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DIMERIZATION ENERGETICS OF DNA MINOR GROOVE BINDERS

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The energy analysis of a dimerization in aqueous solutions of seven biologically active lexitropsins, which are different by structure, was carried out with the use of the molecular simulation method. The main stabilization of dimers was shown to take place owing to hydrophobic and intermolecular van der Waals interactions. The latter are mainly associated with energy-favorable contacts between the aromatic rings of molecules and their peptide groups. Despite the significant dipole moments of the molecules concerned, the electrostatic interactions are relatively weak and destabilize the complexes because of the unfavorable relative arrangement of molecular dipoles. Entropic factors and the dehydration were shown to also hinder the dimerization.

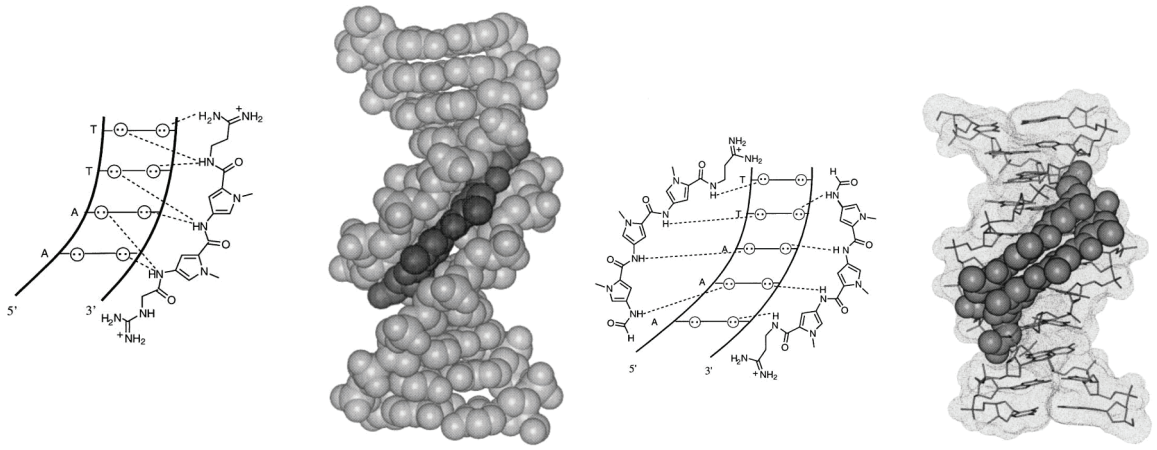
K e y w o r d s: lexitropsins, dimer, peptide group, aromatic ring, energy contributions.

1. Introduction

Small molecules binding with a two-helical DNA one, when being placed into the minor groove of the latter (the so-called Minor Groove Binders or MGB ligands), have a crescent shape that reproduces the groove profile and allows the ligand to be arranged in the groove without substantial conformational changes in both the ligand and the DNA duplex, with hydrogen bonds specific to AT tracts being formed at that [1]. A lot of MGB ligands–first of all, lexitropsins–reveal an antineoplastic action, so that they are widely used in clinical practice for the chemotherapy of cancer diseases [2]. Some MGB ligands are applied as fluorescent DNA markers, e.g., DAPI and Hoechst33258.

The main representatives of lexitropsins are distamycin (DM) and netropsin (NT) (see Fig. 1). They are natural tripeptides possessing a considerable antiviral and antitumoral activity associated with the inhibition of the DNA replication and transcription [2]. The arrangement of a NT molecule in the minor groove of a DNA duplex was discovered for the first time as a new type of binding, alternative to the intercalation [3]. At the monomeric (1:1) binding, the molecule of a MGB ligand occupies the minor groove center and forms the bifurcation H-bonds with each nitrogenous base in the given DNA base pair [4] (see Fig. 1, *a*). Therefore, such a ligand cannot discern between the AT/TA and GC/CG base pairs. Hence, in the case of 1:1 complex, the MGB ligand reads out only half information from the DNA minor groove.

While studying the complex formation of DM with DNA, it was found for the first time that, at DM concentrations much higher than that of the biopolymer, DM binds highly cooperatively with the latter following the dimer mechanism (see Fig. 1, *b*) [5]. The dimer mechanism of complex formation is partly driven by the fact that DM carries a unit positive charge of a diamidine group at one of its ends, whereas NT does it at both ends (see Fig. 1). This circumstance makes



a *b*

Fig. 1. Structures of 1:1 complexes of netropsin (*a*) and distamycin (*b*) with DNA

an antiparallel arrangement of two DM molecules possible. In this case, each of two ligand molecules forms H-bonds with "its own" DNA strand, i.e. each DNA strand becomes "recognized" separately. Therefore, in the context of the molecular recognition efficiency, the dimer binding has two main advantages over the monomer one. First, it enables the AT/TA and GC/CG base pairs to be distinguish from each other. Second, this double read-out of a nucleotide sequence is twice more reliable in comparison with the monomer one. Nowadays, the high toxicity and the restricted specificity of DM stimulates a search for new lexitropsins, also with the dimer mechanism of binding [6].

It was considered till now that a dimer of MGB ligands is formed directly on DNA by the consecutive binding of single ligand molecules with the latter. However, it was shown recently that a dimer can be formed irrespective of DNA in a free solution, and this newly formed dimer gets bound afterward with the biopolymer minor groove [7–9]. There are reasons to believe that such an "algorithm" of complex formation is entropically more beneficial than a consecutive molecule-by-molecule binding of single molecules. Moreover, the binding of an already existing dimer with a DNA molecule actually corrects our representations concerning the molecular mechanism of complex formation between the nucleic acids and compounds belonging to the lexitropsin class and points to a key role of the MGB-ligand dimerization in a free solution. In this connection, there emerges a problem, which has not been considered for MGB ligands till now, namely, the establishment of main regularities in the MGB-ligand dimerization in aqueous solutions and the revealing of physical factors that stabilize those ligands. In some earlier works (see, e.g., works [8, 9]), only general thermodynamic parameters of dimerization were experimentally measured for MGB ligands Hoechst33258 and AIK-18/51; in particular, these are the variations of the Gibbs energy, ΔG, enthalpy, ΔH, entropy, ΔS, and heat capacity, ΔC_P. However the issue concerning the physical factors that stabilize MGB dimers has not been raised till now.

