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ESTIMATION OF INTERACTION ENERGY OF SOME FUNCTIONAL GROUPS OF MEDICAL SUBSTANCES WITH PROTEIN MOLECULES IN WATER CONDITION

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The basic energetic effects when forming organic substance complexes with protein molecules which determine thermodynamics of this process are examines using nonempirical quantum-chemical DFT method. Complex formation is considered as a replacement of water in solvated protein and organic compound molecules. Determinative role of strong hydrogen bonds and hydrophobic effect in strength of the complexes is shown.

The estimation of pharmacological and biological substance activity is of great practical importance. The purpose of our work is to describe quantitavely energetic effects of medical substance complex formation with protein molecules in the conditions of interaction in aquatic environment.

Thermodynamic description permits to consider formation of such complexes as a dynamic equilibrium of different energetic states. Calculation of energies in such states is possible using modern methods of quantum chemistry.

Materials and methods

Nonempirical quantum-chemical calculations of DFT was performed by means of standard GAMESS program package by B3LYP/6-311G* method with exchange Bekke B3 functional and LYP correlation functional [1]. Geometries of calculated molecules were fully optimized, the absence of imaginary vibration frequencies proved their stationary character.

Results and discussion

Complex strength constant of medical substance with protein molecules in aquatic environment can be estimated by thermodynamic equation:

$$K_p = e^{\frac{-\Delta G}{RT}} = e^{\frac{-\Delta H}{RT}} \cdot e^{\frac{\Delta S}{R}}. \quad (1)$$

Entropic constituent has the value $T{\cdot}\Delta S{<}4$ kJ/mol [2].

The process of complex formation we consider as a dynamic equilibrium of two energetic states in the conditions of organism (*in vivo*) at 310 K (37 °C). Initial state – hydrated molecules of medical substance (MS) and protein. Second state – complex of MS molecule and protein formed and interaction of liberated water molecules among each other.

Enthalpy constituent of equation (1) can be calculated by the formula:

$$\Delta H = (\Delta H_{ЛВ-БС} + \Delta H_{В-В}) - (\Delta H_{ЛВ-В} + \Delta H_{БС-В}), \quad (2)$$

where $\Delta H_{ЛВ\text{-}БС}$ – active group interaction energy of medical substance with protein molecule groups; $\Delta H_{В\text{-}В}$ – interaction energy of water molecules with each other; $\Delta H_{ЛВ\text{-}В}$ – hydration energy of active group of medical substance; $\Delta H_{БС\text{-}В}$ – hydration energy of protein active group.

Intermolecular interaction is described by many constituents. Weak Van der Waal's forces of interaction have energy of less than 0,4 kJ/mol, taking into account that in initial state molecules are solvated, i. e. they interact with other molecules, the difference of these energies have an order of magnitude less and is close to zero.

Special role in intermolecular interactions plays hydrogen bond which can have energy in the range of 1...200 kJ/mol comparable with energies of covalent chemical bonds. One distinguishes hydrogen bond with energy up to 20 kJ/mol, average – 20...60 kJ/mol, producing at interaction of neutral molecules and strong hydrogen bond from 60 to 200 kJ/mol which is observed at molecule interaction with charged ions [3].

The calculation of 40 different organic and inorganic molecules including 20 amino acids occurring in organism was made. Also, we performed the calculation of amino acid dimers and trimers that showed that at polymerization effective charges in active centres of aminoacid residuals do not practically change at chain accretion. It permits to model interaction with protein replacing it by interaction with aminoacid residuals. For all molecules involved the energy of interaction of strongly charged centres with water molecules is calculated.

Then energy of replacement for the process of complex formation by equation (2) was calculated. In the

course of the processes the number of hydrogen bonds did not change.

