Hydrophobic Effect on the Protein–Ligand Interaction; Hydrophobic Field-Effect Index and Hydrophobic Correlation Index

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Two empirical indices accounting for the hydrophobic interaction are described. The first index is a "hydrophobic field-effect (Hf) index," which indicates the hydrophobic nature of the binding site of a host molecule such as an enzyme, and the second index is a "hydrophobic correlation (Hc) index," which indicates the hydrophobic correspondency between a host molecule and its guest molecule such as a ligand. Furthermore, a method to calculate the surface area of a molecule is described, in which the molecular surface is treated as a set of area-preserving spherical triangles. The hydrophobic effects on the interaction between papain and its inhibitor benzyloxycarbonyl-L-phenylalanyl-L-alanyl-methylene (Z–Phe–Ala–CH2–), which is covalently bound to catalytic Cys S^{γ} of the enzyme, were investigated by using these indices. It is quantitatively shown that the binding sites interacting with the benzene rings of P2 Phe and P3 Z are more hydrophobic, while the site of the carbonyl group of P1 Ala is more hydrophilic. The substrate specificity of papain can be explained in part by these indices. Both the Hc and Hf indices are visualized by using computer graphics. These indices would be useful as quantitative structure–activity relationship (QSAR) parameters.

Keywords hydrophobic field-effect index; hydrophobic correlation index; hydrophobic interaction; papain; benzyloxycarbonyl phenylalanyl alanyl methylene; QSAR parameter; surface area; drug design

During the last decade much evidence has been accumulated that drugs interact with specific receptors on the target cell. Drug-receptor interaction is a phenomenon which depends on non-covalent interaction forces. These non-covalent interactions can be roughly classified into three categories; hydrophobic interactions, steric interactions and electrostatic interactions. Of these interactions, steric interactions and electrostatic interactions can be estimated by using molecular mechanics or quantum mechanics, and many sophisticated methods combined with computer graphics have been proposed to analyze these aspects.[1-3] Hydrophobic interactions are of great importance for understanding the biological reactions. They participate in stabilization of membranes, folding of macromolecules, and transport of drugs to a target receptor. They also regulate drug activity through receptor–drug interaction. In spite of this importance, no convenient method for analyzing hydrophobic interaction at the molecular level has been well-established because of difficulties in handling; hydrophobic effect is closely related to the structure of the water molecules[4] surrounding a receptor and/or a ligand molecule.

Recently, methods for calculating solvation energy of protein folding were reported.[5,6] We have also reported a method[7] to calculate the hydrophobic interaction energy based upon the assumption that the extent of a hydrophobic effect is proportional to the solvent accessible surface area (ASA) of the molecules responsible for the association. This calculation method provides the hydrophobic energy of the ligand forming a complex, but does not provide information about the hydrophobic character of the binding site surrounding the ligand. This information is useful for understanding the mechanism of the complex formation and its stability, and for designing a ligand.

In order to quantify the hydrophobic effect on the protein-ligand interaction, we propose here two empirical indices. The first index is a "hydrophobic field-effect (Hf) index," which indicates the hydrophobic nature of a binding site of a host molecule such as an enzyme. The second index is a "hydrophobic correlation (Hc) index," which indicates the hydrophobic correspondency between a host molecule and a guest molecule. Furthermore, in order to visualize these indices, they are displayed on the molecular surface with color codes by using computer graphics.

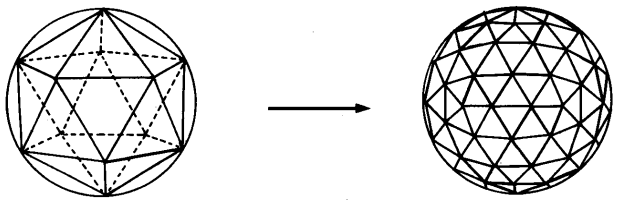
In the previous papers,[7-9] the molecular surface used in the calculation was approximated as an ensemble of plane patches defined by lines of latitude and longitude. In this study, we define the molecular surface as a set of area-preserving spherical triangles with the intention of (i) calculating the surface area accurately and (ii) obtaining a uniform surface.