In this work, we carried out the energy analysis of the molecular binding between seven MGB ligands, the complex structures of which were studied relatively well [10–16], and DNA following the dimer mechanism. This makes it possible to carry out the energy calculations for dimer structures corresponding to nuclear magnetic resonance (NMR) or X-ray diffraction (XRD) analysis data and directly "cut out" from experimentally determined dimer-DNA structures. The following lexitropsins were studied (see Fig. 2): AR1-144 (AR), DM, and thiazotropsin A (TZA), as well as unnamed compounds (marked as PA1 to PA4) synthesized by the Dervan group [14–16].

2. Methods

2.1. General approach to the energy analysis

The energy analysis technique was described in our previous works [17, 18] in detail. In brief, its essence consists in the following. The total change of the

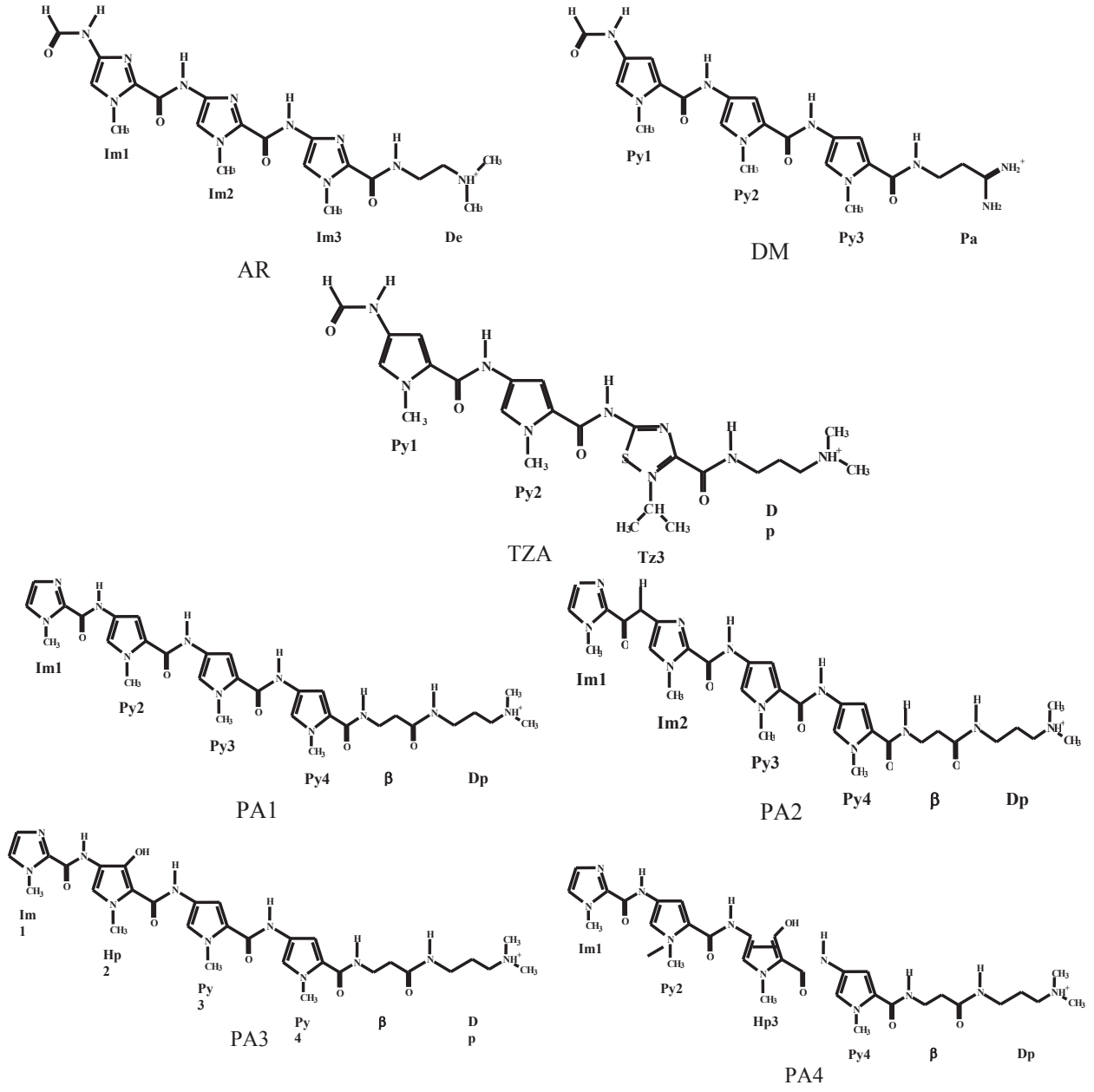


Fig. 2. Structural formulas of the studied lexitropsins

Gibbs energy, ΔG_{total}, in the reaction of molecule dimer formation is composed of energy components that are related to various physical factors,

$$\Delta G_{\text{total}} =$$

$$= \Delta G_{\text{VdW}} + \Delta G_{\text{el}} + \Delta G_{\text{hyd}} + \Delta\Delta G_{\text{HB}} + \Delta G_{\text{entr}}, \quad (1)$$

where the subscripts mark the energy contributions made by van der Waals (VdW), electrostatic, and hydrophobic interactions, hydrogen bonds, and specific factors of the mainly entropic nature, in that order.

Assuming that there are no substantial conformational modifications of lexitropsin molecules in the course of their complex formation in the aqueous so-

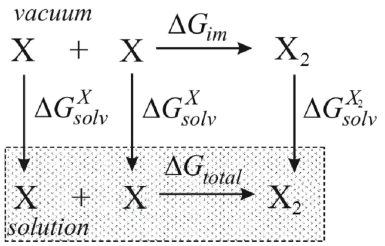


Fig. 3. Thermodynamic cycle for the calculation of the energy parameters of the molecular dimerization in an aqueous solution

lution, each component in Eq. (1) can be calculated with the help of a thermodynamic cycle (Fig. 3). The main feature of the latter consists in the calculation of the free energy separately in vacuum (the intermolecular component $\Delta G_{\rm im}$) and in the aqueous phase (the solvation component $\Delta G_{\rm solv}$). The terms in Eq. (1) were calculated using a combination of empirical and nonempirical techniques [17, 18].

