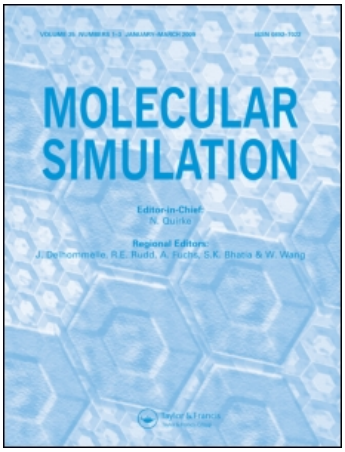

Molecular Simulation
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713644482

Flexible docking of DNA fragments and actinocin derivatives

K. V. Miroshnychenko[a]; A. V. Shestopalova[a]
[a] A.Ya.Usikov Institute of Radiophysics & Electronics of NAS Ukraine, Kharkov, Ukraine

To cite this Article Miroshnychenko, K. V. and Shestopalova, A. V.(2005) 'Flexible docking of DNA fragments and actinocin derivatives', Molecular Simulation, 31: 8, 567 — 574
To link to this Article: DOI: 10.1080/08927020500134276
URL: http://dx.doi.org/10.1080/08927020500134276

Molecular Simulation, Vol. 31, No. 8, July 2005, 567–574

Taylor & Francis
Taylor & Francis Group

Flexible docking of DNA fragments and actinocin derivatives

K.V. MIROSHNYCHENKO* and A.V. SHESTOPALOVA

A.Ya.Usikov Institute of Radiophysics & Electronics of NAS Ukraine, Kharkov, Ukraine

(Received February 2005; in final form March 2005)

We have applied molecular docking methods to systems containing nucleic acids as targets and biologically active substances as ligands. The complexes of DNA fragments and actinocin derivatives with different lengths of aminoalkyl side chains were obtained by molecular docking. It was observed that actinocin derivatives could form energetically favourable complexes with DNA both as intercalators and minor groove binders. It was shown that small changes in the binding energy (~1 kcal/mol) could result in complexes with substantially different structure. The complexes of actinocin derivatives and DNA fragments were stabilized by hydrogen bonding upon intercalation and minor groove binding. It was found that the change of solvent-accessible surface area upon binding of the actinocin derivative to DNA linear increased with the growth of methylene groups' number in ligand side chains. The solvation energy change upon binding of actinocin derivatives to DNA calculated by the WSAS method was favourable in the case of small uncharged ligands and unfavourable for positively charged ligands.

Keywords: Molecular docking; DNA; Actinocin derivatives; Specificity

1 Introduction

Target-ligand interactions play a major role in all biological processes. The study of these interactions is of vital physiological and pharmacological importance. The molecular docking method is one of the most effective computer simulation methods, which makes it possible to construct rapidly all the possible variants of the complexes of the biological macromolecule-target and the ligand of interest. In this method, the target structure is usually kept rigid, whereas, the conformation of ligand undergoes changes during the simulation, i.e. such docking is flexible. The molecular docking method is commonly used for the estimation of specifity of protein–ligand interactions [1–4]. The docking of ligands to DNA molecules-targets is more rarely applied. In such a case, anticancer drugs are used usually as ligands [5].

Actinomycin D (AMD) is a widely used DNA-binding antitumor antibiotic, but its use suffers from its limited solubility and from causing many side effects [6,7]. Therefore, the study of AMD-derivatives is of especial interest. AMD consists of a phenoxazone chromophore substituted with two equivalent cyclic pentapeptide lactone rings (see figure 1). Its biological activity is thought to involve preferential intercalation of the planar phenoxazone chromophore into GC sequences of DNA with the two cyclic pentapeptide rings lying in the minor groove [8]. This structure has been confirmed by detailed studies of crystal structure AMD–DNA complexes using RSA [9], NMR investigations of solution [10] and theoretical calculations [11]. Although the structural significance of the AMD chromophore is established, the role of its side chains is still under discussion. Taking into account that the mutual arrangement of the phenoxazone chromophore and different functional groups can affect the interaction of aromatic compounds with DNA and possibly their antitumor properties [12,13], it was important to design drugs having the actinocin moiety and other functional substituents separated from each other by spaces of different length. It should be noted that new synthetic actinocin derivatives were obtained recently and their anticancer properties examined [14,15]. It is known from the literature that such actinocin derivatives can interact with nucleic acids by two possible mechanisms: intercalation and minor groove binding [16].

The aim of this study was to obtain the complexes of DNA fragments and actinocin derivatives, in which ligands could be both: intercalators and minor groove binders. We studied ligands with different number of methylene groups in side chains in order to determine the influence of side chains length on the binding process.