Energetic effects of intermolecular interaction radically change taking into account that interaction takes place in aquatic environment. It is illustrated by the following examples:

Table 1. *Energetic effect of carbonyl group interaction with hydroxylic group forming hydrogen bond in aquatic environment*

Interaction	Energy, kJ/mol
CH_3–COOH ... $HOCH_2$– CH_3	–59,85
H_2O ... H_2O	–43,47
CH_3–COOH ... H_2O	–63,26
CH_3–CH_2OH ... H_2O	–43,27
Energetic effect of water replacement CH_3COOH ... H_2O+CH_3CH_2OH ... H_2O= =CH_3COOH ... $HOCH_2CH_3$+H_2O ... H_2O	+3,21

In this case the interaction of carbonyl group with hydroxylic one takes place. If the interaction occured in vacuum, energetic effect would amount –59,85 kJ/mol, in aquatic environment these groups are already connected with water molecules with energy –63,26 and –43,27 kJ/mol. As a result, energetic gain has the value +3,21 kJ, i. e. in terms of entropic constituent, is close to zero. I.e. introduction of weak groups cannot increase MS affinity conceptually.

Table 2. *Energetic effects of methylic radicals interaction with each other and with water molecules*

Interaction	Energy, kJ/mol
CH_4 ... CH_4	≈–0,1
H_2O ... H_2O	–43,27
CH_4 ... H_2O	≈–0,1

The second example illustrates hydrophobic effect. In this case the gain is conditioned by high energy of molecule interaction with each other, which makes this process of replacement energetically advantageous. Hydrophobic effect is proportional to the square of hydrophobic segment and amounts –10 kJ/mol·nm^2, that for methyl group is about –2 kJ/mol, benzene ring gives effect of –11 kJ/mol. In vacuum such interaction would have energy of the order of –0,1 kJ/mol [4].

Table 3. *Energetic effect of carboxylic group interaction with carboxylic ion with formation of hydrogen bond in aquatic environment*

Interaction	Energy, kJ/mol
CH_3–COOH ... $^-$OOC– CH_3	–139,24
H_2O ... H_2O	–43,27
CH_3–COOH ... H_2O	–63,26
CH_3–COO^- ... H_2O	–84,37
Energetic effect of water replacement CH_3COOH ... H_2O+CH_3COO^- ... H_2O= =CH_3COOH ... $^-OOCCH_3$+H_2O ... H_2O	–34,88

The third example shows energetic gain when forming strong hydrogen bonds. Though these groups have great energy of interaction with water molecules, energetic gain of replacement process comes to –34,88 kJ/mol.

The process of formation of strong complex can be considered not only as a replacement of water on aminoacids by MS molecules, but also as a replacement by aminoacid water residuals in MS-water complex. To replace water molecule bonded with protein molecule by active centre of MS it is necessary for it to be capable of forming a stronger hydrogen bond than water molecule does. And, vice verse, for active centre of aminoacid residual to replace water molecule bonded with the substance, it should form a stronger hydrogen bond. It is necessary for groups in MS molecules and aminoacids to be simultaneously stronger than water, and then such a replacement could intensify preparation activity. However, not in all systems corresponding to this rule the energetic gain is observed, i. e. it is a necessary, but not obligatory condition. All the rest active centres of both active substance molecule and protein molecule forming less strong hydrogen bond are not capable of giving energetic gain when forming complex in aquatic environment, i.e. to increase its strength.

That fact permits to exclude a great number of variants of interaction not resulting in increase of complex density in aquatic environment. In MS molecule one should consider only some centres, which give energetic gain. Among 20 aminoacids occurring in organism only arginine (Arg), lysine (Lys), asparagic (Asp) and glutaminic (Glu) acids are capable of forming stronger hydrogen bonds, than water molecules. With conjugate and aromatic systems in the molecules involved hydrogen bond can have irregularly high values. Among aminoacids containing conjugate systems tyrosine (Tyr), tryptophane (Trp) and charged form of histidine (His) are capable of having energetic gain in aquatic environment.

Quantum-chemical calculation of model systems was performed. In table 4 the values of internal energy change in the processes of complex formation among organic molecules in aquatic environment are presented.

