Communications to the Editor

[Chem. Pharm. Bull. 34(8)3492—3495(1986)]

A NEW METHOD FOR CALCULATING HYDROPHOBIC INTERACTION ENERGY IN THE BIOLOGICAL SYSTEM[1)]

Kenji Akahane and Hideaki Umeyama*
School of Pharmaceutical Sciences, Kitasato University,
Shirokane, Minato-ku, Tokyo 108, Japan

A method is proposed to estimate the hydrophobic interaction energy for the binding of a ligand to an enzyme. The hydrophobic energy for the association of methotrexate and dihydrofolate reductase was estimated by this method. Compensations were found between the hydrophobic and the electrostatic energies for the constituent functional groups in methotrexate. The association may be unfavorable from the viewpoint of hydrophobicity; it may be electrostatically driven.

KEYWORDS —— hydrophobic interaction energy; dihydrofolate reductase; methotrexate; drug design; inhibitor; computer graphics

Recently, Eisenberg and McLachlan reported a method for calculating the solvation energy of a protein folding.[2)] They assumed that, in the process of protein folding, the overall free energy change was able to be approximated as the sum of individual atomic contributions which are closely related to these solvent-accessible surface areas (ASA).[3)] There are some disadvantages in their treatment, although it is one of the most interesting methods that have made it possible to calculate the hydrophobic effect in a biological system. In their treatment the hydrophobicities of 20 amino acid residues were divided into only five atomic contributions, i.e., C, N/O, O^-, N^- and S. Consequently, the subtle variety of the environment of each atom was not taken into account; the contribution of a carbon in a carboxyl group is considered to be equal to that of hydrocarbon. Because the atomic contributions obtained by such treatment are dependent on the data set used to estimate the values, hydrophobicity can be correctly calculated only for the compounds within the set. This may cause some trouble in calculating the hydrophobic energy of the interaction between a ligand and an enzyme. Here we propose a new method for estimating the hydrophobic interaction energy. To illustrate the method it is used to calculate the hydrophobic energy for the association of dihydrofolate reductase (DHFR) and methotrexate (MTX).

The hydrophobic interaction energy (solvent effect), ΔG_{HI}, is defined in Eq. 1 for the association process of a ligand molecule (L) and an enzyme (E) in an aqueous phase (Fig. 1). Here, ΔG^{w}_{asso} and ΔG^{g}_{asso} are free energy changes for the association process in an aqueous and a gaseous phase, respectively. Since ΔG^{w}_{asso} is thought to be thermodynamically equal to the free energy change for the process I→II→III (Eq. 2), ΔG_{HI} is expressed by Eq. 3. ΔG^{I} and ΔG^{III} are expressed by Eqs. 4a and 4b, respectively. Consequently, Eq. 3 can be converted into Eq. 5. $\Delta G^{w\to g}$ represents the free energy change for transferring a molecule from the aqueous

$$\Delta G_{HI} = \Delta G^{w}_{asso} - \Delta G^{g}_{asso} \quad (1)$$

$$\Delta G^{w}_{asso} = \Delta G^{I} + \Delta G^{g}_{asso} + \Delta G^{III} \quad (2)$$

$$\Delta G_{HI} = \Delta G^{I} + \Delta G^{III} \quad (3)$$

$$\Delta G^{I} = \Delta G^{w\to g}_{L} + \Delta G^{w\to g}_{E} \quad (4a)$$

$$\Delta G^{III} = -\Delta G^{w\to g}_{L-E} \quad (4b)$$

$$\Delta G_{HI} = \Delta G^{w\to g}_{L} + \Delta G^{w\to g}_{E} - \Delta G^{w\to g}_{L-E} \quad (5)$$

$$\Delta G_{HI} = \sum_{i} (ASA^{L}_{i} + ASA^{E}_{i} - ASA^{L-E}_{i}) \cdot f_{i} \quad (6)$$

$$\Delta G_{HI} = \sum_{\text{complex molecules}} \left(\sum_{i} \left(\sum_{\text{groups } j} (\psi \cdot f_{i} \cdot ASA_{i} \cdot S_{j})/SA_{i} \right) \right) \quad (7)$$

$$\psi = \exp(\beta \cdot r_{jk}^{2}) \quad (8)$$

Fig. 1. Schematic Representation of Association of a Ligand (L) and an Enzyme (E)

