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MOLECULAR BIOPHYSICS

Energy Analysis of Non-Covalent Ligand Binding to Nucleic Acids: Present and Future

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Abstract—Generalization of current views on energy analysis of complex formation of biologically active compounds with nucleic acids is represented and the outlook for further developments is determined.

Keywords: nucleic acids, ligand, non-covalent complex formation, physical factors, energy analysis
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INTRODUCTION

In connection with vigorous development of the productiveness of computing techniques and methodology of molecular modeling in the last 15 years in the field of molecular biophysics there has formed a new scientific direction – energetic analysis of biomolecular interactions. Its subject comes to be determination of contribution of various physical factors into experimentally measured thermodynamic potentials $\Delta G/\Delta H/\Delta S/\Delta C_P$ of reactions of noncovalent complex formation of biomolecules in aqueous medium. It is believed that solution of the given problem may essentially supplement the scientific basis of the modern strategy of directed synthesis of new preparations with preset thermodynamic (energetic) parameters of the reaction of complex formation.

As applied to binding of ligands with nucleic acids (NAs), most systematically and consecutively the strategy of energetic analysis was realized first in works of Chaires et al. [1–3], Jayaram et al. [4] and later in works of Evstigneev and Kostjukov [5–9]. At the present time the energetic profile can be held to be more or less established for a group of DNA-intercalators [2, 3, 5–7], ligands binding in the DNA minor groove [4, 8], and some RNA-binding ligands [9]. The cited works contain full-fledged analysis of results obtained by various research groups on each of the types of "ligand–NA" interactions, and further will not be discussed. The aim of the present work comes as generalization of these results with isolation of problematic points and formulation of prospects of further development of energetic analysis.

METHODOLOGY OF ENERGETIC ANALYSIS OF LIGAND–NUCLEIC ACIDS SYSTEMS

The most complete decomposition of Gibbs free energy into energetic members used by various authors (see works [4, 5, 8, 10] and references therein) may be presented in the form of equation (1):

$$\Delta G_{\text{total}} = \Delta G_{\text{conf}} + \Delta G_{\text{VDW}} + \Delta G_{\text{EL}} + \Delta G_{\text{CT}} + \Delta G_{\text{PE}} + \Delta G_{\text{HYD}} + \Delta G_{\text{HB}} + \Delta G_{\text{entr}}, \quad (1)$$

where ΔG_{total} denotes the sum of theoretically calculated energetic terms; ΔG_{conf} [2, 5, 10, 11] – energetic contribution from conformational changes in molecules upon complex formation; ΔG_{VDW} [5, 8, 11], ΔG_{EL} [11, 12] and ΔG_{HYD} [5] – contributions from van der Waals, electrostatic and hydrophobic interactions; ΔG_{CT} [5, 13] – contribution from interactions conditioned by "charge transfer"; ΔG_{PE} [1–3] – polyelectrolytic contribution; ΔG_{HB} [5] – sum contribution from the energetics of loss of hydrogen bonds «to-water» and formation of new intermolecular H-bonds in complex; ΔG_{entr} [14,15] – entropic contribution conditioned by the change of the total number of degrees of freedom of the system (translational, rotational and vibrational) and also change of NA stiffness upon complex formation. Terms ΔG_{VDW}, ΔG_{EL} and ΔG_{HB} further expand into interaction with aqueous medium and interaction of molecules in complex (in vacuo).

Calculation and analysis of each term in equation (1) presents in itself an autonomous problem—a general notion about the problematics of such calculations may be obtained from cited publication and will not be discussed in the present work. Let us note only that the main condition imposed on equation (1) comes to be coincidence of total calculated energy ΔG_{total} with

experimentally measured ΔG_{exp} – in the framework of admissible inaccuracy.

GENERALIZATION OF RESULTS OF ENERGETIC ANALYSIS

By the present time a sufficiently large volume of information has been accumulated on the results of energetic analysis of terms entering into equation (1) for a large number of ligands essentially differing in structure and medicobiological properties (full citation of the corresponding scientific literature on each of the considered types of interactions is presented in works [1–9]), which gives ground for formulation of a generalized notion about the energetics of processes of complex formation in aqueous medium and methodology of its investigation. To our mind, such generalization may be served by the following set of conclusions.