Table 4. *Energetic effects of replacement in aquatic environment*

System	Δ*Et*, kJ/mol	System	Δ*Et*, kJ/mol
HCOOH+$HCOO^-$	–31,19	Phenol + $HCOO^-$	–48,72
CH_3COOH+CH_3COO^-	–34,88	Phenol + CH_3COO^-	–47,83
CH_3COOH+CH_3COOH	34,87	Phenol + Furane	12,61
C_2H_5OH+CH_3COOH	3,21	Phenol + $C_2H_5NH_3^+$	33,03
C_2H_5OH+CH_3COO^-	–0,20	Pyrrole + CH_3COO^-	–37,76
CH_3OCH_3+CH_3COOH	–0,82	C_6H_5COOH + CH_3COO^-	–40,11
$C_2H_5NH_2$+CH_3COO^-	28,01	Furane + HCOOH	19,05
$C_2H_5NH_2$+CH_3COOH	13,92	Pyrrole + $HCOO^-$	–37,12
Pyradine+CH_3COOH	–8,32	Pyrrole + Furane	8,52
Anilin+CH_3COO^-	–31,24	Furane + $C_2H_5NH_3^+$	22,31
Anilin+CH_3COOH	15,82	Indole + CH_3COO^-	–53,45

One should mention that in is observed only at interaction of organic anions with neutral molecules. It should be particularly pointed out the formation of strong hydrogen bonds in aromatic and conjugate sy-

stems. Though cation forms of molecules form stable hydrogen bonds, however, energetic gain are not observes as at their interaction with water the initial state is great with respect to energy. In systems containing only neutral molecules energetic gain is either negligible or absent at all. All these facts permit to say about complementarity at interaction of organic molecules with protein. Only definite combinations of active groups of organic molecule at their interaction with active groups of aminoacid residuals provide complex formation. Only some groups forming molecules of active substance are capable of increasing energy of complex formation process, i. e. increasing stability coefficient.

The second effective way of increasing complex stability is accretion of hydrophobic parts of MS molecules according to hydrophobic parts of protein molecules.

These examples show that energetic effects of complex formation process completely change taking into consideration the fact that interaction takes place in aquatic environment.

This fact permits to treat rationally modelling of medical substances, reducing time and means consumption for predicting activity significantly. Calculating energies of hydrophobic interaction and estimating energetic effects of replacing water molecule with formation of strong hydrogen bonds, we can calculate the maximum possible affinity of the given medical substance to ideally relevant protein, which, in its turn, permits to determine the maximum possible coefficient of MS complex stability with protein. The value of maximum MS affinity to protein substrate serves as a quantitative characteristic caused by its pharmacological activity, it makes possible to estimate necessary quantities of substances to observe pharmacological effect as well as toxic doses.

For example, let us estimate maximum possible affinity for, in figure.

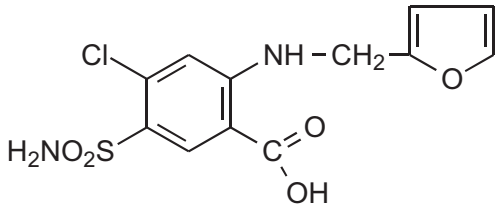

Figure. *Molecule of furosemide*

In this molecule there are some groups capable of forming strong hydrogen bond with protein targets: sulphanilamide, carboxylic and amino group. However, it should be taken into account that amino group forms inner-molecular hydrogen bond and, probably, cannot participate in complex formation.

Of two groups remained – sulphanilamide and carboxylic, only the latter is capable of providing energetic gain and increasing complex stability 35 kJ/mol more. Replacing water molecule on protein sulphanilamide group does not increase complex stability. Hydrophobic effect can raise complex stability approximately 22 kJ/mol more. It makes possible to estimate complex stability of furosemide with ideally relevant protein, it amounting –57 kJ/mol, that is the value of maximum possible affinity.

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