2.2. Molecular dynamics (MD)

The dimer structures of MGB ligands AR, DM, TZA, and PA1 to PA4 were taken from the PDB databank (the parameters of their complexes with DNA – their PDB IDs are 1B0S, 378D, 1RMX, 1CVX, 365D, 407D, and 1CVY, respectively – were selected). The dimers were "cut out" from their complexes with DNA and used as initial structures in the MD procedure. The aqueous environment was set explicitly using the molecules of the model TIP3P located in a cubic box with an edge length of 35 Å. The atomic charges and dipole moments of MGB ligands were calculated by applying the Merz–Kollman method at the level of density functional theory (the B3LYP functional) with the 6-31G basis set and with the help of the software package Gaussian03 [19].

The geometry of complexes in the aqueous medium was optimized by minimizing the potential energy with the use of the conjugate gradient method. The parameters of noncovalent interactions corresponded to the AMBER force field [20]. At the first energy minimization stage, the coordinates of ligand atoms were fixed, whereas the water molecules were allowed to relax at their equilibrium positions. The second stage of energy minimization was carried out with the fixed water molecules. The final stage of the geometry optimization was carried out without imposing any restrictions on the motions of atoms in the system.

After the potential energy had been minimized, the MD procedure was executed according to the Verlet algorithm at a constant temperature of 298 K and with the use of the X-PLOR software package [21]. For a time step of 2 fs to be used, the motions of hydrogen atoms were restricted with the help of the SHAKE procedure. The evolution time for each system was selected to equal 80 ps. The coordinates of all atoms were stored in 1 ps. Earlier, it was demonstrated [18] that, in the case of complexes of small molecules in a solution, the used time parameters of the evolution of the system turned out rather sufficient for the time-averaged values of energy components in Eq. (1) to be determined quite reliably.

2.3. Van der Waals energy

The VdW energy was calculated in the framework of the standard technique with the help of Lennard-Jones potential, which implicitly involves the dispersion, induction, and orientational components, as well as the repulsion of the electron shells of atoms in the form

$$G_{\rm VdW} = \frac{A}{r^{12}} + \frac{B}{r^6}, \qquad (2)$$

where r is the distance between interacting atoms; and A and B are the repulsion and attraction, respectively, parameters, which depend on the type of atoms and their chemical environment and which correspond to the AMBER force field used at the simulation. The component $G_{\rm VdW}$ was calculated using the MD trajectories obtained with the help of the X-PLOR software program by averaging them within the last 40 ps of the evolution of the system.

2.4. Electrostatic energy

The electrostatic energy $\Delta G_{\rm el}$ includes the interactions between the partial charges of lexitropsin atoms, water molecules, and salt ions in a solution. The value of $\Delta G_{\rm el}$ was calculated by solving the nonlinear Poisson–Boltzmann equation (NPBE)

$$\nabla\left[\varepsilon(\mathbf{r})\nabla\varphi(\mathbf{r})\right] - \frac{8\pi^2 I}{kT}\sinh\left[\varphi(\mathbf{r})\right] + \frac{4\pi\rho_{\rm f}(\mathbf{r})}{kT} = 0 \qquad (3)$$

with the help of the software program DelPhi [22] widely used for the calculation of electrostatic interactions in biomolecule complexes. In Eq. (3), φ is the

dimensionless electrostatic potential at a given point (described by the radius-vector $\mathbf{r}$) in terms of kT/e units, k the Boltzmann constant, T the absolute temperature, ε the dielectric permittivity of the medium, $\rho_{\rm f}$ the density of fixed charges in the molecule concerned, and I the macroscopic (far from the molecule) ionic strength of the solution. In this work, we put $I = 0.1$ M, which corresponded to standard physiological conditions.

For a system described by NPBE (3), the quantity $G_{\rm el}$ is calculated by integrating over the volume,

$$G_{\rm el} = \iiint\limits_{\infty} \left\{ \frac{\rho_{\rm f}\varphi_{\rm f}}{2} + \rho_{\rm f}\varphi_{\rm m} + \frac{\rho_{\rm m}\varphi_{\rm m}}{2} - \right.$$
$$\left. - (\rho_{\rm m}\varphi + kTc[2\cosh(\varphi) - 2]) \right\} {\rm d}V, \qquad (4)$$

where $\varphi_{\rm f}$ and $\varphi_{\rm m}$ are the potentials created by fixed and mobile (ionic), respectively, charges (naturally, $\varphi_{\rm f} + \varphi_{\rm m} = \varphi$); c is the salt concentration; and $\rho_{\rm m}$ the density of mobile (ionic) charges.

The NPBE involves changes in the electric properties of the nearest hydration sphere of molecules at the complexation, which makes this method the most preferable, while studying electrostatic interactions in aqueous solutions. The hydration layer separates a region with a low dielectric permittivity in the molecule volume and the solvent with $\varepsilon_{\rm e} = 80$. In the NPBE method, the solvent is specified implicitly, and the finite difference method is used to solve Eq. (3). The polarization of ligands was also taken into account implicitly by putting the internal dielectric permittivity of molecules and their complexes $\varepsilon_i = 4$. While calculating the molecular surface of ligands and their complexes, the values of VdW radii corresponded to the AMBER force field [20].

2.5. Hydrophobic energy

Hydrophobic stabilization of complexes is a result of the water displacement from the complex volume into a free solvent, so that the hydrophobic energy $\Delta G_{\rm hyd}$ has mainly an entropic character. While calculating the hydrophobic contribution, the standard empirical approach was applied. It is based on the existence of a linear correlation between the hydrophobic dissolution energy and the variation of the solvent-accessible surface area (SASA) ΔA, i.e.

$$\Delta G_{\rm hyd} = \gamma \Delta A, \qquad (5)$$

where γ is the microscopic surface tension coefficient [23]. The value $\gamma = 50$ cal/(mol $\cdot$ Å^2) [23] has been "calibrated" by us earlier for DNA-binding ligands [24]. The SASA is defined as a locus of the center of test sphere with the radius equal to the VdW radius of the oxygen of a water molecule (1.4 Å) when the sphere moves over the surface confined by the VdW surfaces of the given molecule. The SASA value A was calculated with the help of the software program GETAREA 1,1 [25].

2.6. Hydrogen bonds

The hydrogen bond energy includes the VdW and electrostatic components, as well as specific factors of the quanum-mechanical origin. Two different types of hydrogen binding should be discriminated in the course of molecular complexation in an aqueous solution:

1) formation of H-bonds between molecules and

2) loss of H-bonds with water because of the dehydration by molecules at the complex formation.