Calculation Method

Definition of the Molecular Surface The molecular surface was successively triangulated to equiareal spherical triangles based upon a regular polyhedron inscribed in a spherical atom. In this report, we use a regular icosahedron or a regular hexahedron as the starting polyhedron.

Triangulation Based upon an Inscribed Regular Icosahedron Each of the 20 spherical triangles on the surface of an atom defined by an inscribed regular icosahedron (see Fig. 1a) is divided into 9 spherical triangles with points Q_1—Q_6 and P′ as shown in Fig. 2. In this figure, P′ is the intersecting point of the spherical triangle ABC and the extension of OP. Point P is the center of the "plane" triangle ABC and O is the center of the atom.

The coordinates of Q_1—Q_6 are determined by a parameter t ($0 \leqq t \leqq 0.5$), and then the surface areas S_1—S_3 are calculated by using the points Q_1—Q_6, A, B, C and P′ (see Eq. 8). The parameter t was determined by the



(a) (b)

Fig. 1. Schematic Representation of Triangulation Based upon an Inscribed Regular Icosahedron

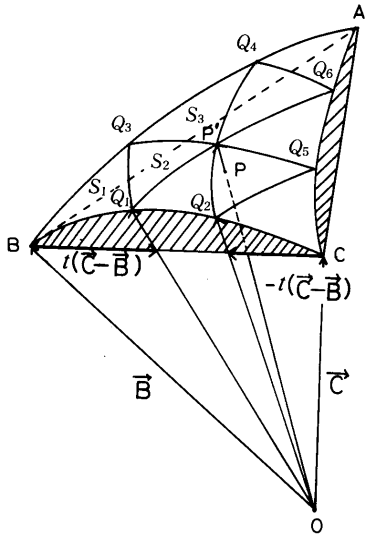


Fig. 2. Schematic Representation of Division of the Spherical Triangle (ABC) into Nine Spherical Triangles

See the text.

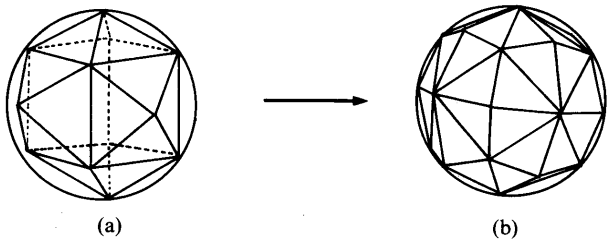


Fig. 3. Schematic Representation of Triangulation Based upon an Inscribed Regular Hexahedron

usual numerical differentiation method, so as to minimize the areal difference among the resulting spherical triangles. That is,

$$\Phi_1(t)=(S_1-S_2)^2+(S_2-S_3)^2+(S_3-S_1)^2 \quad (1)$$

$$\Phi_1(t)\rightarrow\min$$

where

$$S_1=\phi_1(t) \quad (2)$$

$$S_2=\phi_2(t) \quad (3)$$

$$S_3=\phi_3(t) \quad (4)$$

Thus, 180 spherical triangles are obtained from 20 spherical triangles by this operation (20×9). These spherical triangles are further divided into 1620 spherical triangles (180×9) and an appropriate number of spherical triangles can be defined on the surface of the molecule by means of an appropriate number of repetitions of the operation (Fig. 1). The parameter t was calculated to be 0.3519 for the starting regular triangle by the numerical differentiation method, and Φ_1 for the optimized t was 1.545×10^{-4} Å^2 (0.0246% of the starting spherical triangle). The triangles would deviate from the regular triangle in shape through the repetition of the division. Accordingly, the parameter t was set to be 1/3 on and after the second division.