*Corresponding author. E-mail: kateryna_mirosh@ire.kharkov.ua

Molecular Simulation
ISSN 0892-7022 print/ISSN 1029-0435 online © 2005 Taylor & Francis Group Ltd
http://www.tandf.co.uk/journals
DOI: 10.1080/08927020500134276

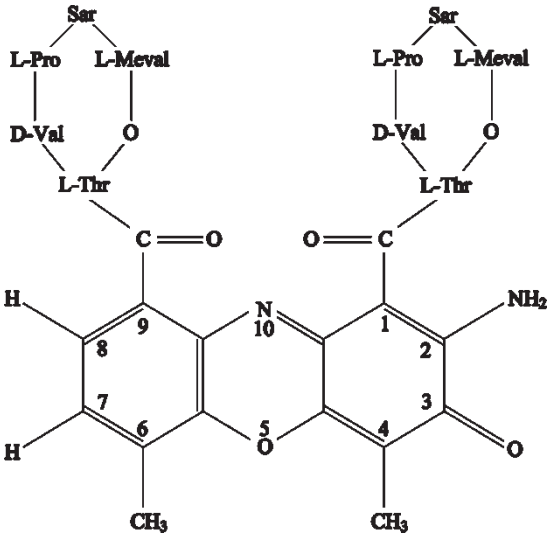

Figure 1. The structure of anticancer antibiotic actinomycin D (AMD).

2 Materials and methods

The objects of investigation (see figure 2) were actinocin derivatives with different length of aminoalkyl side chains synthesized recently by prof. Glibin E.N. from St Petersburg State Technical University. The number of methylene groups in side chains of actinocin derivatives varied from 1 to 8 (act1–8). Ligands were positively charged (+2). We studied also uncharged ligands (act and act0). Ligand atomic charges were obtained by pm3 method. Then the molecular mechanics optimization of ligand geometry in force field AMBER96 [17] was performed: first *in vacuo* and then in the water (model TIP3P [18]). The conjugate gradient method was used for the optimization.

We used DNA fragments d(GAAGCTTC)$_2$ (PDBindex 316D) and d(CGCGTTAACGCG)$_2$ (PDBindex 195D) complexed with AMD and netropsin as targets for intercalation complexes and minor groove binding complexes correspondingly. First, ligands and water molecules were removed from PDB entries. Then hydrogens were added to DNA fragments and standard AMBER96 charges were assigned to target-macromolecule atoms.

Simulation of target–ligand interaction was carried out with program package AutoDock 3.05 [19]. The preparation of DNA and ligands files was performed using AutoDock Tools.[†] First unpolar hydrogens were removed and their charges were added to carbon atoms that held these hydrogens. Then rotatable bonds in ligands were assigned. Bonds in cycles and amide bonds were set non-rotatable. All other bonds were allowed to rotate. The number of rotatable bonds in ligands varied from 1(act) to 21(act8). AutoDock can handle up to 32 rotatable bonds.

Grid maps were calculated using module AutoGrid. The number of grid points in $x \times y \times z$ directions was $70 \times 70 \times 70$. The distance between two adjacent grid points was 0.375 Å. The grid centre was located in the GC-site (the binding site of AMD) in the case of intercalation and in the TTAA-site (the binding site of netropsin) in the case of minor groove binding.

For each target-ligand system, we performed 100 docking runs. The Lamarckian genetic algorithm was used with population size of 50 individuals. The stop criteria were a maximum of 1,500,000 energy evaluations or a maximum of 27,000 generations.

The solvent-accessible surface area (SAS) of actinocin derivatives and their complexes with DNA fragments was calculated using two probe radii: 1.4 and 0.6 Å. The SAS obtained with the probe radius of 0.6 Å was used for the solvation energy calculation by weighted solvent-accessible surface area (WSAS) method [20].

Table 1. The results of the test calculations performed by rigid and flexible docking method.

	Rigid docking		*Flexible docking*	
System	E_b *(kcal/mol)*	*RMSD (Å)*	E_b *(kcal/mol)*	*RMSD (Å)*
AMD + DNA	−23.8	0.21	−20.3	1.24
DAPI + DNA	−12.2	0.61	−12.1	1.07
Netropsin + DNA	−15.2	0.56	−15.1	1.29

E_b is the binding energy of the ligand conformations obtained by docking method that had the minimal RMSD from the reference PDB structures.

3 Results and discussion

3.1 Test calculations

We performed test calculations for three systems: actinomycin D-d(GAAGCTTC)$_2$ (intercalation, PDBindex 1DSC), DAPI-d(CGCGAATTCGCG)$_2$ (minor groove binding, PDBindex 1D30) and netropsin-d(CGCGTTAA-CGCG)$_2$ (minor groove binding, PDBindex 195D) complexes. The results of rigid and flexible docking calculations for test systems are listed in table 1. Here, E_b is the free energy of binding for structures that had the minimal RMSD from the reference PDB structure. It can be seen that both rigid and flexible docking methods enable to reproduce the original complex structure with sufficiently high accuracy (RMSD $<$ 1.3 Å) (see figure 3).

Relying on the results of our test calculations, the application of molecular docking method with program package AutoDock 3.05 to systems, containing nucleic acids as targets, was confirmed.