The analysis carried out for the structures of lexitropsin complexes showed that no intermolecular H-bonds are formed in them. In this work, to determine the H-bonds of MGB ligands with the aqueous medium, we calculated the average number of water molecules that form hydrogen bonds with hydrophilic atoms (N, O, S) in the examined molecules (the hydration index $N_{\rm solv}$) within last 40 ps of MD. A hydrogen bond was assumed to emerge if the distance between the electronegative atoms of a ligand and the oxygen or hydrogen atoms of water molecules did not exceed 3.2 or 2.4 Å, respectively [26]. The energy of H-bond with water was evaluated on the basis of the empirical expression

$$\Delta\Delta G_{\rm HB}({\rm kcal/mol}) = -2.25\Delta N_{\rm solv}. \qquad (6)$$

Being applied to Eq. (1), this relation demonstrated a good agreement with the experiment for a large number of various DNA-binding ligands [17, 18]. The energy contribution $\Delta\Delta G_{\rm HB}$ has meaning of a correction to the energy of VdW and electrostatic interactions in Eq. (1), which is necessary to make allowance for the hydrogen binding with the aqueous medium in the calculated total energy.

2.7. Entropic contribution

The total entropic contribution $\Delta G_{\rm entr}$ to the Gibbs free energy of molecular complexation is a sum of three major components,

$$\Delta G_{\rm entr} = \Delta G_{\rm tr} + \Delta G_{\rm rot} + \Delta G_{\rm vib}, \tag{7}$$

corresponding to the variations in the free energies of translational, rotational, and vibrational, respectively, degrees of freedom at the complex formation. The terms $\Delta G_{\rm tr}$ and $\Delta G_{\rm rot}$ stem from a partial transformation of the translational and rotational degrees of freedom at the complexation, and they were calculated in the framework of classical statistical thermodynamics [27],

$$\begin{aligned}
&\Delta G_{\rm tr} = \Delta H_{\rm tr} - T\Delta S_{\rm tr},\ \Delta G_{\rm rot} = \Delta H_{\rm rot} - T\Delta S_{\rm rot},\\
&\Delta H_{\rm tr} = \Delta H_{\rm rot} = -\frac{3}{2}RT,\\
&S_{\rm tr} = R\left[\frac{5}{2} + \frac{3}{2}\ln\frac{2\pi mkT}{h^2} - \ln\frac{N_A}{V}\right],\\
&S_{\rm rot} = R\left[\frac{3}{2} + \frac{1}{2}\ln\pi I_x I_y I_z \frac{3}{2}\ln\frac{8\pi^2 kT}{h^2}\right],
\end{aligned} \tag{8}$$

where $N_{\rm A} = 6.02\times10^{23}$ mol^{-1}, $V = 10^{-3}$ m^3, h is Planck's constant, m the molecule mass, and I_x, I_y, and I_z are the main inertia moments of molecules or their complexes calculated using the X-PLOR software package.

The term $\Delta G_{\rm vib}$ corresponds to a variation of the vibrational energy at the complexation. As a result, there emerge new high- and low-frequency vibrational modes associated with changes in the characters of chemical bond vibrations ($\Delta G_{\rm vib1}$) and mechanical vibrations of the ligand, as a whole, regarded as a part of the complex ($\Delta G_{\rm vib2}$): $\Delta G_{\rm vib} = \Delta G_{\rm vib1} + \Delta G_{\rm vib2}$.

In the harmonic approximation, the expressions for the entropy and the enthalpy of first-kind vibrations (vibrations of chemical bonds) look like [27]

$$\begin{aligned}
&S_{\rm vibl} = \frac{1}{T}\sum_{j=1}^{3N-6}\left[\frac{h\nu_j}{e^{h\nu_j/kT}-1} - kT\ln\left(1-e^{h\nu_j/kT}\right)\right],\\
&H_{\rm vibl} = \frac{1}{T}\sum_{j=1}^{3N-6}\left(\frac{h\nu_j}{e^{h\nu_j/kT}-1} + \frac{h\nu_j}{2}\right),
\end{aligned} \tag{9}$$

respectively. Here, N is the number of atoms, and ν_j are the frequencies of normal modes calculated with the help of the Gaussian03 software package and using the Dreiding method [28].

It should be noted that the calculation procedure for the vibrational contribution according to formulas (9) was based on the assumption concerning the harmonic character of chemical bond vibrations. We analyzed this issue in detail for the class of MGB ligands in work [29]. The corresponding results testify to an insignificant variation of the $\Delta G_{\rm vib}$-value if the vibration anharmonicity is taken into account. This fact evidences the correctness of the use of formulas (9) to estimate the energy parameters of the vibrational contribution.

Expressions for the thermodynamic parameters of second-kind vibrations (low-frequency mechanical ones) were derived earlier, while considering the dimerization of aromatic ligands [17, 27]. They can be used here if we suppose that the residual rotational motions of molecules in lexitropsin complexes are little significant, and the vibrations are harmonic. Therefore,

$$H_{\rm vib2} = RT, \quad S_{\rm vib2} = R\ln\frac{kT}{h\nu_r} + R. \tag{10}$$

The parameter ν_r in Eq. (10) is the classical frequency of mechanical vibrations along the coordinate axes $r \in (r, y, z)$,

$$\nu_r = \frac{1}{2\pi}\sqrt{\frac{2K_r}{m_{\rm red}}}, \tag{11}$$

where K_r is the force constant, and $m_{\rm red}$ the reduced mass of interacting molecules determined from the relation $\frac{1}{m_{\rm red}} = \frac{1}{m} + \frac{1}{m} = \frac{2}{m}$.

The magnitude of K_r can be evaluated, by using the square-law approximation for the potential energy $U(r)$ in the case of small vibrations along the direction r:

$$U = U_0 + K_r(r - r_0)^2. \tag{12}$$

Calculations of the dependence $U(r)$ of the intermolecular interaction (VdW + electrostatics) energy in the dimer were carried out with the help of the software package X-PLOR. The results obtained were approximated by Eq. (12) to obtain the K_r-values. The further calculation of ν_r according to Eq. (11) and the application of Eqs. (10) allowed us to obtain the thermodynamic parameters of second-kind vibrations.

