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MOLECULAR BIOPHYSICS

# General Features of the Energetics of Complex Formation between Ligand and Nucleic Acids

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**Abstract**—The analysis of the energy contributions of various physical factors to the complex formation between biologically active compounds and nucleic acids in aqueous solution was performed. A comparison of the energy parameters was made for ligand–ligand, intercalator–DNA, MGB–DNA and ligand–RNA groups. It was shown that the energetics of these reactions is of compensatory nature. Physical factors exerting the most pronounced influence on the energy parameters were identified. Correlation of the energy contributions to MGB–DNA complex formation and its biological effect was found.

*Keywords*: nucleic acids, biologically active compounds, complexation, free energy decomposition, energetic contributions, physical factors, aqueous solution

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## INTRODUCTION

In the course of the last two decades in molecular biophysics, energetic analysis of biomolecular interactions has emerged and got powerful development. Its appearance as a new direction in molecular biophysics is conditioned by substantial growth of the output of computing techniques, and also development of methods of molecular modeling. In the basis of energetic analysis, there lies theoretical calculation and comparison with each other of the contributions of all possible physical interactions (energetic components) into the experimentally measured Gibbs energy $\Delta G$ of reactions of noncovalent complex formation of biomolecules. Elucidation of the role of each of these contributions has important significance during directed synthesis of new compounds with required energetic characteristics of binding.

For complex formation of biologically active compounds with nucleic acids (NAs) the methodology of energetic analysis was developed most systematically first in works [1–4], and later by authors of the given work [5–10]. By the present day for DNA intercalators [2, 3, 5–7, 10], compounds binding in the DNA minor groove (MGB) [4, 8, 10], some RNA-binding ligands [9, 10] and aromatic π-stacking [10, 11] the energetic analysis factually was already executed. The aim of the given work is analysis and revelation of the regularities of distribution of energetics over various physical factors giving a contribution into total Gibbs energy of reactions of binding of ligands with various types of bioreceptors, on the basis of material accumulated in literature.

## METHOD OF ENERGETIC ANALYSIS

The most heretofore complete decomposition of Gibbs free energy into energetic components may be presented in the form of the following equation [5–11]:

$$\Delta G_{\text{total}} = \Delta G_{\text{conf}} + \Delta G^{\text{im}}_{\text{VDW}} + \Delta G^{\text{solv}}_{\text{VDW}} + \Delta G^{\text{im}}_{\text{EL}} + \Delta G^{\text{solv}}_{\text{EL}} + \Delta G_{\text{HYD}} + \Delta G^{\text{im}}_{\text{HB}} + \Delta G^{\text{solv}}_{\text{HB}} + \Delta G_{\text{entr}}, \quad (1)$$

where superscripts “solv” and “im” denote respectively interaction with aqueous medium and interaction of molecules in complex (in vacuo); $\Delta G_{\text{total}}$ denotes the sum of theoretically calculated energetic components; $\Delta G_{\text{conf}}$ – energetic contribution from conformational changes in molecules upon complex formation; $\Delta G_{\text{VDW}}$, $\Delta G_{\text{EL}}$ and $\Delta G_{\text{HYD}}$ – contributions from van der Waals (VDW), electrostatic (EL) and hydrophobic (HYD) interactions; $\Delta G_{\text{HB}}$ – contribution from the energetics of loss of hydrogen bonds (HB) “to-water” ($\Delta G^{\text{solv}}_{\text{HB}}$) and formation of new intermolecular HB in the complex ($\Delta G^{\text{im}}_{\text{HB}}$); $\Delta G_{\text{entr}} = \Delta G_{\text{TR}} + \Delta G_{\text{VIB1}} + \Delta G_{\text{VIB2}}$ – entropic contribution conditioned by the change of the total number of degrees of freedom of the system: translational + rotational – $\Delta G_{\text{TR}}$ (TR), vibrations of chemical bonds – $\Delta G_{\text{VIB1}}$ (VIB1) and residual mechanical vibrations of ligand in the binding site – $\Delta G_{\text{VIB2}}$ (VIB2). (Equation (1) in explicit form does not take into account two more components – polyelectrolytic contribution and contribution from “charge transfer”, their magnitude in