3.2 Flexible docking of DNA fragments and actinocin derivatives

As a result of 100 flexible docking runs for each target–ligand system, a set of 100 possible complexes was

[†]AutoDock Tools http://www.scripps.edu/%7Esanner/python/index.html

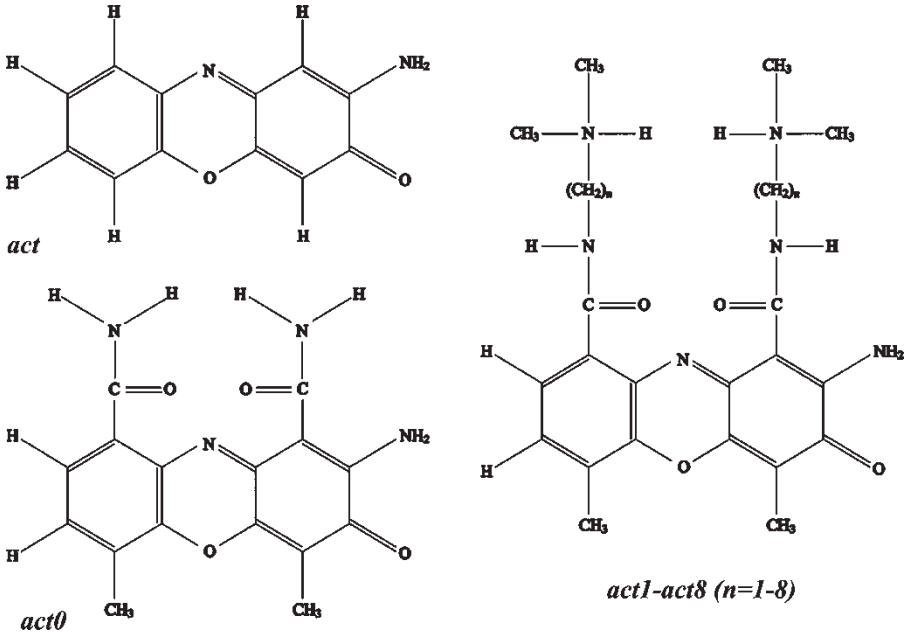

Figure 2. The structure of actinocin derivatives (*n*—number of methylene groups in ligand side chains). Ligands act1–8 are positively charged (+2). Ligands act and act0 have zero charge.

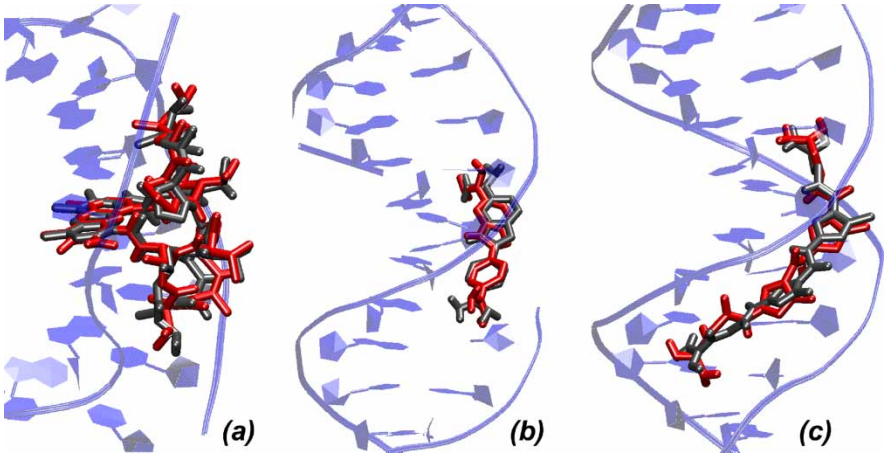

Figure 3. A comparison of the ligand configurations from PDB structures (dark grey) with the ligand configurations obtained by flexible docking method (light grey; red in online version): (a) AMD–DNA complex, (b) DAPI–DNA complex, (c) netropsin–DNA complex. Ligands are shown using stick rendering. DNA is shown using ribbons rendering.

obtained. Some complexes from this set had almost the same energy but a substantially different structure. We considered complex with the minimal binding energy as the most probable and performed analysis of its structure.

The data on the binding energy of actinocin derivatives to DNA fragments are shown in figure 4. It was received that actinocin derivatives could form energetically favourable complexes with DNA both as intercalators and minor groove binders. It can be seen (see figure 4) that in the case of intercalation, the absolute value of the binding energy of actinocin derivatives to DNA fragments was higher than in the case of minor groove binding. Complexes of the same type (intercalation or minor groove binding) of act1–8 with DNA had a little difference in binding energy among

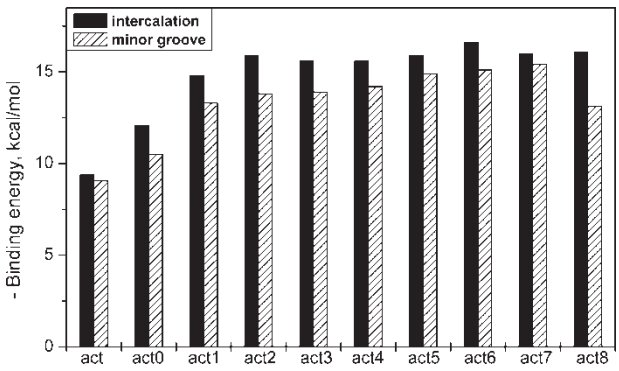

Figure 4. The energy of binding of actinocin derivatives to DNA (black—the energy of intercalation complexes, hatched—the energy of minor groove binding complexes).