3. Results and Their Discussion

3.1. General structural parameters of lexitropsins and their dimer complexes

Let us emphasize some important features in the structure of MGB ligands examined in this work, which are necessary for the following analysis of dimerization energy parameters. Lexitropsin molecules belong to oligopeptides, in which C^{α} atoms are substituted by various aromatic rings. While constructing MGB ligands with the dimer type of binding in the DNA minor groove, the rings of four main types are used the most often: imidazole (Im), pyrrolic (Py), hydroxypyrrolic (Hp), and thiazine (Tz) ones; with each of them performing a specific functional role. In particular, an AR molecule includes three imidazole (Im) rings, whereas an DM one contains three pyrrolic (Py) rings (see Fig. 2). A shortcoming of the Py cycle is its incapability to form intermolecular H-bonds with the DNA minor groove because of the absence of donor and acceptor centers of hydrogen binding in the latter [30]. Therefore, Py antibiotics (besides DM, they include, e.g., NT) form H-bonds with DNA only by means of the imine groups of peptide bonds or end amino groups (Fig. 2), which are hydrogen donors. Accordingly, they can form H-bonds only with acceptor groups of DNA and, as a consequence, reveal their specificity to AT sequences in nucleic acids, but do not discriminate between AT/TA and GC/CG base pairs at that. Hence, it the case of pure Py preparations, the change from the monomer binding (NT) to the dimer one (DM) does not give rise to a pronounced cooperativity and specificity of binding with DNA. At the same time, Py cycles (they are inefficient from the viewpoint of their specificity to DNA) in lexitropsin molecules are substituted by imidazole, hydroxypyrrolic, and thiazine ones, which makes it possible to recognize guanine and discern between the AT and TA base pairs. Therefore, the type of an aromatic ring in the structure of an MGB ligand plays a crucial role in the dimer recognition of a primary DNA structure.

Another important feature of the lexitropsins exhibited in Fig. 2 and forming dimers on DNA consists in that a positively charged chain is attached to the C-end of their oligopeptide sequence. In polyamides PA1 to PA4 synthesized by the Dervan group [14–16], the chain is composed of β-alanine (β) and dimethylaminopropylamide (Dp), and only of Dp in

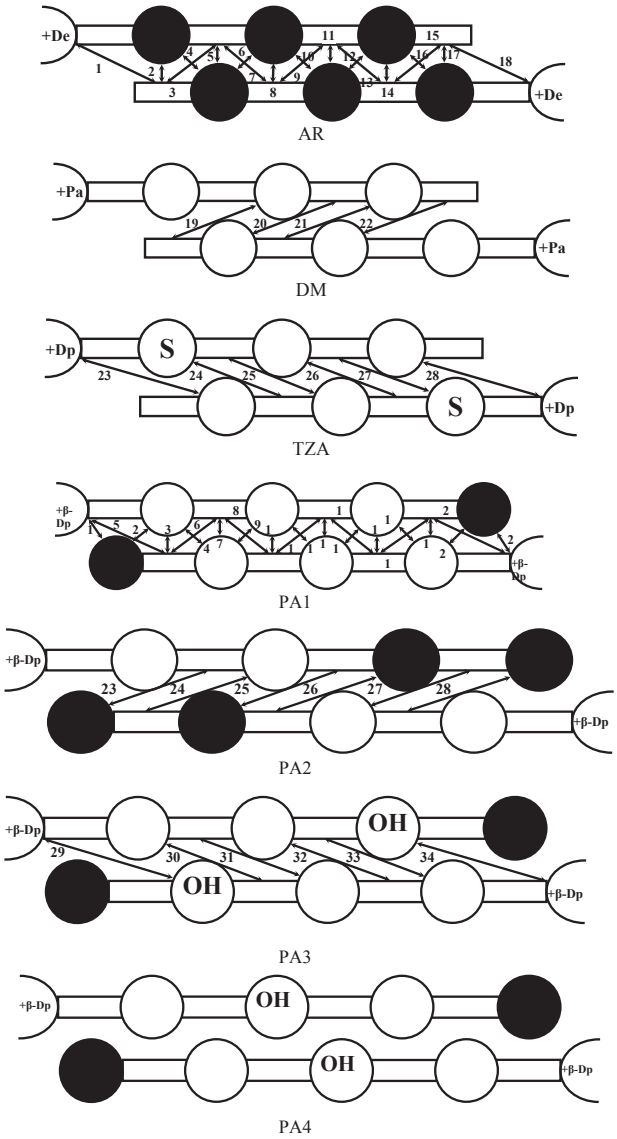


Fig. 4. Diagrams of dimer lexitropsin complexes and van der Waals contacts in them. Black circles stand for imidazole (Im), white circles for pyrrol (Py), S for thiazole (Tz), rectangles for peptide bonds (CONH), and OH for hydroxypyrrole (Hp)

TZA. AR has dimethylaminoethylamide (De) at its end, and DM has propyldiamidine (Pa). The positive charge of MGB ligands plays an important role in their electrostatic interaction with the negatively charged DNA [1–3]. At the same time, the lengths of end chains are selected long enough to weaken their electrostatic repulsion that destabilizes the dimer.

Table 1. **Contributions of various physical factors to the total energy of lexitropsin dimerization in the aqueous solution (kcal/mol)**

Dimer	$\Delta G_{\rm hyd}$	$\Delta G_{\rm el}^{\rm solv}$	$\Delta G_{\rm el}^{\rm im}$	$\Delta G_{\rm vdw}^{\rm solv}$	$\Delta G_{\rm vdw}^{\rm im}$	$\Delta\Delta G_{\rm HB}^{\rm solv}$	$\Delta G_{\rm tr}$	$\Delta G_{\rm rot}$	$\Delta G_{\rm vib1}$	$\Delta G_{\rm vib2}$	$\Delta G_{\rm total}$
AR	–21.7	–1.8	3.5	22.3	–28.7	12.1	11.7	9.9	–3.8	–8.4	–4.9
DM	–20.2	–3.1	5.3	22.2	–24.7	0.0	11.7	9.9	–1.6	–7.7	–8.3
TZA	–20.8	–1.2	3.0	24.4	–27.1	10.0	11.8	10.0	–3.5	–7.7	–1.1
PA1	–27.7	–0.7	2.7	32.6	–37.8	7.1	12.0	10.8	0.5	–9.4	–9.8
PA2	–28.5	–2.4	4.2	31.1	–36.9	9.8	12.0	10.7	–0.2	–9.3	–9.5
PA3	–28.0	–1.4	4.0	35.3	–39.0	5.5	12.0	10.7	–3.0	–8.3	–12.2
PA4	–30.2	–1.3	5.0	29.7	–39.7	14.7	12.0	10.6	–2.5	–9.4	–11.1

In Fig. 4, the schematic structures of dimer complexes constructed in this work are depicted. They were obtained by "cutting" them out from NMR or XRD structures dimer-DNA, and the following minimization by energy in the aqueous box was carries out (see Section 2). The following common features of those complexes can be marked.