Triangulation Based upon an Inscribed Regular Hexahedron Each of the 24 spherical triangles (Fig. 3a), which is derived from an inscribed regular hexahedron, is divided into 2 equiareal spherical triangles. The division of a starting spherical triangle is shown in Fig. 4, where O is the center of the atom and FG is the longest side of the triangle EFG. The spherical triangle EFG is divided into two area-preserving spherical triangles by the point Q which is defined by using a parameter t (see Fig. 4). That is, the parameter t is determined as follows.

$$\Phi_2(t)=(S_4-S_5)^2 \quad (5)$$

$$\Phi_2(t)\rightarrow0$$

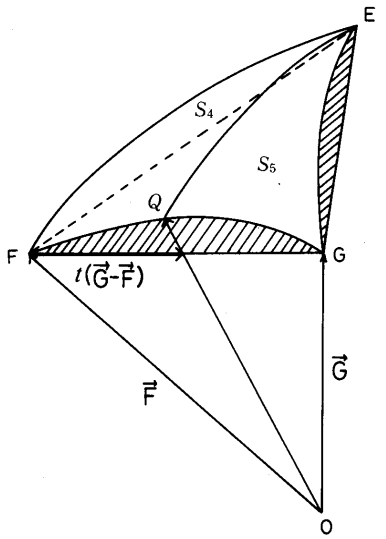


Fig. 4. Schematic Representation of Division of the Spherical Triangle (EFG) into Two Spherical Triangles

See the text.

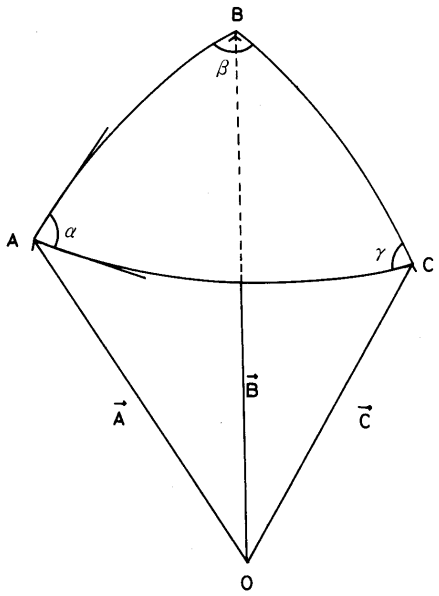


Fig. 5. The Surface Area (S) of the Spherical Triangle (ABC) is Calculated by Using the Formula $S=R^2(\alpha+\beta+\gamma-\pi)$

R is the radius of the sphere. The evaluation of the interior angles (α—γ) is described in the text.

where,

$$S_4=\phi_4(t) \quad (6)$$

$$S_5=\phi_5(t) \quad (7)$$

S_4 and S_5 are the surface areas of the resulting spherical triangles. The parameter t was determined for every division by using the numerical differentiation method ($\Phi_2\sim0$). These spherical triangles are able to be further divided into an appropriate number of spherical triangles by means of an appropriate number of repetitions of the operation (Fig. 3).

Calculation of the Molecular Surface Area The surface area S of a spherical triangle is calculated by applying the well-known formula

$$S=R^2(\alpha+\beta+\gamma-\pi) \quad (8)$$

where α, β and γ are the interior angles, and R is the radius of the sphere (see Fig. 5). The interior angles are determined as follows.

$$\cos\alpha=\cos(\pi-\theta_a)=-\vec{e}_b\cdot\vec{e}_c \quad (9)$$

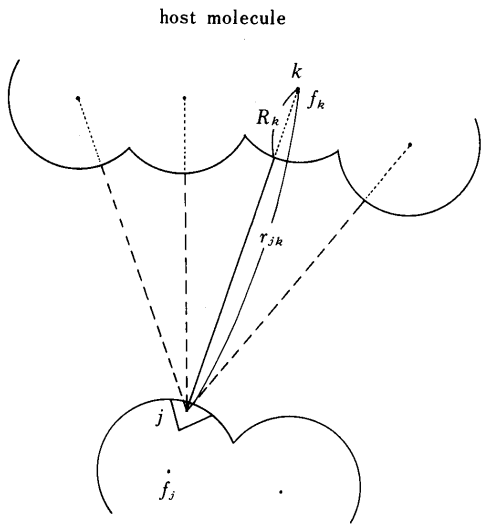


Fig. 6. The Projection of Hydrophobic Effect from the k-th Atom on the j-th Spherical Triangle is Defined as $f_k \cdot \phi$ and the Total Effect of the Host Molecule on the j-th Spherical Triangle is Calculated by $\sum f_k \cdot \phi$