**Table 1.** Investigated types of ligands

| Ligand [10] | Intercalator–DNA [5, 6 ] | MGB–DNA [8] | Ligand–DNA [9] |
|---|---|---|---|
| Acridine orange | Actinomycin D | Berenil | Adenosinemonophosphate |
| Actinomycin D | Daunomycin | DAPI | Acetylpromazine |
| Caffeine | Ethidium bromide | DB293 | Argininamide |
| Daunomycin | Nogalamycin | Distamycin | Biotin |
| Doxorubicin | Novantrone | DB75 (Furamidine) | Flavin mononucleotide |
| Ethidium bromide | Proflavine | Hoechst33258 | Gentamycin |
| Flavin mononucleotide | Trioxatriangulene | Netropsin | Malachite green |
| Nicotinamide | Fascaplysin | Pentamidine | Novantrone |
| Nogalamycin | Phenosaphranine | Propamidine | RBT203 |
| Norfloxacin | Thionine | SN6999 | Theophylline |
| Novantrone | Ellipticine | | Tobramycin |
| Proflavine | | | |
| Propidium iodide | | | |
| Topotecan | | | |

aqueous medium turns out to be essentially smaller as compared with the rest of energetic components [5].) Inasmuch as reliable separation of the energy of HB- and EL-factors is problematic, in the quality of an index of efficiency of hydrogen bonding it is expedient to use not Gibbs energy but the number of intermolecular hydrogen bonds in complex ($N_{im}$ instead of $\Delta G_{HB}^{im}$) and the change in the number of hydrogen bonds "to-water" upon complex formation ($\Delta N_{solv}$ instead of $\Delta G_{HB}^{solv}$) (see more detailed discussion of the specifics of estimation of the constituent from hydrogen bonds in works [5, 8]).

Calculation and analysis of each component in equation (1) presents in itself an autonomous problem—a general notion about the problematics of such calculations may be obtained from primary source-works [5–9, 11] or from monograph [10]. Let us note only that the main condition imposed on equation (1) comes to be coincidence of total calculated energy $\Delta G_{total}$ with experimentally measured $\Delta G_{exp}$ – in the framework of admissible inaccuracy. Component $\Delta G_{conf}$ gives no immediate contribution into stabilization of ligand–NA complexes, was earlier investigated in detail in works [1, 4, 5, 8] and presents no interest in the context of energetic analysis. The aggregate of the rest of components in equation (1) are the energetic parameters of complex formation considered in the present work.