First, no appreciable structural transformations in the dimer were observed at the minimization by energy or in the course of MD calculations to determine the VdW energy and the hydration indices. This fact testifies that the dimer structures in the free solution and in the complex with DNA are similar to each other.

Second, in a lexitropsin dimer, the aromatic ring of one dimer is always located over the polar peptide bond of the other (Fig. 4). The specific interaction between them and the physical reasons for such an arrangement remain almost obscured. Some authors mentioned the importance of their mutual polarization [31]. At the same time, it is known that similar interactions play an important role in the stabilization of protein structures (see review [32]). Two types of overlapping between aromatic rings and peptide bonds are possible in dimers: stagger and maximum [31]. In the case of the examined lexitropsins, the former is realized in three-ring molecules (AR, DM, and TZA) with a peptide group at the N-end, and the latter in four-ring ones (PA1 to PA4) without this group (see Fig. 4). Apparently, the stagger overlapping makes it possible to reach a larger distance between the positively charged ends of molecules, provided the same number of contacts "ring–peptide bond", which is important for the stabilization of complexes of shorter (three-ring) lexitropsins.

3.2. General energy analysis of dimerization

The calculated results for the contributions of various physical factors to the total dimerization energy of MGB ligands in the aqueous solution are quoted in Table 1. A direct comparison between the numerically obtained dimerization energy, $\Delta G_{\rm total}$, and the experimental one, $\Delta G_{\rm exp}$, is not possible, since there is a lack of experimental data on the dimerization of the examined MGB ligands in the free solution. Nevertheless, the obtained energies can be estimated quite adequately. In the literature, there are the experimental values concerning the total Gibbs energy $\Delta G_{\rm exp}$ only for the dimerization of four-ring MGB ligands AIK-18/51 (an analog of TZA) [9] and Hoechst33258 [8]; they are equal to -6.0 and -5.0 kcal/mol, respectively. A typical calculation error for the total Gibbs energy of DNA-binding ligands has an order of magnitude of the energy itself [18, 33], because each contribution to $\Delta G_{\rm total}$ in Eq. (1) is a difference between large values. For this reason, the analysis of the calculated $\Delta G_{\rm total}$ value has no sense. However, the $\Delta G_{\rm total}$ values quoted in Table 1 fall within the interval from -1 to -12 kcal/mol, i.e. they correspond to the range of $\Delta G_{\rm exp}$ values for typical MGB ligands. Therefore, the further analysis of the Gibbs energy components calculated with a smaller error of about 1–3 kcal/mol [18] becomes reasonable.

From the data in Table 1, it follows that the main stabilization of complexes is fulfilled by intermolecular VdW interactions $\Delta_{\rm VdW}^{\rm im}$ and, to a lesser extent, by hydrophobic ones $\Delta G_{\rm hyd}$. In this case, the magnitudes of $\Delta G_{\rm vdw}^{\rm im}$ and $\Delta G_{\rm hyd}$ for the four-ring lexitropsins turn out, on the average, larger than those for the three-ring ones. It is interesting to note that the magnitude of $\Delta G_{\rm vdw}^{\rm im}$ for MGB ligands relatively

weakly depends on the ring type – cf. the values for AR (Im_3) and DM (Py_3) – taking the error of its calculation of about 1–3 kcal/mol into account. At the same time, it is well known that, for typical DNA-binding aromatic molecules, the stacking parameters depend rather strongly on the type of their chromophores [18]. Apparently, this is a consequence of the diagonal arrangement of aromatic rings in lexitropsin dimers, which makes the stacking between them less intense and specific and, as a consequence, weakly dependent on the ring type. Note that the magnitudes of $\Delta G_{\rm hyd}$ and $\Delta G^{\rm solv}_{\rm vdw}$ for three- and four-ring molecules also have rather a narrow spread and do not depend practically on the type of aromatic rings that form ligands (see Table 1).

The destabilization of the complexes of MGB ligands occurs owing to a number of entropic factors ($\Delta G_{\rm entr}$) and the dehydration ($\Delta\Delta G^{\rm solv}_{\rm HB}$) at the dimer formation (see Table 1). The transformation of the translational, $\Delta G_{\rm tr}$, and rotational, $\Delta G_{\rm rot}$, degrees of freedom is an entropically unfavorable process, whereas the complexation results in the appearance of new vibrational modes of chemical bonds (vibrations of the first kinds). This process is favorable entropically but not enthalpically, because the extra energy is required to create those vibrations. As a result of such an enthalpic-entropic compensation, the magnitude of $\Delta G_{\rm vib1}$ turns out close to zero, being positive for PA1 and negative for other analyzed lexitropsins. Vibrations of the second kind have a similar physical interpretation. However, the entropic contribution for them unambiguously dominates over the enthalpic one. Therefore, $\Delta G_{\rm vib2} < 0$ and the dimer complexes are considerably stable (see Table 1). But the entropic contribution is energy-unfavorable in general because of the components $\Delta G_{\rm tr}$ and $\Delta G_{\rm rot}$.

Note also that the dehydration contribution to $\Delta\Delta G^{\rm solv}_{\rm HB}$ correlates with the number of hydrophylic atoms (N and S) in the aromatic cycles of three-ring molecules: AR > TZA > DM (see Table 1 and Fig. 3). For the four-ring lexitropsins, each consisting of different types of rings, this regularity is not observed.

As follows from Table 1, the electrostatic intermolecular interactions ($\Delta G^{\rm im}_{\rm el}$) and the interactions with the aqueous medium ($\Delta G^{\rm solv}_{\rm el}$) make rather a small contribution to the dimerization energy of MGB ligands in comparison with other components of the total Gibbs energy. This conclusion was not expectedly evident, because it is usually considered that electrostatics plays a crucial role in the binding of MGB ligands with DNA [34]. This also means that the factor of electrostatic stabilization of the dimers of MGB ligands in the complex with DNA owing to the interaction between the static dipole moments of molecules, which was studied by some authors, is seemingly little significant. In order to elucidate the nature of this effect, we carried out quantum-mechanical calculations of the atomic charges with the use of the software package Gaussian03 (see Section 2) and used them to obtain the dipole electric moments P of molecules entering the dimer complexes (Table 2). Supposing that the positive charge of the dipole is localized at the corresponding edge of the molecule and representing the dipole as a classical system of positive and negative charges ($P = eL_P$), we can estimate the dipole length L_P within the limits of the own size L of the molecule, the overlapping x of molecules in the dimer, and the smallest distance between the molecule axes $z \approx 3.4$ Å (Fig. 5).