Compensational effect. Data obtained by various authors point to that all without exception processes of complex formation of ligands with NAs in aqueous medium are characterized by a vividly expressed compensational effect, manifesting itself both on the level of summation of energetics of various physical factors and on the level of summation of intermolecular interactions with water surroundings. More concretely, the manifestations of this compensational effect come as three main regularities:

– summation of large-magnitude (tens–hundreds kcal/mol) energetic terms responsible for the contribution into ΔG_{total} from various physical factors (see equation (1)) gives a relatively small total Gibbs energy for complex formation reaction ($\Delta G_{exp} \sim -10$ kcal/mol);

– desolvation of ligand and receptor in complex formation, expressed in large positive values of energies of interaction with solvent, is compensated by negative energies of intermolecular interactions in complex;

– depending on the sign of charge of ligand and receptor the magnitude of the energy of electrostatic interactions can be immense (hundreds of kcal/mol – for interaction "+" and "–", and units of kcal/mol – for interaction of neutral molecules). However therewith the total electrostatic energy (ΔG_{EL}) and total experimental energy (ΔG_{exp}) of complex formation always come to be comparatively small and in many cases independent of the sign of charge of interacting molecules.

The formulated regularities are traced in many publications and for different molecular complexes and, as it appears, reflect the general properties of energetics of biomolecular interactions in aqueous medium.

Problem of analysis of full Gibbs energy. The relatively small value of total Gibbs energy for binding of small ligands with NAs ($\Delta G_{total} \sim 10$ kcal/mol) comes as a result of summation of large quantities (tens–hundreds kcal/mol) entered by contributions of concrete physical factors with opposite signs (consequence of compensational effect). The error of calculation of each such "great number" for molecules of NA-binding ligands, in the framework of modern methods of molecular modeling, in a rough approximation has an order of the very ΔG_{total}. This signifies that analysis of the calculated ΔG_{total} value itself has no physical sense, consequently, prediction of the equilibrium constant of complex formation in aqueous medium for a class of NA-binding ligands also turns out to be hardly possible. In essence it is just for this reason that the strategies of energetic analysis known to us [1–9], as a rule, operate just with the members of decomposition of ΔG_{total} into components calculated with smaller relative error than ΔG_{total} itself. Hence it also follows that a solution of the decomposition problem for NA-binding ligands must be recognized as "successful" if the difference of total calculated Gibbs energy ΔG_{total} and the experimentally measured energy of the reaction of complex formation fits into the following interval [5–9]:

$$\left|\Delta G_{total} - \Delta G_{exp}\right| \in 0 \ldots \Delta G_{exp}. \quad (2)$$

It is necessary, however, to underline that this conclusion is valid only for ligands with insignificant scatter of ΔG_{total} energy relative to ΔG_{total} itself. Modern methods of molecular docking, based on the method of criterion functions (see, for example, [16]) and used, in particular, in express screening of potential ligands, sufficiently reliably can predict ΔG_{total}, but in the limits of a ten of kcal/mol.

Necessity of correcting the "paradigm" of modern energetic analysis. Dominating at the present time in works of some authors [1–3], the «paradigm» of energetic analysis in the main boils down to interpretation of sum energies of the process of complex formation responsible for the contribution of various physical factors (i.e. sum of the contributions of the given physical factor at all levels of interactions: separately with water surrounding, intermolecular interaction, interactions at various stages of the process of complex formation), and namely: analysis of total van der Waals energy (ΔG_{VDW}), electrostatic energy (ΔG_{EL}), energy of hydrogen bonds (ΔG_{HB}) etc. Recent results [4–9], however, testify to that such an approach in a general case does not come to be correct. The cause of this is dual. Firstly, in a majority of cases the total energies do not demonstrate any correlation with the properties of the very ligand (for example, with charge and type/ramification of side groups [12]) and are therefore useless for interpretation of binding energetics and also during solving problems of QSAR type (directed modification of ligand with an aim of optimization of its medicobiological effect). Secondly, in consequence of the compensational effect the value of total energy of each separately taken physical factor may be close to zero (for example, $\Delta G_{VDW} \approx 0$ for intercalators [5] and $\Delta G_{EL} \approx 0$ for DNA minor groove bind-

