) was noted at the end of the dynamics, and the maximum (equal to 321) - was at the initial steps of the dynamics. In this

case, 12.15% of dynamic steps have up to 200 atom pairs with a distance between ≤3.4 Å, 66.76% - from 200 to 250 atom pairs, 20.39% - from 250 to 300 atom pairs, 0.7% - from 300 and more pairs of atoms. If we consider the number of atom pairs in the time of dynamics, then 1) the number of pairs of atoms falls in the initial half of the dynamics, 2) on average, 20 atom pairs less in the second half of the dynamics, after a pronounced change in the RMSD of the lipid.

To analyze the mutual orientation of molecules in the PS–DNAcomplex, we selected six pairs of atoms of this complex (Figure 6) because the average distances between them during the entire dynamics were less than 3.4 Å: H4'-O10, O2-H61, H4'- O2, O4'-H60, N3-H72, O4'-H50.

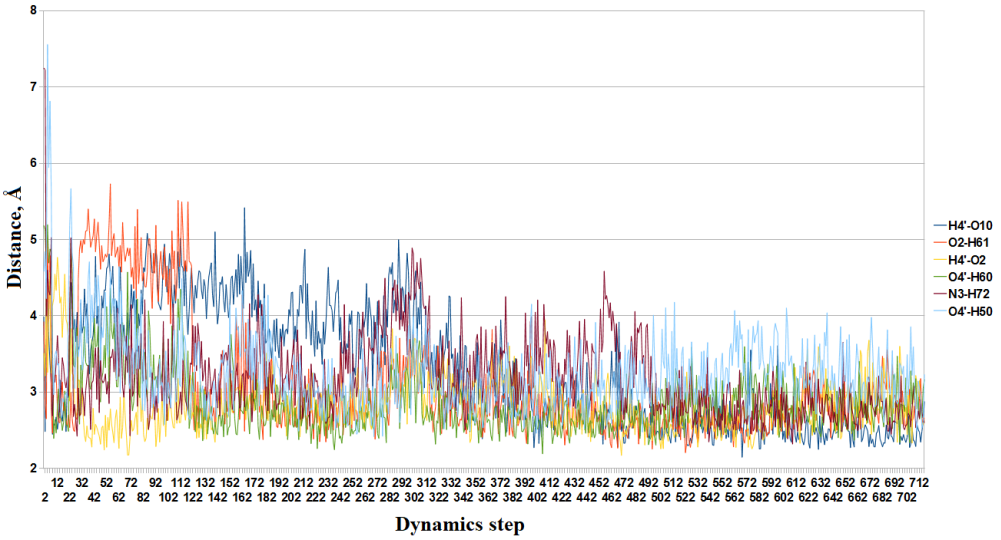

Figure 6. MD&MDS of phosphatidylserin-DNA complexes. Changes in the distances between atom pairs in the course of dynamics with the distance between ≤3.4 Å in the starting and last steps of the dynamics.

3.2. Discussion.

Using MD&MDS of phosphatidylserin-DNA complexes, we proposed a relatively stable complex structure. In addition, low conformational mobility of the PS ligand in oligonucleotide $(A)_{20}$ $(T)_{20}$ was found. This can be due to the strong PS bounding by two fatty acid residues, as we proposed earlier [4].

The energy of PS binding to $(A)_{20}$ $(T)_{20}$ in the minor groove was about 6.3 kcal/mol. Such structure of the phosphatidylserin-DNA complexes (Fig. 1) was firstly obtained by us and could be the third example of the structure of molecular complexes of DNA and phospholipid. Our previous works presented the structures of the complexes of DNA- phosphatidylglycerol [3] and DNA-phosphatidylethanolamine [4]. Our three alternative hyposises of PS positions in the DNA before the MD&MDS experiment were the following: in the major groove or in the minor groove, as well as the compromised position when each of the fatty acid residues is located in the minor and major grooves simultaneously. The minimization of the energy of the DNA-PS complex showed that, in the structure with the minimum energy, both fatty acid residues bound to the DNA minor groove (Figure 1). However, no significant changes are observed in the values of the RMSD parameter in the case of PS in the DNA-PS complex. Nevertheless, there are regions in which there are symmetric changes in the RMSD values of DNA and PS: for example, there is a rise in the 572 steps (3 Å for phospholipid, 1.5 Å for DNA), and for a phospholipid, the value of such a rise is greater than for DNA by 1.5 Å (Figure

2), by 644 steps - a decline in the curve of this parameter for DNA and lipid (1 Å - for lipid, 2 Å - for DNA), and lipid, the value of such a drop is less than for DNA by 1 Å (Figure 2).