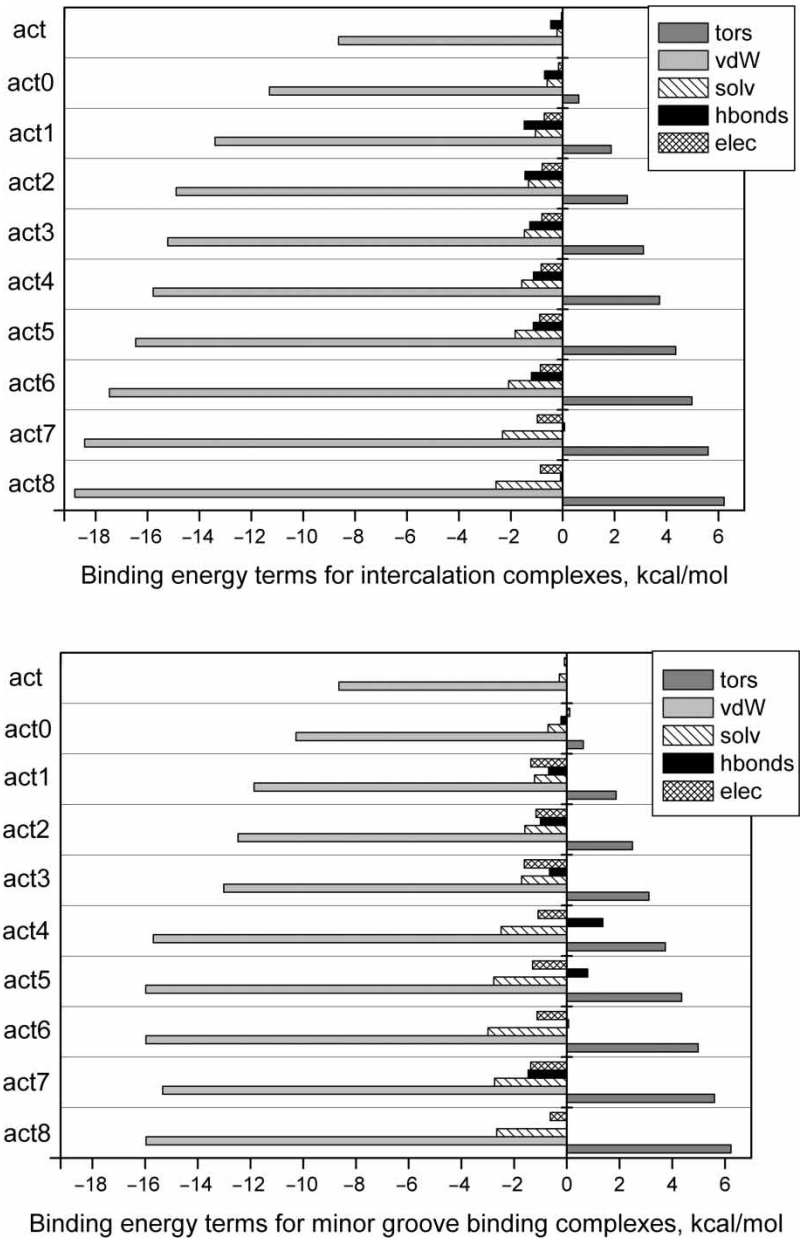

Figure 5. The Autodock's binding energy terms for actinocin derivatives–DNA intercalation complexes (5a) and minor groove binding complexes (5b). Here, tors is a torsional energy term that describes the loss of torsional degrees of freedom of ligand upon its binding to target-molecule; vdW is van der Waals energy term; solv is a ligand desolvation energy; hbonds represents hydrogen bonding; elec is an electrostatic energy term.

themselves (about 1.8–2.3 kcal/mol). The contribution of phenoxazone chromophore (act) to the binding energy of act1–8 to DNA was about 60% both in the case of intercalation and in the case of minor groove binding.

The complex of act2 with DNA, which is considered to be the most stable according to literature data, had no evident advantage in comparison with the other actinocin derivatives-DNA complexes. May be the reason of this is the implicit water representation (as a distance-dependent dielectric function) in the AutoDock 3.05 program. It is known from the IR-spectroscopy data and from the Monte Carlo simulation that water molecules play an important role in the stabilization of act2-DNA complex [21]. Therefore, explicit water representation may be critical for energy evaluation.

The docking method has shown that actinocin derivatives did form two types of complexes with DNA structures and they could be both intercalators and groove binders.

To understand what interactions play the key role in the binding of actinocin derivatives to DNA fragments we decomposed the binding energy into AutoDock energy terms (see figure 5a and b). It can be seen that van der Waals interactions made the greatest contribution to the stabilization of both intercalation and minor groove binding actinocin derivatives-DNA complexes. The electrostatic term that is supposed to be important for such highly charged systems as DNA molecules in our case was rather small (about −1 kcal/mol). The ligand desolvation free energy term also gave small favourable

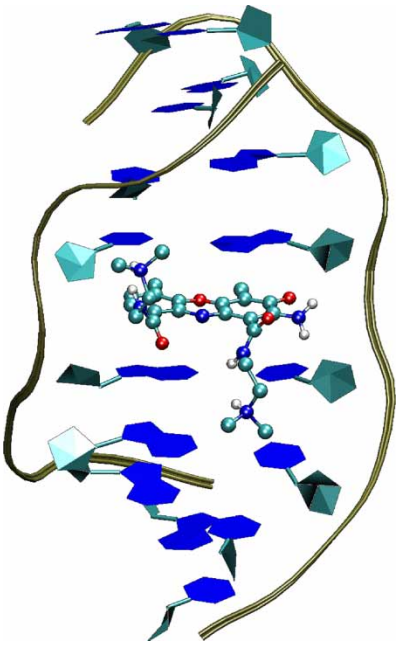

Figure 6. The act2–DNA intercalation complex. Ligand is shown using balls and cylinders rendering. DNA is shown with ribbons. View into the minor groove of DNA.