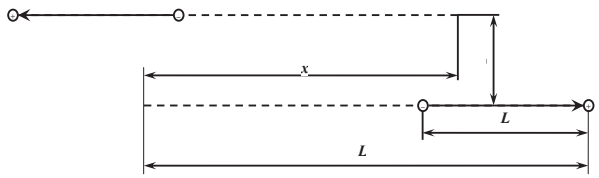


Fig. 5. Model of two dipoles, with AR as an example. L is the length of the molecule, other explanations see in the text. The shift of dipoles and the distance between them are adequately reproduced

Figure 5 demonstrates that the dipoles are actually shifted with respect to each other. The estima-

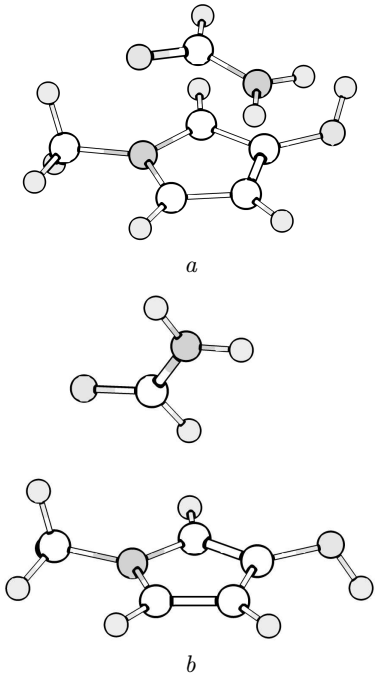
Table 2. **Dipole moments (P) of lexitropsin molecules, their dipole (L_P, Å) and geometrical (L, Å) lengths, overlapping between them (x, Å), and electrostatic energies of dipole-dipole interaction ($U_{\rm el}$, kcal/mol)**

Molecule	P	L_p	L	x	U_{++}	U_{--}	U_{+-}	$U_{\rm el}$
AR	37.2	7.8	≈20	≈14	3.2	7.6	–9.0	1.8
DM	42.4	8.8			3.2	9.2	–9.5	2.9
TZA	38.0	7.9			3.2	7.7	–9.0	1.9
PA1	63.0	13.1	≈26	≈19	2.5	10.9	–8.2	5.2
PA2	51.2	10.7			2.5	6.9	–7.4	2.0
PA3	64.1	13.4			2.5	11.7	–8.3	5.9
PA4	45.8	9.5			2.5	5.8	–7.0	1.3

Table 3. **Average energies of pair VdW contacts between structural elements of lexitropsins (kcal/mol)***

Ring-peptide bond		Ring-ring		With end groups	
Py-CONH	-1.8 ± 0.2	Im-Im	-2.1 ± 0.8	$CONH_2$-De	-1.3 ± 0.01
Im-CONH	-1.9 ± 0.4	Py-Py	-1.9 ± 0.5	$CONH_2$-Pa	-0.4 ± 0.03
Hp-CONH	-1.9 ± 0.1	Hp-Hp	–1.6	$CONH_2$-Dp	-0.6 ± 0.19
Tz-CONH	-2.0 ± 0.6	Tz-Py	-1.7 ± 0.03	Im-(β-Dp+)	-2.7 ± 0.4
Bond-bond		Im-Py	-2.9 ± 0.8	CONH-(β-Dp+)	-0.5 ± 0.1
CONH-CONH	-0.5 ± 0.2	Py-Hp	-2.7 ± 1.3		

*The errors quoted in Table are average intervals of the spread of corresponding energies over various ligands for the given type of contact.



a

b

Fig. 6. Parallel (*a*) and perpendicular (*b*) relative arrangements of the aromatic ring and the peptide group in lexitropsin dimers

tion of the energy of Coulomb interaction between dipole charges in the dimer (see Table 2, $U_{\rm el} = U_{++} + U_{--} + U_{+-}$) with the use of the indicated geometrical parameters shows that the interaction between dipoles is mainly governed by the repulsion between their negative charges, which is the origin of a low significance of the interaction between static dipoles in the dimers of MGB ligands. The corresponding values of $U_{\rm el}$ qualitatively agree with the values $\Delta G_{\rm el}^{\rm im} > 0$ quoted in Table 1. At the same time, an increase of the charge density at the center of a complex near two negative poles of dimers results in an energy-favorable interaction with the aqueous environment, $\Delta G_{\rm el}^{\rm solv} < 0$, owing to the polarization of the latter (Table 1); this effect is well-known for the binding of charged molecules–intercalators with DNA [35].

3.3. Analysis of interaction between structural components of MGB ligands in dimers

As was marked above, the intermolecular VdW interactions make the largest contribution to the stabilization of lexitropsin dimers. In this connection, it is of interest to elucidate the role of VdW interactions between various structural components of MGB ligands in dimers. This problem can be reformulated as follows. Above, we considered the decomposition of the Gibbs energy into contributions according various physical factors. Below, we will consider its decomposition into contributions given by different structural components of MGB ligands.

Let us consider the total VdW energy of intermolecular interactions in a dimer as a sum of terms over VdW contacts connecting two main structural elements of MGB ligands, aromatic rings and peptide bonds (see Fig. 4). The calculated energies of pair VdW contacts are quoted in Appendix (see Table A1) and their averaged values in Table 3.