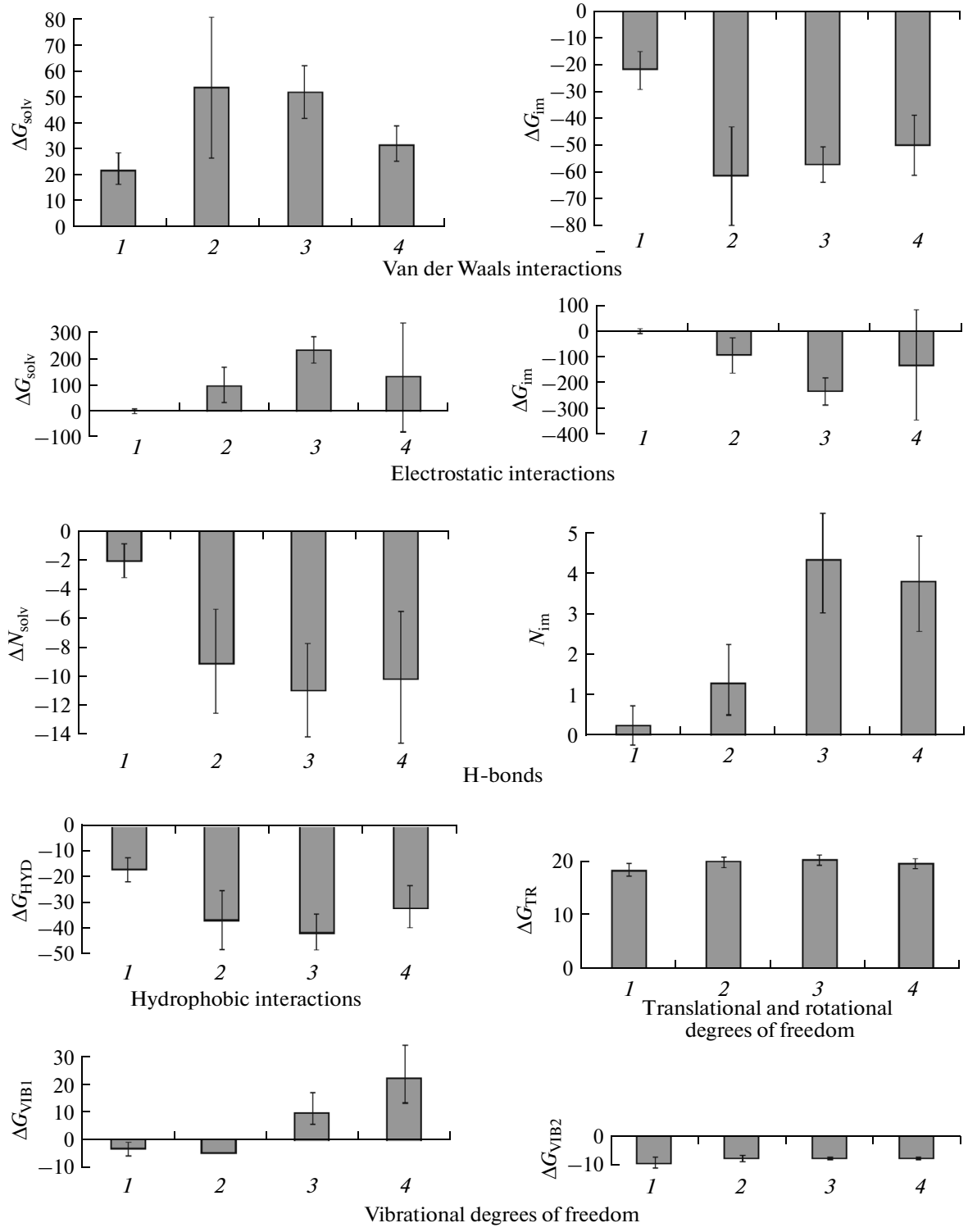
## RESULTS AND DISCUSSION

In the present work we considered four groups (types) of biologically important interactions: intercalation into DNA, minor groove binding (MGB–DNA), binding with RNA and complex formation of aromatic molecules (π-stacking "ligand–ligand"). In works [5–11] a complete calculation is given for components of decomposition of Gibbs energy by equation (1) with the use of a uniform method of molecular modeling and giving a possibility of conducting comparative analysis of energetic parameters both in the limits of a given group and for different groups of interactions. Inasmuch as a characteristic of energetic parameters in the limits of each of the four regarded groups has already been given earlier by various authors [1–13], of interest is a comparison of the components of decomposition just between various groups of interactions – such analysis for a class of ligands binding with NAs has heretofore not been conducted.

In Table 1 we have listed ligands used earlier for calculation of the energetics of complex formation and possessing comparable molecular masses (from 300 to 1000 Da). Each component in equation (1) was averaged over all ligands in the limits of the regarded group of interactions. The results are presented in the figure.

As will be shown below, additional variability introduced by the distinction of ligands in mass does not give a substantial contribution into the results of interpretation of energetic parameters.

**Comparison of energetic parameters of various types of complex formation.** Analysis of the figure




Distribution of energetics in various types of complex formation of NA-binding ligands. Vertical lines indicate intervals of scatter of values of components over investigated ligands.

allows making the following main conclusions about the regularities of the energetics of various types of complex formation with participation of NA-binding ligands.

1. Comparison of averaged components of interaction with aqueous medium (superscripts "solv") and intermolecular interactions (superscripts "im") for VDW, EL and HB points to availability of a vividly

expressed compensation effect, manifesting itself in that the energetics of desolvation of a ligand ($\Delta G^{solv}_{VDW}$, $\Delta G^{solv}_{EL}$, $\Delta N_{solv}$) roughly compensates the energetics of intermolecular interaction in complex ($\Delta G^{im}_{VDW}$, $\Delta G^{im}_{EL}$, $N_{im}$), giving a relatively small value of summary VDW-, EL- or HB-energy. Earlier a similar effect was noted by many authors both for NA-binding ligands [5, 8, 13] and for ligands binding with proteins [4, 14], and, as it appears, reflects a general regularity of energetics of complex formation in aqueous medium.

2. The sign of components of decomposition in the limits of the given type of interaction in a majority of cases turns out to be identical, which points to qualitative similarity of the distribution of energetic parameters for various types of interaction. The only vividly expressed distinction in sign is possessed by the component of chemical bond vibrations $\Delta G_{VIB1}$, assuming relatively large positive values for "ligand–RNA" and "MGB–DNA" systems and relatively small negative values for "intercalator–DNA" and "ligand–ligand" systems. Here we can state a definite tendency: systems with dominance of stacking interactions are characterized by negative values $\Delta G_{VIB1}$, while systems without stacking – by positive ones. The peculiarity of sign of this component has earlier been discussed by various authors in relation to separately taken systems (see, for example, [5, 8, 15]).