contribution to the total binding energy. In AutoDock, the desolvation energy term is only calculated for aliphatic and aromatic carbon atoms in the ligand. Therefore, the favourable negative value of this term can be explained by the favourable hydrophobic interactions between ligand's carbons and DNA molecule. To take into account, the unfavourable effects of the desolvation of charged ligand tails upon binding to the DNA we performed further detailed analysis of the solvation energy change (see section 3.3). The torsional term in AutoDock scoring function is responsible for the loss of conformational degrees of freedom of ligand upon its binding to the molecule-target. This term is proportional to the number of rotatable bonds in the ligand that rotate heavy atoms, therefore, it's unfavourable contribution linearly increased with the ligand side chains length growth. The contribution of the hydrogen bonding term was favourable for the intercalation complexes of actinocin derivatives and DNA fragments. In the case of minor groove binding, the value of this term was highly dependent on the complex type.

The most probable intercalation type complexes for all actinocin derivatives with DNA had the similar structure with phenoxazone chromophore located among the GC-base pairs of the DNA-target molecule and side chains of ligand lying in the minor groove (see figure 6). The NH_2-group of the phenoxazone chromophore, that is known to be critically important for the biological activity of AMD, formed hydrogen bonds with sugar phosphate backbone of the DNA. Nitrogen atoms in the ligand side chains also took part in the complex stabilization due to hydrogen bonding with DNA. The number of hydrogen bonds between the actinocin derivative and DNA fragment was equal to six at the average in the case of intercalation.

In the case of minor groove binding, there were three types of complexes of actinocin derivatives and DNA fragments. They are shown in figure 7. Actinocin derivatives act, act0, act4–6 formed with DNA fragments minor groove complex of (a) type. In this type of complex, the longitudinal axis of phenoxazone chromophore was oriented along the minor groove and the chromophore itself was outside the groove, whereas, the ligand side chains lied inside the minor groove. This interesting structure can be used for an explanation of UV–visible spectrophotometry results that showed a possibility for ligand stacking aggregates formation on the DNA matrix.

The (b) type complex was formed upon binding of act1–3 and act8 in the minor groove of the DNA. Actinocin derivative act7 formed (c) type minor groove binding complex. In both (b) and (c) type complexes, chromophore was perpendicular to the minor groove and one of the ligand side chains lied in the groove. The NH_2-group of the phenoxazone chromophore was oriented towards the minor

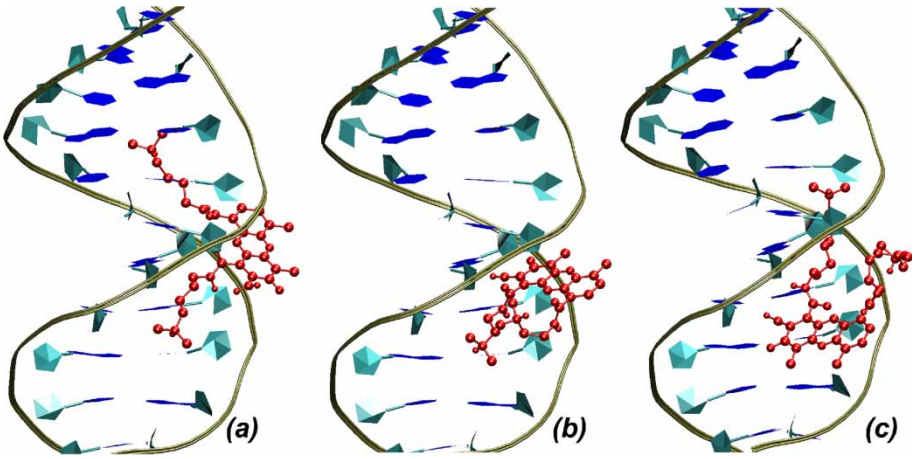

Figure 7. The actinocin derivatives-DNA minor groove binding complexes: (a) act4–DNA complex, (b) act2–DNA complex, (c) act7–DNA complex. Ligands are shown using balls and cylinders rendering. DNA is shown with ribbons. In (b) and (c) type complexes, the amino group of ligand's chromophore is oriented towards the minor groove.

