Biophys. Struct. Mech. 7, 101–106 (1980)

Biophysics of Structure and Mechanism

Hydrophobic Bond Energy of Non-Polar Molecules: Application to β-Ionone and 11-*cis* Retinal

T. Kakitani, H. Kakitani*, and S. Yomosa

Department of Physics, Nagoya University,
Furo-cho, Chikusa-ku, Nagoya 464, Japan

Abstract. A method for calculating the hydrophobic bond energy (HPE) of a nonpolar molecule at room temperature was presented by assuming that the whole HPE is the sum of HPE of each group or atom composing the molecule. This method was applied to β-ionone and 11-*cis* retinal and those HPE's were found to be considerable. Some comparison of the present method with Chothia's method was made.

Key words: Hydrophobic bond energy – Rhodopsin – β-Ionone – 11-*cis* Retinal – Binding energy

Introduction

In general, the hydrophobic bond energy (hereafter abbreviated as HPE) may be defined as a decrease of the free energy due to the transfer of a nonpolar molecule from an aqueous environment to the nonpolar liquid solution or the interior of proteins. Kauzmann (1959) was the first who used this concept in interpreting the denaturation of proteins. Nemethy and Scheraga (1962a, b) made microscopic calculations of thermodynamic properties of hydrocarbons in aqueous solutions and hydrophobic bonds in proteins, and clarified many important features of the hydrophobic bond. Recently, Chothia (1974) noticed a linear relationship between accessible surface area in proteins and hydrophobicity of amino acid residues. Estimating the accessible surface area of proteins, Chothia demonstrated an important contribution of the HPE to the stability of globular protein (1975a), and to the protein-protein association together with the large contribution from the translational/rotational free energy (1975b, 1976). By this semiempirical method, the HPE has become to be treated quantitatively.

Under such a situation it is desirable to develop a feasible method to calculate HPE also of substrates in the binding to enzymes. For this object, we

* *Present address:* Department of Physiology and Biophysics, University of Illinois

0340-1057/80/0007/0101/$ 01.20

are going to present a simple method by which HPE of hydrocarbon parts can be calculated semiempirically.

HPE of Hydrocarbons

In the calculation of HPE of hydrocarbons, an additivity rule that the whole HPE of a molecule is the sum of HPE of each group or atom composing the molecule is assumed to hold. The validity of this rule is empirically verified in the aliphatic hydrocarbons as we see later. The linear relationship between the accessible surface area and hydrophobicity as denoted by Chothia almost corresponds to this rule. (However, it should be noticed that the quantity of the accessible surface area also includes the steric effect of the complicated conformation of a molecule which is important in large molecules such as proteins.) Throughout this paper, we consider HPE at room temperature.

The HPE of benzene is 4.61 kcal/mol (Nemethy and Scheraga 1962a). From this we estimate the HPE contributed from a CH group is 4.61 kcal/mol $\div$ 6 $\simeq$ 0.8 kcal/mol. The HPE of toluene is 5.33 kcal/mol (Nemethy and Scheraga 1962a). Toluene molecule is obtained by the substitution of a methyl group for a hydrogen atom of benzene. So, we estimate the HPE contributed from a $\geqslant C-CH_3$ group is (5.3 $-$ 0.8 $\times$ 5) kcal/mol $\simeq$ 1.3 kcal/mol. The HPE's of aliphatic hydrocarbons are as follows (Nemethy and Scheraga 1962a); methane (2.51 $\sim$ 3.15 kcal/mol), ethane (3.22 $\sim$ 3.86 kcal/mol), propane (4.90 $\sim$ 4.91 kcal/mol) and butane (5.82 $\sim$ 6.00 kcal/mol). When this energy is plotted against the number of carbon atoms of hydrocarbons, roughly straight line is obtained. From its gradient we estimate the HPE of a $>CH_2$ group as 1.0 kcal/mol. As we obtained before, the HPE of $\geqslant C-CH_3$ is larger than that of $\geqslant CH$ by 0.5 kcal/mol. Considering the effect of this methyl substitution is universal, we estimate the HPE of $>C<^{CH_3}_{CH_3}$ as (1.0 + 0.5 $\times$ 2) kcal/mol = 2 kcal/mol. All these values are summarized in Table 1.

The total HPE of a given hydrocarbon is calculated by using these values for each atomic group composing the molecule.

Application to β-Ionone and 11-*cis* Retinal

We are going to apply the method presented in the previous section to the chromophore (11-*cis* retinal) of visual pigment rhodopsin and its inhibitor (β-ionone). Their molecular structures are shown in Fig. 1. The values of HPE contributed from each hydrocarbon group of 11-*cis* retinal are written in Fig. 2. For simplicity we assume that the oxygen atom of the aldehyde group plays no role in the hydrophobicity. The total HPE's of 11-*cis* retinal, β-ionone and ionone ring are calculated to be 15.1 kcal/mol, 9.8 kcal/mol, and 6.9 kcal/mol, respectively. It is found these HPE's are considerably large.