As follows from Table 3, the interactions of aromatic rings with one another (along the diagonal) and between the aromatic rings and the peptide bonds are approximately identical on the average. It should also be noted an intensive VdW contact between the N-end Im and the C-end chain (β-Dp+) in lexitropsins PA1 to PA4. VdW interactions of the peptide bonds with one another, as well as between the end peptide

Table 4. **Energies of interaction between aromatic rings and a peptide group (kcal/mol)**

CONH+	Parallel arrangement	Perpendicular arrangement (optimized structure)
Hp	–4.09	–16.51
Im	–4.06	–20.89
Py	–4.00	–22.05
Tz	–2.58	–44.24

Appendix. **Energies of pair VdW contacts between structural elements of lexitropsins***

Contact	AR	DM	TZA	PA1	PA2	PA3	PA4
1	–1.3	–0.4	–0.5	–3.3	–2.7	–2.6	–3.6
2	–1.9	–2.0	–1.6	–3.7	–3.2	–2.7	–3.4
3	–0.3	–0.6	–0.6	–1.9	–1.0	–1.7	–1.7
4	–2.8	–1.6	–1.7	–1.4	–1.5	–1.2	–1.7
5	–1.9	–1.9	–1.9	–0.3	–0.3	–0.3	–0.3
6	–1.3	–1.1	–2.5	–0.6	–0.7	–0.8	–1.0
7	–0.6	–0.4	–0.5	–1.6	–1.8	–1.8	–1.9
8	–1.9	–1.7	–2.1	–0.2	–0.3	–0.2	–0.4
9	–0.3	–0.5	–0.5	–3.0	–3.0	–4.0	–2.9
10	–2.3	–1.8	–2.0	–1.7	–2.0	–1.7	–2.1
11	–1.9	–2.0	–2.2	–0.6	–0.5	–0.8	–0.5
12	–1.3	–2.4	–2.4	–1.4	–1.7	–1.5	–1.6
13	–0.6	–0.3	–0.5	–1.9	–1.9	–1.6	–1.8
14	–1.9	–1.9	–1.8	–0.3	–0.3	–0.2	–0.3
15	–0.3	–0.3	–0.5	–2.9	–2.5	–3.9	–2.9
16	–2.8	–2.0	–1.7	–1.8	–1.9	–1.7	–1.7
17	–1.9	–1.9	–2.5	–0.7	–0.6	–0.7	–0.7
18	–1.3	–0.4	–0.7	–1.8	–1.4	–1.1	–1.7
19	–0.05	–0.3	–0.2	–1.8	–1.6	–1.6	–1.4
20	–0.06	–0.2	–0.2	–0.3	–0.4	–0.2	–0.4
21	–0.06	–0.2	–0.1	–3.2	–2.9	–4.1	–2.9
22	–0.05	–0.1	–0.2	–2.6	–3.1	–2.2	–2.7
23	–0.3	–0.03	–0.05	–0.3	–0.2	–0.3	–0.3
24	–0.3	–0.05	–0.06	–0.3	–0.2	–0.3	–0.5
25	–0.3	–0.08	–0.09	–0.3	–0.2	–0.4	–0.2
26	–0.3	–0.07	–0.09	–0.3	–0.2	–0.4	–0.2
27	–0.3	–0.08	–0.06	–0.3	–0.2	–0.3	–0.3
28	–0.3	–0.08	–0.05	–0.2	–0.1	–0.3	–0.2
29				–0.03	–0.04	–0.02	–0.04
30				–0.02	–0.05	–0.02	–0.05
32				–0.03	–0.05	–0.02	–0.04
33				–0.04	–0.07	–0.01	–0.03
34				–0.04	–0.06	–0.01	–0.04

*The numbers in column 1 correspond to contact designations in Fig. 4.

groups and the rings, are rather weak. In this case, every VdW contact, without any exclusion, stabilizes dimer complexes.

The values calculated for the ring-ring energies fall within the interval from −1.6 to −2.9 kcal/mol. However, no pronounced relation was found between those values and the type of rings participating in the interaction (see Table 3). This fact agrees with the conclusion made above about the absence of correlation between the energy of VdW interactions and the type of aromatic rings. In general, the results obtained agree with the available ideas concerning the specificity of interaction of aromatic compounds [36]. At the same time, the stacking of aromatic systems with peptide groups remains rather little-studied. In lexitropsin dimers, the planes of aromatic rings and peptide groups are almost parallel and arranged one over the other (Fig. 6,*a*), which is not typical of such interactions in protein systems. The latter are usually characterized by the perpendicular orientation of an NH-group with respect to the aromatic ring plane [32]. Nevertheless, the results of our quantum-mechanical calculations in the Gaussian (MP2/6-31++G**) environment showed that the energy of interaction between the aromatic rings and the peptide groups at their plane-parallel arrangement still remains negative, although small by absolute value (Table 4), which is in qualitative agreement with the results of molecular-mechanical calculations (Table 3).

Most likely, interactions between the rings and the peptide groups stem from the overlapping of their π-electron clouds. In this case, the interaction energy weakly depends on the ring type (except for Tz, where the ring is distorted by a massive sulfur atom). At the same time, the free geometry optimization for the complexes "peptide bond–ring" expectedly gave rise to the perpendicular arrangement of the planes of those elements (Fig. 6,*b*), in total agreement with their behavior in protein systems [32].

4. Conclusions

In this work, the role of various physical factors in the stabilization of dimer complexes of a promising class of MGB ligands, lexitropsins, as a preliminary phase providing a highly cooperative and highly specific binding between biologically active compounds of this type and DNA, has been studied for the first time. The main stabilization of dimer lexitropsin

complexes in the aqueous solution is shown to occur owing to the intermolecular Van der Waals interactions and, to a less extent, to the hydrophobic ones. The VdW contribution is associated with the energy-favorable contacts between the aromatic rings of molecules, as well as between the rings and the peptide groups. Despite the substantial dipole moments of molecules, the electrostatic interactions are rather weak and destabilize the complex owing to the disadvantageous relative positions of molecular dipoles. The dehydration of molecules at the dimerization and the entropic contributions also turn out energy-disadvantageous.

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ЕНЕРГЕТИКА ДИМЕРИЗАЦІЇ ЛІГАНДІВ,
ЩО ЗВ'ЯЗУЮТЬСЯ В МАЛИЙ ЖОЛОБОК ДНК

Р е з ю м е

Методами молекулярного моделювання виконано енергетичний аналіз димеризації у водному розчині семи різних за структурою біологічно-активних лекситропсинів. Показано, що основна стабілізація димерів відбувається за рахунок гідрофобних і міжмолекулярних ван-дер-ваальсовських взаємодій. Останні зумовлені в основному енергетично вигідними контактами між ароматичними кільцями молекул, а також кілець з пептидними групами. Електростатичні взаємодії, незважаючи на значні дипольні моменти молекул, вельми слабкі і дестабілізують комплекс в силу невигідного взаємного розташування молекулярних диполів. Ентропійні фактори і дегідратація також перешкоджають димеризації.