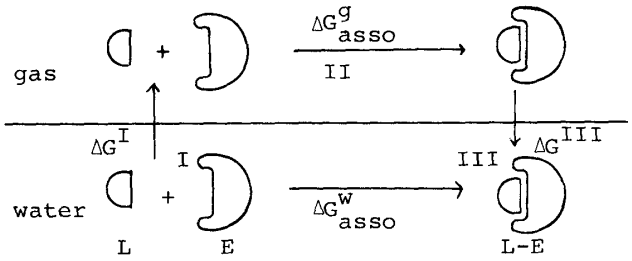


Table 1. f-Values for DHFR

Group	ASA(Å^2)	F*)	f_i(cal/mol·Å^2)
$-CONH_2$	108.9	-0.35	4.40
$-Gua^+$	162.5	-1.71	14.33
$-COO^-$	95.5	-1.34	19.15
-SH	70.6	0.66	-12.73
$-SCH_3$	124.2	0.94	-10.31
-OH(ali.)	51.8	-0.27	7.10
-OH(aro.)	56.4	-0.58	13.99
$-Imi^+$	146.0	-1.00	9.33
-Ind	230.3	1.25	-7.39
$-NH_3^+$	93.0	-2.28	33.39
-H(Gly)	26.6	0.17	-8.69
Benzene ring	-	-	-12.64
Hydrocarbon	-	-	-17.23
-CONH-	64.8	-2.02	42.47

*) Calculated from partition coefficients of amino acids (ref. 7a) by the least squares method, in which contribution of hydrogen attached to α-carbon was assumed to be 0.17 (ref. 7b).

Table 2. f-Values for MTX

Group	ASA(Å^2)	F	f_i(cal/mol·Å^2)
2,4-Diamino pteridinyl	288.6	-2.79*)	13.17
-N-(aro.)	1.6	-0.91	792.12
-CONH-(aro.)	54.8	-1.15	28.48
$-COO^-$ (α)	112.5	-4.59	55.53
$-COO^-$ (γ)	112.5	-5.01	60.61
Benzene ring	-	-	-22.81**)
Hydrocarbon	-	-	-24.42**)

*) Calculated from partition coefficients of MTX derivative (ref. 7c).
**) Based on partition coefficients taken from ref. 7d.

phase to the gaseous phase, and can be estimated from solubility data. Assuming (a) the overall free energy change of transfer for a molecule or a complex is obtained as the summation of all the individual energy changes of the groups which constitute the molecule or the complex and (b) the contributions of the groups to the overall energy change of the molecule or the complex are proportional to their ASA, ΔG_{HI} can be approximately represented by Eq. 6. Where ASA_i is the ASA of the *i*-th group, and f_i is the free energy per unit ASA for transferring the *i*-th group from the aqueous phase to the gaseous phase. This is our first formula to calculate the hydrophobic energy, and is formally similar to the equation proposed by Eisenberg and McLachlan.[2]

However, three problems arise. Firstly Pangali *et al.*[4] found two minima in the potential curve obtained by Monte Carlo simulation. The two minima are separated by about the diameter of a water molecule. This was predicted by theoretical treatment by Pratt and Chandler.[5] These results suggest that, considering a dynamic feature, two solutes separated by one water molecule are in metastable states and still have hydrophobic interaction energy. However, for two solutes separated by one water molecule, the hydrophobic interaction energy is calculated to be zero according to Eq. 6. Secondly, in the calculation of the

hydrophobic energy from Eq. 6, one can not obtain microscopic information about the direction of intermolecular interaction. This infromation is of great importance for the analysis of enzymatic reactions or designing new inhibitors. Thirdly a hydrophobic correlation index[6] can not be obtained from such treatment. Considering these points, we propose here a new formula to calculate the hydrophobic interaction energy, which directly considers the interacting surface (Eq. 7).