In the above calculation, we neglected the effect as to how each group is connected in a molecule. This effect will be somewhat important as the molecule becomes large. The number of water molecules forming cage structure around

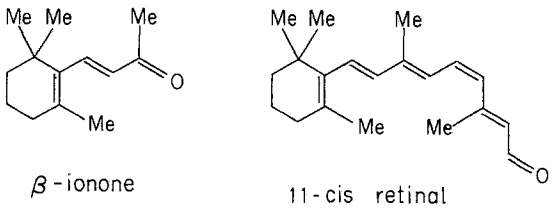

Fig. 1. Molecular structures of β-ionone and 11-*cis* retinal

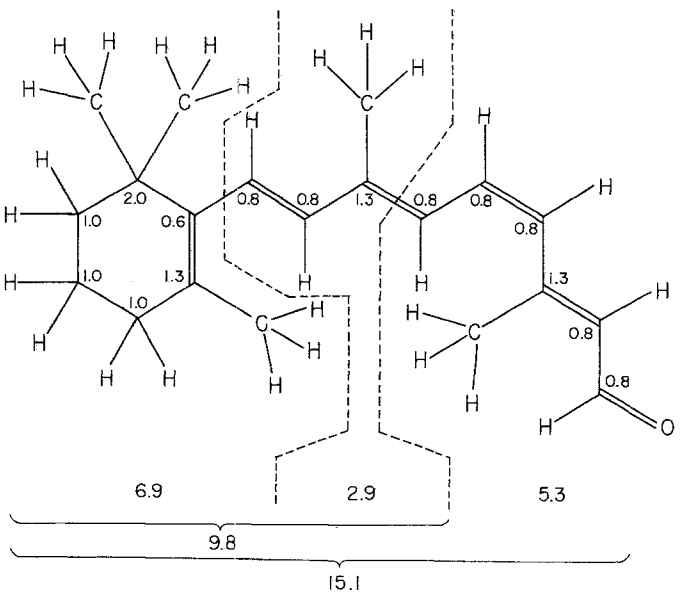

Fig. 2. The HPE of each group of 11-*cis* retinal. The calculated HPE's of ionone ring, β-ionone and 11-*cis* retinal are 6.9 kcal/mol, 9.8 kcal/mol, and 15.1 kcal/mol, respectively

the hydrocarbon will be small when hydrocarbon groups connect in a ring form as compared with that when they connect in a linear form. The HPE of saturated hydrocarbon group in Table 1 was obtained from the chain aliphatics. So that, our obtained HPE of the ionone ring may be a little overestimated. As to the part of the linear conjugated chain of 11-*cis* retinal, the opposite situation holds. That is, the HPE of these groups was estimated from aromatic molecules. So, if we apply those values to the linear polyene, the HPE will be underestimated. For β-ionone, the conjugated part is small compared with the bulky part of the ionone ring. Thus, the calculated value of HPE of β-ionone will become a little overestimated. In the case of 11-*cis* retinal, the contributions from the ionone ring and the polyene chain are roughly the same. So that, we expect that the overestimation at the ionone ring and the underestimation at the polyene chain will roughly compensate each other. From these we can expect that our estimated value of 15.1 kcal/mol for the HPE of 11-*cis* retinal is reasonable.

Group[a]	HPE (Kcal/mol)
⩾CH	0.8
⩾C−CH_3	1.3
>CH_2	1.0
>C(CH_3)(CH_3)	2.0

Table 1. The hydrophobic bond energy of some hydrocarbon groups at room temperature. For the derivation, see the text

[a] If we assume that the additivity of the HPE holds not only for the atomic group but also for each atom, we can consistently partition the HPE for each atom as follows: HPE (⩾C — or >C<) = 0.6 Kcal/mol and HPE(—H) = 0.2 Kcal/mol. In this, it should be mentioned that we cannot divide the HPE of the methyl group into the contribution from constituted atoms due to the extreme closeness of each atom. In this case, we can assign HPE(—CH_3) = 0.7 Kcal/mol only as a set

Discussion

It will be valuable to compare our calculation method of HPE of hydrocarbons with that of Chothia. By a rough calculation using a molecular model of rotatory ellipsoid, the accessible surface area is estimated to be 450 Å for β-ionone and 700 $Å^2$ for 11-*cis* retinal. If we use the empirical law of Chothia that the HPE per 1 $Å^2$ of the accessible surface area is 26 calorie for hydroxyl groups of amino acids, the HPE of β-ionone is calculated to be 11 kcal/mol and that of 11-*cis* retinal 18 kcal/mol. These values are close to our previously obtained values. Thus, the both methods are expected to give fairly consistent result. As our method is applicable to any hydrocarbon molecule, it may be used complimentally to the Chothia's method.