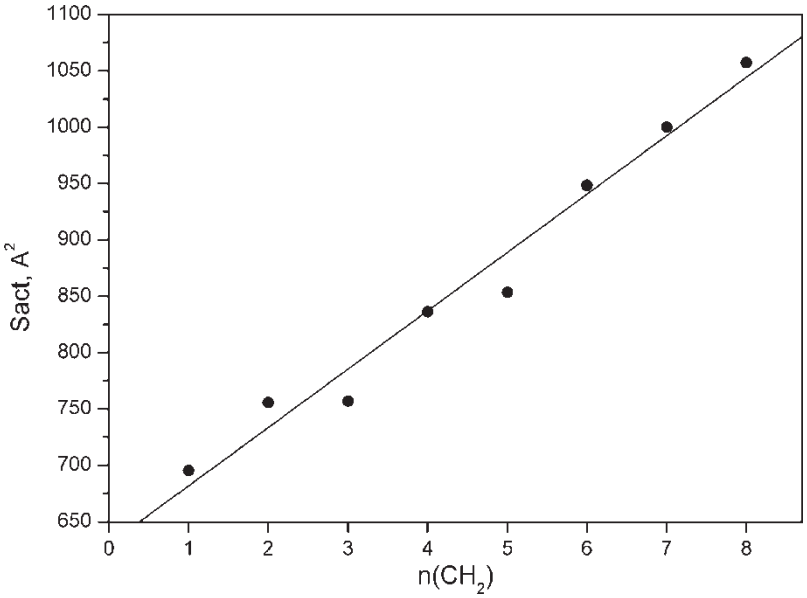

Figure 8. The SAS of actinocin derivatives (*n*—number of methylene groups in ligand side chains).

groove and took part in hydrogen bonding with DNA molecule.

The number of hydrogen bonds between actinocin derivative and DNA fragment varied from 2 to 6 depending on the complex structure and was equal to 5 at the average. The complexes of (a) type had less number of hydrogen bonds than (b) and (c) complexes. This correlates with the positive value of hydrogen bonding term for act4 and act5 minor groove binding complexes (see figure 5b). The unfavourable contribution of the hydrogen bonding term to the total binding energy can be described as energy penalty for oxygen and nitrogen ligand's atoms that did not take part in the hydrogen bonding with DNA molecule.

The complexes of actinocin derivatives and DNA fragments were stabilized by hydrogen bonding upon intercalation and minor groove binding.

3.3 SAS and solvation energy calculations

It is known that hydrophobic interactions play an important role in the stabilization of structure of biological molecules. The SAS area changes upon binding of two molecules in water. The change of SAS can be used as an additional criterion of complex stability: the more is the surface change the more stable complex is formed. We used this criterion for the characteristic of actinocin derivatives–DNA complexes obtained by docking method.

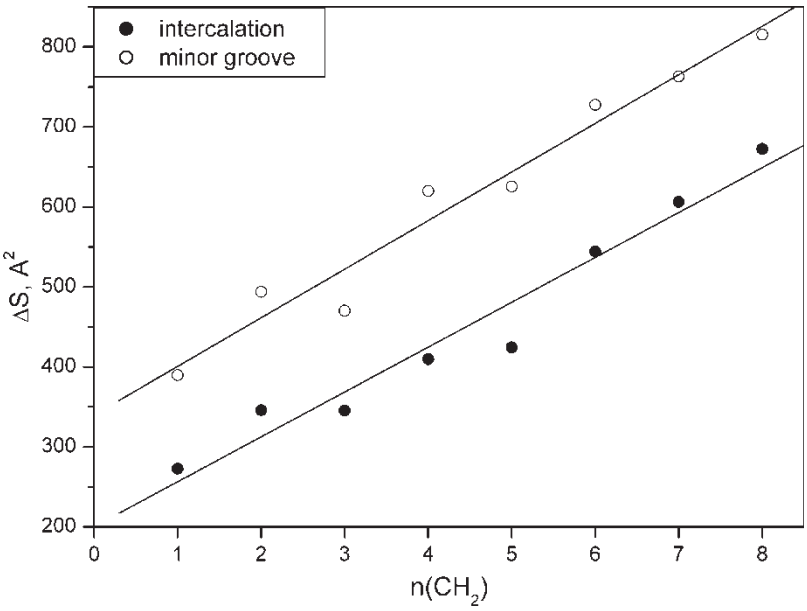

Figure 9. The SAS change upon binding of actinocin derivative to DNA (*n*—number of methylene groups in ligand side chains).