The number and types of interacting atoms at all steps of dynamics reflect the following structures of DNA-PS complexes (Figure 5): a) from 212 to 342 pairs of atoms at the initial steps of dynamics, b) from 164 to 220 at the final steps of dynamics, c) the maximum in intermediate steps is 300. Qualitative analysis shows an increase in the number of OH pairs. It should be noted that the distances between atom pairs of DNA and PS at the starting steps of dynamics undergo significant changes during MD&MDS (Figure 5).

During the dynamics of six selected pairs of atoms, it was revealed (Figure 6) that the average minimum distance was observed between pairs of H4'-O2 atoms; the average distance between them was 2.85 Å, the minimum was 2.17 Å, and the maximum was 4.57 Å. The distance between other pairs of atoms is not much greater. For a pair of H4'-O10 atoms, the distance ranges from 2.25 Å to 5.1 Å, the average being 3.3 Å. For the O2-H61 pair - from 2.25 Å to 5.51 Å, average 3.07 Å, for O4'-H60 - from 2.25 Å to 5.19 Å, average 2.87 Å, for N3-H72 - from 2.35 Å to 7.23 Å, average 3.2 Å, for O4'-H50 - from 2.42 Å to 6.82 Å, average 3.25 Å.

The complex (DNA-PS) was analyzed by reference points of interaction of the PS fatty acid residues with the $(A)_{20}$ $(T)_{20}$ (Figure 7).