Matsumoto and Yoshizawa (1975) investigated the nature of the binding site of opsin which is the apoprotein of rhodopsin, with use of β-ionone. They observed that β-ionone has strong binding ability competitive with 11-*cis* retinal but does not form Schiff base linkage with lysine residue of opsin. (The binding energy was found to be 5.6 kcal/mol.) From this, they anticipated that the ionone ring would be bound by the hydrophobic bond to the binding site of opsin. Then, we try to estimate the binding energy of β-ionone by calculation assuming that the binding force is due to the hydrophobic bond. The great importance of the loss of the translational/rotational free energy due to the binding, bond linkage or association among medium or large molecules was pointed out by Page and Jencks (1971), and its important role in the case of the protein-protein association was evidently demonstrated by Chothia and Janin (1975b). Following the usual way of calculation of translational and rotational free energies in gas phase (Pitzer and Brewer 1961), the sum of those free energies of

β-ionone is calculated to be about 18 kcal/mol. This value is about a half of that in the above protein-protein association. If β-ionone is tightly bound to opsin, the above free energy should be lost. In addition to this, there is the free energy loss of 2.4 kcal/mol due to the term of the mixing entropy (Kauzman 1959). These free energy losses are too large compared with the HPE of β-ionone itself which is expected to be gained by the binding of β-ionone to the anhydrous binding site of opsin. In order to realize a considerable positive value of the binding energy as found in the experiment, either or both of the following two possibilities must be realized. One is that the β-ionone molecule in the binding site of opsin can still librate or slide rather easily and thus considerable part of the entropic term of the whole motion of β-ionone will be reserved. This implies that the binding site of opsin does not fit the conformation of β-ionone so rigidly. Such a notion is consistent with the recent experimental knowledge that the binding site is considerably flexible as investigated from the binding ability of some retinal analogues (Matsumoto et al. 1979). The other is that the binding site of opsin is hydrophobic and some water molecules are immersed in this region when β-ionone molecule is absent and those water molecules are expelled out from the binding region when β-ionone is bound. In such a manner the binding site of opsin itself is also possible to acquire some HPE by the binding of β-ionone.

In the binding of the native chromophore, 11-*cis* retinal, all the above factors will work similarly. In addition to this, The Schiff base linkage with lysine residue is formed (Bownds 1967) and probably the Coulombic interaction between the protonated retinylidene Schiff base and the counter anions will exist (Honig et al. 1979a, b). Although many factors work in the binding of 11-*cis* retinal to opsin, it can be said that the HPE greatly contributes to its binding energy. Since the HPE mostly originates from the entropic contribution, it will be valuable to investigate the temperature dependence of the binding energy experimentally and so we shall be able to ascertain the important role of the hydrophobic bond effect.

It will be valuable to evaluate the HPE for a group including heteroatoms, so that we can calculate the HPE of any molecule by the method given in this paper. This seems to be possible by subtracting the HPE due to the hydrocarbon part from the total HPE's of amino acids as used by Chothia, and obtain HPE's of some heteroatom groups. Such an investigation will become the future task.

References

Bownds D (1967) Site of attachment of retinal in rhodopsin. Nature 216: 1178–1181

Chothia C (1974) Hydrophobic bonding and accessible surface area in proteins. Nature 248: 338–339

Chothia C (1975a) Structural invariants in protein folding. Nature 254: 304–308

Chothia C, Janin J (1975b) Principles of protein-protein recognition. Nature 256: 705–708

Chothia C, Wodak S, Janin J (1976) Role of subunit interfaces in the allosteric mechanism of hemoglobin. Proc Natl Acad Sci USA 73: 3793–3797

Honig B, Ebrey T, Callender RH, Dinur U, Ottolenghi M (1979a) Photoisomerization, energy storage, and charge separation; A model for light energy transduction in visual pigments and bacteriorhodopsin. Proc Natl Acad Sci USA 76: 2503–2507

Honig B, Dinur U, Nakanishi K, Balogh-Nair V, Gawinowicz MA, Arnaboldi M, Motto MG (1979) An external point-charge model for wavelength regulation in visual pigments. J Am Chem Soc 101: 7084–7086

Kauzmann W (1959) Some factors in interpretation of protein denaturation. Adv Protein Chem 14: 1–63

Matsumoto H, Yoshizawa T (1975) Existence of a β-ionone ring-binding site in the rhodopsin molecule. Nature 258: 523–526

Matsumoto H, Asato AE, Liu RSH (1979) 7-*cis*-porphyropsin from 7-*cis*-3-dehydroretinal and cattle opsin. Photochem Photobiol 29: 695–698

Nemethy G, Scheraga HA (1962a) Structure of water and hydrophobic bonding in proteins. II. Model for the thermodynamic properties of aqueous solutions of hydrocarbons. J Chem Phys 36: 3401–3417

Nemethy G, Scheraga HA (1962b) Structure of water and hydrophobic bonding in proteins. III. The thermodynamic properties of hydrophobic bonds in proteins. J Phys Chem 66: 1773–1789

Page, MI, Jencks W (1971) Entropic contribution to rate accelerations in Enzymic and intramolecular reactions and the chelate effect. Proc Natl Acad Sci USA 68: 1678–1683

Pitzer KS, Brewer L (1961) Thermodynamics, chapt 27. McGraw-Hill Book, New York

Received February 13, 1980/Accepted September 29, 1980