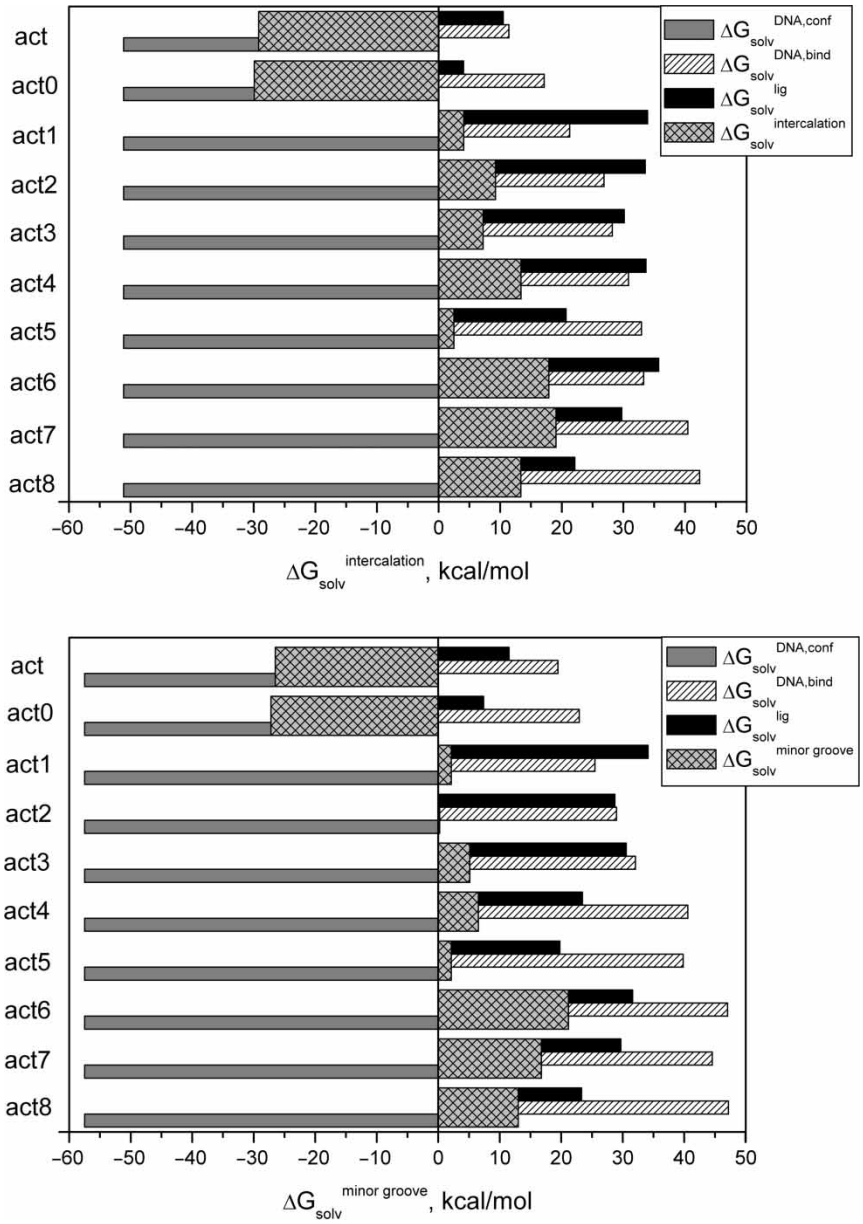

Figure 10. The solvation energy change upon intercalation of the actinocin derivative in the DNA (10a) and upon binding of the actinocin derivative in the minor groove of the DNA (10b). Here $\Delta G_{solv}^{DNA,conf}$ is the change of the solvation energy caused by the conformational rearrangement of the DNA; $\Delta G_{solv}^{DNA,bind}$ is the energy penalty due to the desolvation of the DNA upon ligand binding; ΔG_{solv}^{lig} is the solvation energy change of ligand upon its binding to the DNA.

The data on the SAS of actinocin derivatives are shown in figure 8. It can be seen that the surface of ligand linear increased with the side chains length growth. We also calculated SAS of actinocin derivatives–DNA complexes. It was obtained that SAS of complexes was ligand side chains length-independent: the SAS of complexes varied in the range of 1–2% for different actinocin derivatives (data not shown).

The data on the SAS change upon binding of actinocin derivative to DNA are shown in figure 9. One can see that SAS change upon ligand–DNA binding linear increased with the ligand side chains length growth both in the case of intercalation and minor groove binding.

An additional characteristic of a target–ligand complex stability is the change of solvation energy upon binding of two molecules. Interactions with water are of great importance especially for charged systems such as DNA molecules. The solvation energy could be evaluated using WSAS data [20]. We calculated the solvation energy change upon binding of the actinocin derivative to the DNA according to the following equation:

$$\Delta G_{solv} = \Delta G_{solv}^{lig} + \Delta G_{solv}^{DNA} \quad (1)$$

where ΔG_{solv}^{lig}—is the solvation energy change of ligand upon its binding to the DNA; ΔG_{solv}^{DNA}—is the solvation energy change of DNA upon ligand binding. In its turn,

ΔG_{solv}^{DNA} could be expressed as:

$$\Delta G_{solv}^{DNA} = \Delta G_{solv}^{DNA,conf} + \Delta G_{solv}^{DNA,bind} \quad (2)$$

The fist term in this equation represents the change of the solvation energy caused by the conformational rearrangement of the DNA. The second term describes the energy penalty due to the desolvation of the DNA upon ligand binding.

The results on the solvation energy change are shown in figure 10a and b. It can be seen that uncharged ligands (act and act0) have favourable solvation energy change upon binding to the DNA. This can be explained mainly by hydrophobic interactions. As for the charged ligands (act1–8), the solvation energy change is unfavourable and it increases with the ligand side chains length growth. This can be caused possibly by the destruction of the DNA and ligand solvation shells and partial desolvation of charged ligand tails upon its binding to the DNA.

4 Conclusions

The PDB structures of actinomycin D–DNA, DAPI–DNA and netropsin–DNA complexes were reproduced by rigid and flexible docking methods with program package AutoDock 3.05. It was shown that docking with AutoDock 3.05 could be applied to the systems containing nucleic acids as targets.

Flexible docking of DNA fragments and actinocin derivatives (act, act0, act1–8) was carried out. It was obtained that actinocin derivatives could form energetically favourable complexes with DNA both as intercalators and minor groove binders.

It was shown that small change in binding energy (~1 kcal/mol) could result in complexes with substantially different structure. The complexes of actinocin derivatives and DNA fragments were stabilized by hydrogen bonding upon intercalation and minor groove binding.

It was obtained that the change of SAS area upon binding of the actinocin derivative to DNA linear increased with the growth of methylene groups' number in ligand side chains.