3. The components of loss of translational and rotational motions, and also component of residual vibrations $\Delta G_{VIB2}$ are practically invariant to the type of complex formation and type of ligand. In this connection the given components may be regarded in the quality of a systematic additive and do not come to be significant in the context of interpretation of energetic parameters. Earlier by some authors an analogous conclusion was made upon consideration of concrete types of complex formation [2, 10, 16] and, as it appears, cones to be just for ligands possessing comparable molecular masses. In this way, the main components determining the energetics of complex formation come to be VDW, EL, HB, VIB1 and HYD.

4. "Ligand–ligand" systems are characterized on average by the least energetics in the main components. The greatest energetics and roughly in an equal degree (in the limits of scatter) characterizes the "intercalator–DNA" and "MGB–DNA" systems. "Ligand–RNA" systems are characterized in the whole by intermediate energetics. The only component somewhat dropping out of this regularity comes to be the energy of intermolecular H-bonds Nim, which plays the greatest role in "MGB–DNA" and "ligand–RNA" systems, and a smaller role in "intercalator–DNA" and "ligand–ligand" systems. This result is consistent with the notion well known from structural studies about that the specificity of binding of MGB-ligands with DNA and aromatic ligands with RNA is largely determined just by H-bonds, while stacking of aromatic chromophores is the main component of binding specificity in "intercalator–DNA" systems. An additional confirmation to the said cones as that, according to the figure, on average the VDW-factor turns out to be the most significant in "intercalator–DNA" systems, while the EL-factor – in "MGB–DNA" systems. Let us note that this result is obtained by us just as a consequence of energetic analysis over a large number of systems without ties to specifics of separately taken complexes and is fully consistent with results of previous investigations of the distribution of energy over various components but conducted in the limits of separately taken types of interactions [5, 8, 11]. Here, however, it is worth noting that the conducted comparison of energetic parameters comes as exclusively qualitative and on the strength of a statistically small sample of the number of ligands does not allow executing such analysis on the level of quantitative relationship of various energetic components. Possibly, expansion in the future of the volume of a sample of ligands for which solution of the decomposition problem has been successfully conducted will allow refining the revealed regularities. Also it is important to underline that the formulated regularities are valid only for an aggregate of ligands with close molecular masses.

**Correlation of energetic parameters with biological effect.** At the present time in the field of thermodynamic analysis of biomolecular interactions it is customary to believe that every class of ligands can be uniquely characterized not only by its medicobiological profile or physicochemical properties of the very molecules but also by a unique "thermodynamic signature" usually presented in the form of relationship of $\Delta H_{exp}$ and $\Delta S_{exp}$ for different types of ligands and interactions [3]. Such analysis allows obtaining important information about an integral character of dominating forces involved in complex formation, and revealing enthalpic-entropic compensation. For example, intercalation into DNA appears as prevalently enthalpic-controlled while minor groove binding – entropic-controlled [3]. However these conclusions in essence turn out to be useless in an attempt at identification of the main stabilizing and destabilizing factors and in part come to be a source of debates lasting now more than half a century about which factor is the most important in stacking of aromatic systems – HYD or VDW, and what is the relationship of HYD- and EL-factors in stabilization of "MGB–DNA" complexes? The energetic analysis in this sense comes to be a more informative characteristic of the process of complex formation, inasmuch as it gives an answer to the question "Which physical factors and in what mutual relationship stabilize the complexes of ligands with NAs?", and also "Which factor in the greatest degree does influence the ligand affinity to NAs?" (see also discussion in works [5, 8, 11]). However this alone

**Table 2.** Correlation ($r$) of energetic components (kcal/mol), number of hydrogen bonds and equilibrium complex formation constant $K$ ($M^{-1}$) with index of ligand biological activity $ID_{50}$