The ASA_i and f_i in Eq. 7 have the same meaning as in Eq. 6. The f-value used in this communication was calculated, based upon the well-established fragmental constants or the partition coefficients between n-octanol and water, taken from the literature.[7] For the hydrocarbon moieties and aromatic portions, the f-value was determined from the slope of the linear relationship between log P[7a,d] and the ASA. The f-values for other functional groups were obtained simply by dividing the fragmental constant (F) by its ASA. These values are listed in Tables 1 and 2. In Eq. 7, SA_i is the van der Waals surface area of the i-th functional group interacting with a solvent in the free state. S_j is the area of j-th patch defined on the van der Waals surface of the i-th group. Only those patches are taken into account which are in contact with a solvent in the free state and then come into intermolecular-contact in the complex. The factor ψ in Eq. 7 is defined by Eq. 8, where r_{jk} is the distance between the patch j and van der Waals surface of the atom k interacting with this patch.[8] For two particles separated by one water molecule, the found potential should be one third of that of the contact particles.[4] For two particles in contact with each other, the hydrophobic interaction energy calculated from Eq. 7 would be in agreement with the value calculated from Eq. 6. Taking these points into account, the β in Eq. 8 was determined to be -0.1312.

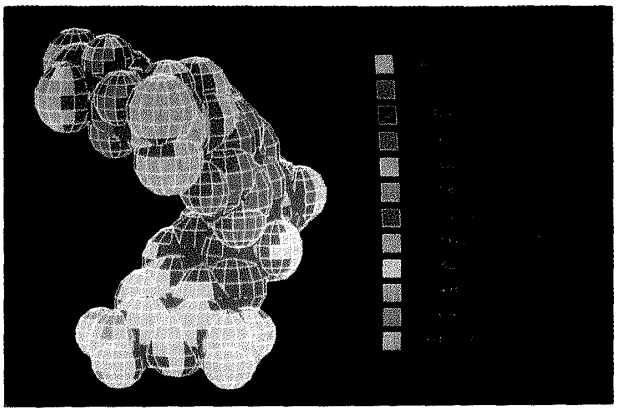


Fig. 2. Hydrophobic Energy on the van der Waals Surface of MTX
For each patch the value calculated from $f_i \cdot ASA_i/SA_i$ (see Eq. 7) is represented by the color code.

This method was applied to the association of DHFR and MTX.[9] Figure 2 shows the contributions of each patch in MTX to the hydrophobic interaction energy. The directions of intermolecular interaction are clearly understandable from Fig. 2. The contributions of the constituent groups to ΔG_{HI} are listed in Table 3 with electrostatic interaction energies which were calculated based on Debye-Hückel theory by considering the solvent accessibilities of each group.[10] From Table 3, it seems likely that the hydrophobically unfavorable parts, such as the pteridine ring, the amide group, and the carboxyl group, gain electrostatic energies. On the contrary, the benzene ring and the hydrocarbon moieties, which are hydrophobically favorable groups, do not gain electrostatic energies. Thus, the compensations were found to be between the hydrophobic and electrostatic energies. For the binding, the hydrophobic energy of MTX was 9.0 kcal/mol and that of DHFR was -3.8 kcal/mol, so that the overall hydrophobic energy was 5.2 kcal/mol. The association may be unfavorable from the viewpoint of hydrophobicity. The electrostatic inter-

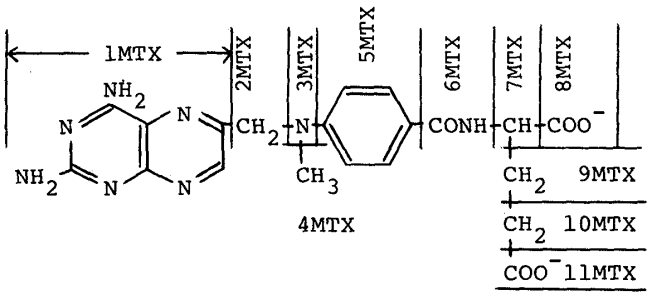
action energy was -18.0 kcal/mol, so the total energy of the system was -12.8 kcal/mol. Although it is necessary to take into account additional factors such as dispersion forces, etc., these results suggest that the binding of MTX to the enzyme is electrostatically driven. Subramanian *et al.*[11] reported that for the binding of MTX to DHFR, ΔH was -13.3 kcal/mol, ΔS was -5.4 cal/K·mol, and ΔG was -11.7 kcal/mol. They stated that the binding was enthalpy-driven and entropy-compensated.