The solvation energy change upon binding of actinocin derivatives to DNA calculated by WSAS method was favourable in the case of small uncharged ligands (act and act0) and unfavourable for positively charged ligands (act1–8).

References

[1] H.A. Gabb, R.M. Jackson, M.J.E. Sternberg. Modeling protein docking using shape complementary, electrostatic and biochemocal information. *J. Mol. Biol.*, **272**, 106 (1997).

[2] G. Schneider, H-J. Böhm. Virtual screening and fast automated docking methods. *Drug Discov. Today*, **7**, 64 (2002).

[3] I. Halperin, B. Ma, H. Wolfson, R. Nussinov. Principles of docking: an overview of search algorithms and a guide to scoring functions. *Proteins*, **47**, 409 (2002).

[4] G.R. Smith, M.J.E. Sternberg. Prediction of protein–protein interactions by docking methods. *Curr. Opin. Struct. Biol.*, **12**, 28 (2002).

[5] A. Lauria, P. Diana, P. Barraja, A. Montalbano, G. Dattolo, G. Cirrincione. Docking of indolo- and pyrrolo-pyrimidines to DNA. New DNA-interactive polycycles from amino-indoles/pyrroles and BMMA. *ARKIVOC*, 5, 263 (2004).

[6] S.J. Farber. Chemotherapy in the treatment of leukemia and Wilm's tumor. *J. Am. Med. Assoc.*, **198**, 826 (1996).

[7] F. Takusagawa, R.G. Carlson, R.F. Weaver. Anti-leukemia selectivity in actinomycin analogues. *Bioorg. Med. Chem.*, **9**, 719 (2001).

[8] J. Feigon, W.A. Denny, W. Leupin, D.R. Kearns. Interactions of antitumour drugs with natural DNA: ^{1}H-NMR study of binding mode and kinetics. *J. Med. Chem.*, **27**, 450 (1984).

[9] S. Kamitory, F. Takusagawa. Crystal structure of the 2:1 complexes between d(GAAGCTTC) and the anticancer drug actinomycin D. *J. Mol. Biol.*, **225**, 445 (1992).

[10] H. Chen, X. Liu, D.J. Patel. DNA binding unwinding associated with actinomycin D antibiotics bound to partially overlapping sites on DNA. *J. Mol. Biol.*, **258**, 457 (1996).

[11] X. Liu, H. Chen, D.J. Patel. Solution structure of actinomycin-DNA complexes: drug intercalation in isolated G–C sites. *J. Biomol. NMR*, **1**, 323 (1991).

[12] J. Mazerski, I. Antonini, S. Martell. The role of side chains in the interaction of new antitumor pyrimidoacridinetriones with DNA: molecular dynamics simulations. *Acta Biochem. Polonica*, **47**, 47 (2000).

[13] B. Wellenzohn, W. Flader, R.H. Winger, A. Hallbrucker, E. Mayer, K.R. Liedl. Significance of ligand tails for interaction with the minor groove of B-DNA. *Biophys. J.*, **81**, 1588 (2001).

[14] L. Karawajew, E.N. Glibin, V.Ya. Maleev, G. Czerwony, B. Droken, D.B. Davies, A.N. Veselkov. Role of crown-like side chains in the biological activity of substituted-phenoxazone drugs. *Anti-Cancer Drug Des.*, **15**, 331 (2000).

[15] A.N. Veselkov. Structure–activity relation for synthetic phenoxazone drugs. Evidence for a direct correlation between DNA binding and proapoptotic activity. *Eur. J. Biochem.*, **270**, 4200 (2003).

[16] V.Ya. Maleev, M.A. Semenov, E.B. Kruglova, T.V. Bolbukh, A.I. Gasan, E.G. Bereznyak, A.V. Shestopalova. Spectroscopic and calorimetric study of DNA interaction with a new series of actinocin derivatives. *J. Mol. Struct.*, **645**, 145 (2003).

[17] W.D. Cornell, P. Cieplak, C.I. Bayly, I.R. Gould, K.M. Merz, D.M. Ferguson, D.C. Spellmeyer, T. Fox, J.W. Caldwell, P.A. Kollman. A second generation force field for the simulations of proteins and nucleic acids. *J. Am. Chem. Soc.*, **117**, 5179 (1995).

[18] W.L. Jorgensen, J. Chandrasekhar, J. Madura, R.W. Impey, M.L. Klein. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.*, **79**, 926 (1983).

[19] G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew, A.J. Olson. Automated docking using a Lamarckian genetic algorithm and empirical binding free energy function. *J. Comput. Chem.*, **19**, 1639 (1998). http://www.scripps.edu/pub/olson-web/dock/autodock.

[20] J. Wang, W. Wang, S. Huo, M. Lee, P.A. Kollman. Solvation model based on weighted solvent accessible surface area. *J. Phys. Chem. B*, **105**, 5055 (2001).

[21] E.G. Bereznyak, M.A. Semenov, T.V. Bolbuch, E.V. Duchopelnikov, A.V. Shestopalova, V.Ya. Maleev. The study of the water influence on the interaction of DNA and actinocin derivatives with different length of aminoalkyl side chains by IR-spectroscopy and computer simulation methods. *Biofizika*, **47**, 1005 (2002).