See the text. The summation is carried out over all atoms which face the j-th spherical triangle.[22]

$$\cos\beta = \cos(\pi - \theta_b) = -\vec{e}_c \cdot \vec{e}_a \tag{10}$$

$$\cos\gamma = \cos(\pi - \theta_c) = -\vec{e}_a \cdot \vec{e}_b \tag{11}$$

where $\vec{e}_a$, $\vec{e}_b$ and $\vec{e}_c$ are unit vectors of $\vec{a}$, $\vec{b}$ and $\vec{c}$, respectively, defined as

$$\vec{a} = \vec{B} \times \vec{C} \tag{12}$$

$$\vec{b} = \vec{C} \times \vec{A} \tag{13}$$

$$\vec{c} = \vec{A} \times \vec{B} \tag{14}$$

θ_a is the angle determined by $\vec{e}_b$ and $\vec{e}_c$, θ_b is the angle determined by $\vec{e}_c$ and $\vec{e}_a$, and θ_c is the angle determined by $\vec{e}_a$ and $\vec{e}_b$.

The overall molecular surface area is obtained by summing up the areas of the spherical triangles. The overlapping spherical triangle placed on the boundary between the neighboring atoms is further divided into an appropriate number of spherical triangles, and final overlapping spherical triangles are treated as plane triangles.

Hf-Index The hydrophobic field-effect index Hf^j is empirically defined by Eq. 15 at the j-th spherical triangle on the guest molecule (Fig. 6).

$$Hf^j = \sum_k f_k \cdot \phi \tag{15}$$

$$\phi = \exp(\beta \cdot (r_{jk} - R_k)^2) \tag{16}$$

where f_k is the transfer free energy per unit surface area of the k-th atom of the host molecule.[7-9] ϕ is a factor that depends on the interacting distance as shown in Eq. 16. In Eq. 16, r_{jk} is the distance between the center of the j-th patch and the k-th atom, R_k is the van der Waals radius of the k-th atom, and β is set to be -0.1312.[7] From the viewpoint of calculation cost, an appropriate cut-off value can be set for $r_{jk} - R_k$. The $f_k \cdot \phi$ values are summed up for all of the host atoms facing this spherical triangle as shown in Eq. 15. This index is a quantitative indication of hydrophobicity of the binding site in a host molecule. According to Eq. 15, those patches that interact with hydrophobic binding sites show negative Hf values.

Hc-Index Hydrophobic correlation index (Hc) between the j-th spherical triangle on the guest molecule and its binding site in the host molecule is empirically defined as follows,

$$Hc^j = \sum_k f_j \cdot f_k \cdot \phi$$

$$= f_j \cdot Hf^j \tag{17}$$

where f_j is the transfer free energy per unit surface area of the atom having the j-th spherical triangle (see Fig. 6). In Eq. 17, Hf^j is the hydrophobicity of the binding site interacting with the j-th patch and f_j is that of the j-th patch. Both Hf^j and f_j for the hydrophobic part are negative, while those for hydrophilic parts are positive. Thus, when a hydrophobic part of the ligand interacts with a hydrophobic binding site or a hydrophilic one interacts with a hydrophilic one, the product of $f_j \cdot Hf^j$ shows a positive value. This is a quantitative indication of the hydrophobic correspondency between the interacting molecules. It should be noted that the Hc index is different from the "correlation coefficient" as can be seen from Eq. 17.