| MGB–ligand | $ID_{50}$ [16] | $G^{solv}_{VDW}$ | $G^{im}_{VDW}$ | $G^{solv}_{EL}$ | $G^{im}_{EL}$ | $\Delta G_{HYD}$ | $\Delta N_{solv}$ | $N_{im}$ | $\Delta N_{solv} + N_{im}$ | $K$ [11] |
|---|---|---|---|---|---|---|---|---|---|---|
| SN6999 | 0.02 | 54.0 | –64.9 | 258 | –255 | –45.7 | –11.5 | 1 | –10.5 | 2.0–106 |
| Hoechst33258 | 1.50 | 52.7 | –64.1 | 141 | –140 | –46.3 | –14.1 | 4 | –10.1 | 3.2–106 |
| Distamycin | 9.00 | 61.4 | –65.8 | 138 | –136 | –53.0 | –16.4 | 10 | –6.4 | 2.0–105 |
| Netropsin | 10.00 | 75.4 | –63.0 | 266 | –260 | –44.6 | –11.2 | 11 | –0.2 | 1.0–105 |
| Berenil | 10.40 | 43.3 | –45.9 | 267 | –264 | –34.7 | –7.7 | 2 | –5.7 | 1.3–107 |
| $r$ | | 0.27 | 0.48 | 0.20 | –0.20 | 0.25 | 0.24 | 0.58 | 0.83 | 0.27 |

does not exhaust the usefulness of regarding the energetic parameters.

Let us consider on the example of MGB-ligands the possibility of correlation of energetic parameters with an index of biological activity of a ligand. In Table 2 we present the values of components of decomposition from work [8] and index $ID_{50}$ for these same ligands from work [17], representing itself a micromolar concentration of a preparation necessary for 50% suppression of growth of the number of leukemic cells L1210. Regretfully, for the quite limited data ser presented in Table 2 there is no speaking of reliable quantitative correlation, however a qualitative correspondence of energetic components and $ID_{50}$ factor may in principle be analyzed. From Table 2 it follows that the most pronounced correlation of ID50 takes place with an integral effect of change in the number of hydrogen bonds: $N_{im} + N_{solv}$ (a search for analogous correlation with summary energies of VDW ($\Delta G_{VDW}$) and EL ($\Delta G_{EL}$) components cannot be regarded as significant inasmuch as the latter are formed by a sum of two large numbers ($\Delta G_{solv} + \Delta G_{im}$) and are determined with a relatively high error (see more detailed discussion of the problem of analysis of sum energies in works [5, 8, 10, 11]). This result is fully consistent with the conclusion obtained above from analysis of energetic parameters about that it is exactly the H-bonds that come to be among the most important in the distribution of energetics over components upon complex formation of MGB-ligands with DNA. Therewith it is important to underline that correlation with $ID_{50}$ is not observed in relation to the constant of complex formation "MGB–DNA" (as a measure of summary energetics of the given process, see Table 2), but takes place only for the components of decomposition. Moreover, this correlation turns out to be comparatively small for such components as EL, VDW and HYD, giving the greatest contribution into $\Delta G_{total}$ in absolute value.

In essence, the above-obtained result indicates a means of directed modification of the structure of ligand with the aim of optimization of its biological effect, and namely the tendency to increasing the total number of H-bonds forming upon complex formation. To our regret, a search for the same correlation for other types of interactions considered in the work does not yet appear possible on the strength of the absence of a suitable set of data on energetics and biological activity for a coincident series of ligands. However let us note that analogous correlation with biological effect with subsequent formulation of a recommendation to synthesis is impossible to disclose on the basis of traditional thermodynamic analysis on the level of $\Delta G$, $\Delta H$ and $\Delta S$, which testifies to potential practical significance of conducting energetic analysis for complex formation of biologically active compounds with NAs.

## CONCLUSIONS

The subject of investigation in the present works comes to be the distribution of energy over various components responsible for the contribution of various physical factors into total Gibbs energy of reactions of binding of small ligands with DNA and RNA, with the use of earlier published calculated values of the components of decomposition of experimental Gibbs energy. The basic distinction of the used methodology of investigation from previous works of other authors consists not in comparative analysis of components over various physical factors (as it has already been done in works [1–12]) but in comparative analysis of components over various types of complex formation: "ligand–ligand", "intercalator–DNA", "MGB–DNA", "ligand–RNA". By way of qualitative comparison it is established that on average the energy of van der Waals interactions turns out to be the most significant factor in "intercalator–DNA" systems, while electrostatic energy and hydrogen bonds – in "MGB–DNA" systems, therewith "ligand–RNA" systems are characterized on average by intermediate energetics, while "ligand–ligand" systems – the smallest energetics of all investigated types of complex formation. Disclosed is a correlation of the sum change in the number of hydrogen bonds with an index of biological activity of ligand $ID_{50}$ in "MGB–DNA" systems. This result points to potential practical significance of conducting energetic analysis for complex formation of biologically active compounds with

NAs as applied to directed synthesis of new preparations with elevated biological activity.

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