ers DNA [8]). From this one can make a physically senseless conclusion about that the given physical factor does not give a contribution into stabilization of the investigated complexes (see discussion of the problem in work [5]). On the contrary, by a series of authors it has been shown that only the members of decomposition not formed by compensation of various interactions may correlate with charge/structural properties of a ligand and with experimental energy of complex formation (ΔG_{exp}) [11, 12]. Consequently, energetic analysis of the process of complex formation in aqueous medium must operate just with the components of total Gibbs energy on the level of separate physical factors (van der Waals, electrostatic etc.), stages of complex formation (preliminary formation of a binding site and binding itself) and type of interactions (intermolecular and with aqueous medium). Most brightly the justness of such an approach has manifested itself in relation of ligand binding into DNA minor groove, prevalently "governed" by intermolecular electrostatic interaction [8], and in a lesser degree, in relation of intercalation of biologically active compounds into DNA, "governed" prevalently by intermolecular van der Waals energy [5]. All the said point to the necessity of correcting the modern "paradigm" of energetic analysis, i.e. refusal of analysis of total energies corresponding to a contribution of a concrete physical factor.

What comes to be the final result of energetic analysis? The main "product" of energetic analysis must consist in obtaining some new knowledge about the investigated process, which further may be applied in theory and practice. Proceeding from this, most laconically the final "product" of energetic analysis, in our opinion, can be formulated in the form of an expanded answer to two questions: "Which physical factors and in what mutual relationship stabilize/destabilize the of ligand–NA complexes?" and "Which physical factors do in the greatest degree influence the magnitude of ligand affinity to a bioreceptor?". An answer to the first question is given by a theoretical notion about the role of various physical factors in stabilization of complexes of biomolecules in the form of a series of calculated terms of Gibbs energies ordered by the degree of diminution of the contribution of one or another factor [5, 8]. An answer to the second question, from our point of view, is more important, because it has a concrete practical output. If it is known which physical factor modulates the affinity of a ligand to a bioreceptor, then it becomes clear which type of atomic group it is necessary to add to the ligand for strengthening the contribution into the energetics of exactly this factor. Most brightly the informativity of the given approach has been demonstrated on the example of a group of medicinal preparations manifesting their biological activity by means of binding into the DNA minor groove: in work [17], pronounced correlation was disclosed of the factor of biological activity with term ΔG_{HB}, at that any correlation with experimental binding energy ΔG_{exp} was not observed. In essence, such strategy of energetic analysis essentially supplements the modern scientific basis of directed synthesis of new medicinal preparations possessing elevated selectivity to bioreceptor – a fundamental problem at the junction of biophysics and organic chemistry being solved by scientific research groups all over the world.

PROSPECTS OF FURTHER DEVELOPMENT OF ENERGETIC ANALYSIS

The heretofore published results of various authors cited above demonstrate a possibility of conducting energetic analysis on the level of Gibbs energy, giving good agreement with experiment for a large number of ligands different in structural properties. Such an approach, however, does not come to be full-fledged: judgment about agreement of calculation with experiment is made only on the basis of one experimental value ΔG_{exp}. At the same time the presently existing methods allow with a sufficient degree of accuracy experimentally measuring two more thermodynamic potentials: change of enthalpy ΔH_{exp} and heat capacity ΔC_p^{exp} in the process of complex formation. However, by our data, decomposition of enthalpy and heat capacity into components for NA-binding ligands has yet not been conducted with the exception of single works [2, 17, 18], not allowing formation of a general notion about peculiarities of energetics on the level of ΔH_{exp} and ΔC_p^{exp}. Moreover, there are grounds for supposing that solution of the given problem may be more problematic than decomposition on the level of ΔG_{exp}: an attempt at decomposition of ΔC_p^{exp} into components actualized in work [17] has led to noticeable disagreement with experiment, while upon solving the problem of decomposition of enthalpy in work [18] we have managed to attain only qualitative correspondence of theory and experiment. The key causes of this come to be [17, 19]: (1) complexity of separating enthalpic and entropic components in the contribution of separate physical factors, (2) significantly greater error of estimating enthalpy as compared with ΔG and (3) insufficient elaboration of the methodology of analysis of ΔC_p^{exp} components.

Taking into account the problem points indicated above, let us note that full-fledged use of the possibilities of energetic analysis is realized only then when agreement with experiment is reached simultaneously on the level of ΔG_{exp}, ΔH_{exp} and ΔC_p^{exp}.

This implies a necessity of solving the problem of decomposition of ΔH_{exp} and ΔC_p^{exp} into contributions from separate physical factors and, in our opinion, constitutes the strategy of future development of energetic analysis.

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