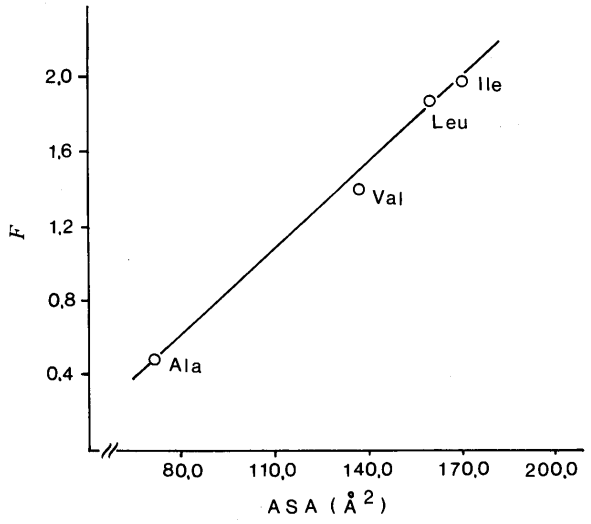


Fig. 7. Relationship of Solvent Accessible Surface Area (ASA) of the Side Chain and Hydrophobic Fragmental Constant (F) for a Side Chain of Aliphatic Amino Acids

A solvent water molecule is assumed to be a sphere with radius of 1.4 Å. $F = \log P(CH_3CONHCHCONH_2) - \log P(CH_3CONHCHCONH_2) + 0.182$, in which the value of 0.182 (contribution of a hydrogen atom) is taken from ref. 11.

These values for the individual spherical-triangle patches are weighted by the surface area and summed up at the atom, the group and the molecule, and they lead to the indices for the atom, the group and the molecule, respectively (Eqs. 18—23).

$$Hf^{atom} = \sum_j^{atom} Hf^j \cdot S^j \tag{18}$$

$$Hf^{group} = \sum_j^{group} Hf^j \cdot S^j \tag{19}$$

$$Hf^{molecule} = \sum_j^{molecule} Hf^j \cdot S^j \tag{20}$$

$$Hc^{atom} = \sum_j^{atom} Hc^j \cdot S^j \tag{21}$$

$$Hc^{group} = \sum_j^{group} Hc^j \cdot S^j \tag{22}$$

$$Hc^{molecule} = \sum_j^{molecule} Hc^j \cdot S^j \tag{23}$$

where S^j is the surface area of the j-th patches. Hf^{atom}, Hf^{group} and $Hf^{molecule}$ have dimensions of cal/mol. It should be noted, however, that these values are just indices and do not indicate the hydrophobic interaction energy defined in reference 7, because the surface area S in Eqs. 18—23 is not for the binding site but for the ligand.

These indices are visualized by using a computer program BIOCES.[10]

***f*-Values** In this report, transfer free energy per unit surface area (f-value) is used as a scale of the hydrophobicity. Other indications such as a hydrophobic fragmental constant[11] *etc.* instead of the f-value can be directly applied. The f-values are calculated according to the previous papers.[7-9] In this study, we use the partition data[12] of N-acetyl amino acid amides instead of the data for free amino acids[13] described in the previous papers. The hydrophobic fragmental constants (F) for the amino acids were calculated by the least-squares method according to the previous papers.[7-9] In the least-squares method, the contribution of a hydrogen atom was assumed to be 0.182.[11] Solvent accessible surface areas (ASA) of hydrocarbon moieties in the amino acid molecules correlate with the hydrophobic fragmental constants (F) as shown in Fig.