In conclusion, a new method has been developed to calculate the hydrophobic interaction energy as shown in Eq. 7.[12] And the free energy calculated for the binding of MTX to DHFR is in good agreement with the experimental value.

Table 3. Hydrophobic (HI) and Electrostatic (ES) Interaction Energies in MTX

Group	HI energy*)	ES energy*)
1MTX	3.4	-10.0
2MTX	-0.8	0.0
3MTX	0.6	0.4
4MTX	-1.5	-0.2
5MTX	-2.4	-0.2
6MTX	1.2	-0.8
7MTX	-0.2	0.1
8MTX	4.8	-6.2
9MTX	-0.5	0.0
10MTX	-0.2	-0.1
11MTX	4.7	-1.0

*) kcal/mol



REFERENCES AND NOTES

1) This research was presented earlier at the 13th Symposium on Structure-Activity Relationships before the publication of ref. 2; K. Akahane, K. Komatsu, and H. Umeyama, Abstructs of Papers, 13th Symposium on Structure-Activity Relationships, Kanazawa, Oct. 1985, p. 218.
2) D. Eisenberg and A. D. McLachlan, Nature, 319, 199 (1986).
3) B. Lee and F. M. Richards, J. Mol. Biol., 55, 379 (1971).
4) C. Pangali, M. Rao, and B. J. Berne, J. Chem. Phys., 71, 2975 (1979).
5) L. R. Pratt and D. Chandler, J. Chem. Phys., 67, 3683 (1977).
6) K. Akahane and H. Umeyama, the 106th Annual Meeting of the Pharmaceutical Society of Japan, Chiba, April, 1986.
7) In this study, the f-value was calculated from the transfer free energy, not from water to the gaseous phase but from water to n-octanol. Accordingly, it should be noted that as a first approximation the interaction of a solute with n-octanol was neglected. The partition data were taken from: a) V. Pliska, M. Schmidt, and J.-L. Fauchere, J. Chromatogr., 216, 79 (1981); b) R. F. Rekker and H. M. de Kort, Eur. J. Med. Chem., 14, 479 (1979); c) A. Rosowsky, J. Med. Chem., 16, 1190 (1973); d) C. Hansch and A. Leo, "Substituent Constants for Correlation Analysis in Chemistry and Biology," Wiley-Interscience, New York (1979).
8) Only the atom facing the patch was regarded as the interacting atom; K. Akahane and H. Umeyama, Enzyme (1986), in press.
9) The coordinates of DHFR and MTX were taken from the protein data bank; where it is listed as 3DFR. Hydrogen atoms were generated with standard geometry. In the calculation of the hydrophobic interaction energy, only the protein atoms within 8 Å from MTX were taken into account. Ionizable residues of the protein were assumed to be in ionized form. Two carboxyl groups in MTX were assumed to be in ionized form, while the 1-nitrogen was assumed to be neutral.
10) K. Komatsu, S. Nakagawa, and H. Umeyama, Abstructs of papers, 13th Symposium on Structure-Activity Relationships, Kanazawa, Oct. 1985, p. 214.
11) S. Subramanian and B. T. Kaufman, Proc. Natl. Acad. Sci. USA, 75, 3201 (1978).
12) At the present calculation, because each patch on a molecular surface can not be identified after the molecule changes its conformation, equation 7 is applicable to a system with no conformational change. On the other hand, equation 6 can be applied to a system with conformational change.

(Received April 21, 1986)