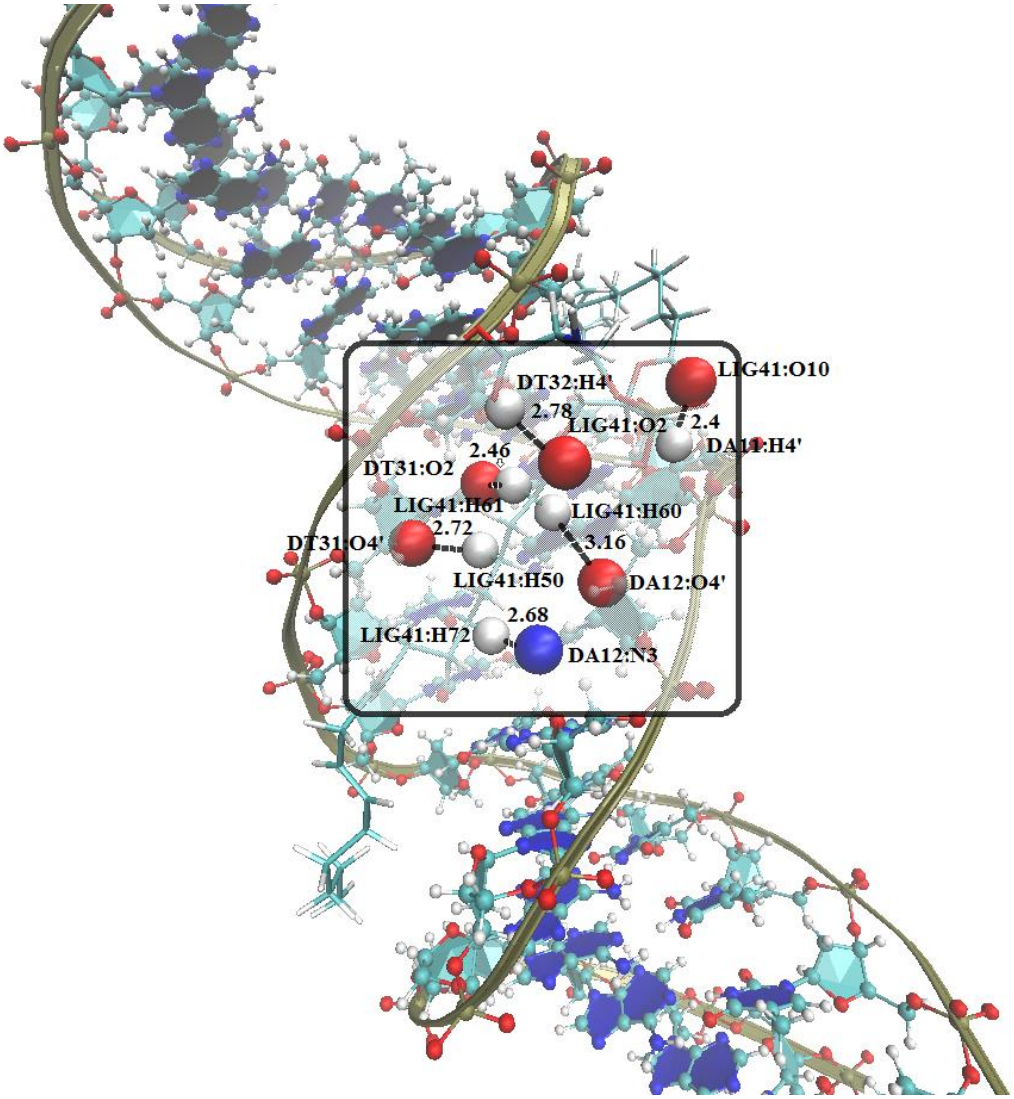

Figure 7. The structure of the complex of oligonucleotide $(A)_{20}$ $(T)_{20}$ and PS (neutral form). The oligonucleotide chain orientation appeared to be changed to provide a better viewing angle of the complex.

The distance between the fatty acid's and oligonucleotide atoms must be reduced by more than 2 angstroms compared to free fatty acids. Presumably, this is due to the presence of two fatty acid residues in PS, linked through the glycerophosphate backbone. When a free fatty acyl interacts, the distance between atoms of fatty acid and DNA is similar in space (≤2 Å). It seems to be due to the steric hindrances created by the glycerol group; that is, the close contact of the hydrophilic lipid head is complicated by the glycerol residue, but the hydrophilic tails interact closely with DNA.

The molecular dynamics of the DNA-lipid complex made it possible to reveal one of the variable complexes of the interaction between a double-stranded DNA molecule and phosphatidylserine. Despite the possibility of DNA interaction with the serine residue in the phospholipid, lipid does not converge with DNA in this area. Frequent repulsion of the serine-containing region of the phospholipid from DNA is observed. This may be due to the disadvantageous location of this lipid region relative to the DNA fragment. We can assume the possibility of the existence of another location of this lipid region relative to DNA, in which the interaction of these parts will be significant. The hydrophobic tails of the PS were more mobile than the PE [4]. This is due to the structural features of these phospholipids. In the course of the dynamics, a clearer interaction of the lipid with nitrogenous bases in the minor groove of DNA was revealed.

4. Conclusions

The interactions between oligonucleotide $(A)_{20}$ $(T)_{20}$ and phosphatidylserine (as a ligand) are found and displayed. It is important to highlight that the PS molecule is arranged in the "DNA-groove". The PS-DNA(oligonucleotide) complex parameters are determined, and the differences in comparison with the interaction of the same oligonucleotide with individual fatty acids and other phospholipid molecules are discussed in detail. The features of the PS arrangement in the nucleic acid double helix are presented. The types of interactions in the PS-DNA(oligonucleotide) complex are suggested as hydrogen bonds, hydrophobic, and van der Waals forces. The present study is of interest due to the importance of such complexes for applied biochemistry and biotechnology, genetics and molecular biology, cytology and pharmacology, nanotechnology, and self-assembly smart materials.

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Conflicts of Interest

The authors declare no conflict of interest.

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