TABLE I. *F*-Values and *f*-Values for a Protein

Group	*F*	*f* (cal/(mol·Å²))
Guanidinium	−2.13	19.30
–SH	1.29	−24.10
–S–	−0.02	0.71
Imidazolium	−0.12	1.27
Indolyl	2.00	−12.56
$-NH_3^+$	−2.54	45.28
$-C_6H_5$	1.54	−12.88
$-CONH_2$	−0.87	11.30
$-COO^-$	−1.17	18.63
–OH (aliphatic)	−0.37	11.26
–OH (aromatic)	−0.65[a]	15.78
Hydrocarbon	—	−20.87[b]
Back-bone amide	−1.58[c]	29.34

a) This value is estimated from the log *P* values of tyrosine and phenylalanine. *b*) See the text. *c*) This value is estimated from Rekker's compilation.[11]

TABLE II. *F*-Values and *f*-Values for ZFA

Group	*F*	*f* (cal/(mol·Å²))
–OCONH–	−1.50	40.46
–CO–	−1.26	31.36
Aliphatic hydrocarbon	—	−24.42[a]
Aromatic hydrocarbon	—	−22.81[a]

a) From ref. 7. This value was obtained by using the slope of the linear relationship between the partition coefficient and the solvent surface area.

7. The *f*-value for these moieties is calculated to be −20.87 cal/(mol·Å²) based upon the slope of the line by using the factor of 2.3 RT at 25 °C. For other functional groups, the fragmental constant (*F*) is divided by its ASA and converted into the *f*-value by using 2.3 RT at 25 °C. R is the gas constant. The *f*-values used in this study are listed in Tables I and II with the hydrophobic fragmental constants (*F*).

Calculation of the Hydrophobic Indices for the Inhibitor Benzyloxycarbonyl-L-phenylalanyl-L-alanylmethylene (Z–Phe–Ala–CH_2–; ZFA) Interacting with Papain The coordinates of papain and the inhibitor ZFA[14] were taken from the protein data bank[15] (ID code = 6PAD). ZFA is covalently bound to catalytic Cys 25 S^γ of the enzyme. Hydrogen atoms were generated with standard geometry. The *f*-values for ZFA are listed in Table II, in which the hydrophobic fragmental constants are taken from Rekker's compilation.[11] The proximity correction described in reference 11 as the C_M (0.289) is divided in equal parts and assigned to each of the proximate groups. The hydrophobic fragmental constants for the amino acid residues in the inhibitor (Phe and Ala) were taken from Table I. Cut-off value for $r_{jk} - R_k$ (see Eq. 16) was 5 Å.

Results and Discussion

Accuracy of the Surface Area The accuracy of the surface area computed by this method depends upon the degree of subdivision of the overlapping spherical triangles, and the computer time required also depends upon this degree. As shown in Fig. 8, both methods give the surface areas with reasonable accuracy (0.4—0.2%) even though subdivision of the overlapping spherical triangles is not performed.

One of the advantage of this triangulation method over the previous method,[7-9] in which a "patch" was defined by using lines of latitude and longitude, is the uniform distribution of the patches. In the previous method, patches near to the pole were more densely distributed than those near to the equator, and this nonuniformity of patch distribution caused some problems in analysis of the interacting mode.

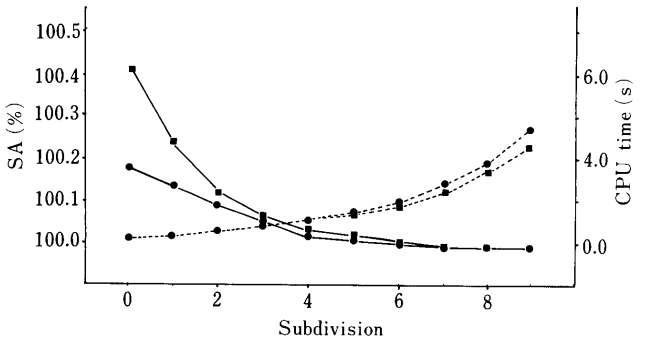


Fig. 8. Relationship of Accuracy of Surface Area, SA (%), and Degree of Subdivision for a Biatomic-Molecule Model System

In this figure, the atomic surface is defined by 190 spherical triangles for the "icosahedron method" (■—■) and 192 spherical triangles for the "hexahedron method" (●—●). Relation to CPU time is represented by using a broken line. Surface area was calculated by using an NEC ACOS430 general-purpose computer (1 mips).

Hydrophobic Effects on the Papain–ZFA Interaction Papain is a thiol protease isolated from the latex of the green fruit of *Carica papaiya*. Enzymes such as cathepsins B, H, L *etc.* are intracellular proteases that originate from animals, and are classified into the same group as papain, because they have a cysteine residue in the catalytic site. These cathepsins are thought to be concerned in the processing of essential proteins such as enzymes,[16] insulin[17] and immunoglobulin,[18] and in diseases such as cancer[19] and muscular dystrophy.[20] Accordingly, a great deal of effort has been spent on the development of inhibitors of these proteases. Structural information about the target enzyme would be very useful to design inhibitors.[21-23] The three-dimensional structures of the cathepsins have not been determined, though that of papain was determined by X-ray diffraction measurements.[14] Consequently, it is possible to analyze the binding mode of the inhibitor in the active site at the molecular level. From the viewpoints of the electrostatic and steric interactions, we have studied the interacting mode of inhibitors with papain and cathepsin B with the intention of obtaining ideas for the design of inhibitors of these enzymes. In this paper we describe the hydrophobic aspect of the interaction between papain and ZFA.

A space-filling molecular model of ZFA is shown in Fig. 9, represented in the same direction as in Figs. 10—12. Figure 10 shows the interacting distance between a patch of ZFA and the van der Waals' surface of the corresponding interacting atom of papain with the use of a color code on the molecular surface of ZFA.[22] The Hf calculated by Eq. 15 is represented on the molecular surface of ZFA by using the color code (Fig. 11). The regions interacting with a hydrophobic binding site of the enzyme are shown in blue (negative value), and the regions interacting with a hydrophilic one are shown in red (positive value). It can be understood visually through Fig. 11 that the peptide backbone of the inhibitor interacts with hydrophilic residues in the binding sites of papain; the Hf values for these regions of ZFA are relatively high positive values because of the influence of the backbone region of Gly 65—Gly 66 of the enzyme. On the contrary, most of the benzene ring of P2 Phe[24] is surrounded by a hydrophobic environment. The high positive values on the benzene ring of P2 Phe (see Fig.

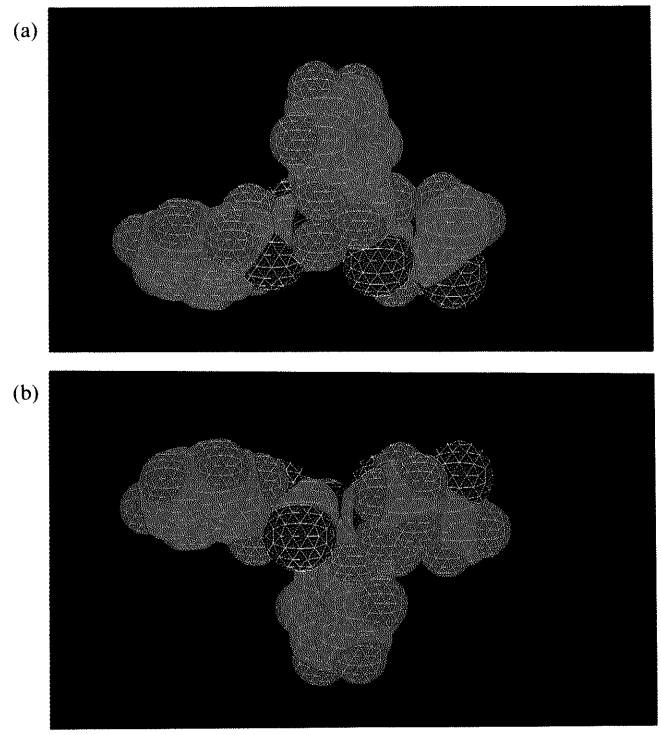


Fig. 9. Space-Filling Display of ZFA

(b) is a view of (a) rotated by 180 around the x-axis. The benzene ring of the benzyloxycarbonyl group is represented on the left, and that of the phenylalanine residue is represented at the center of the figure. These illustrations are represented in the same direction as in Figs. 10—12 (red = oxygen, blue = nitrogen, light blue = carbon, green = hydrogen).

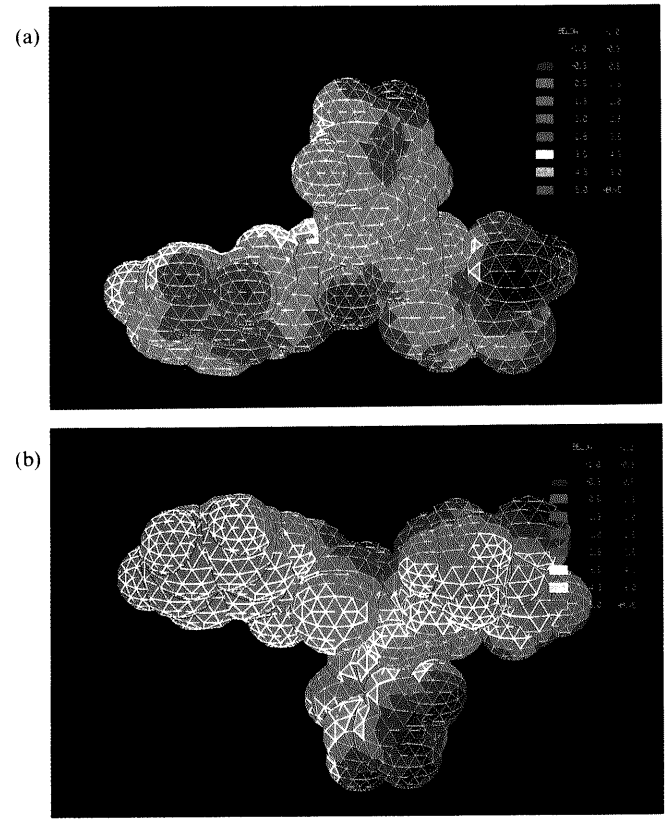


Fig. 10. Graphical Representation of Distance[22] between van der Waals Surface of ZFA and Papain

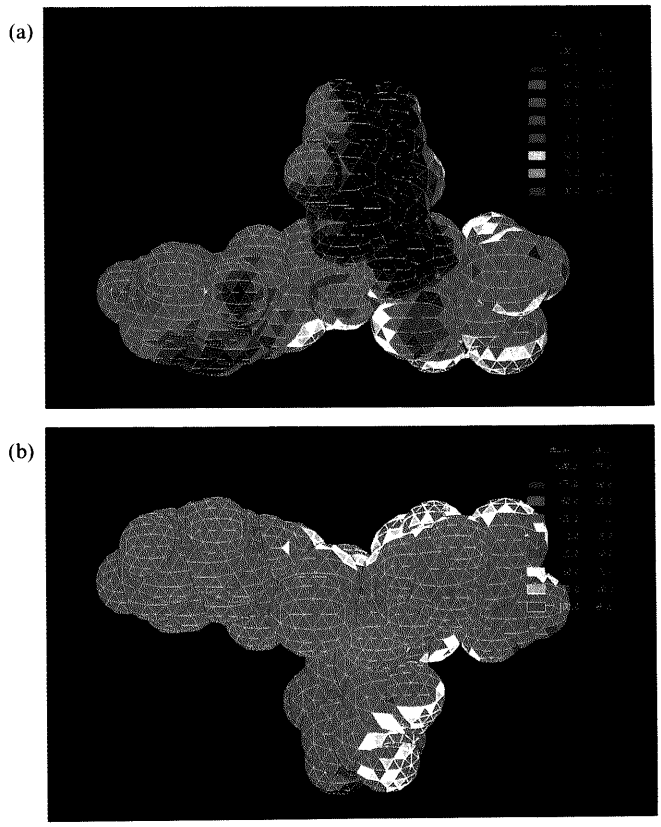


Fig. 11. Hf on the van der Waals Surface of ZFA

For each patch the value is calculated from Eq. 15.

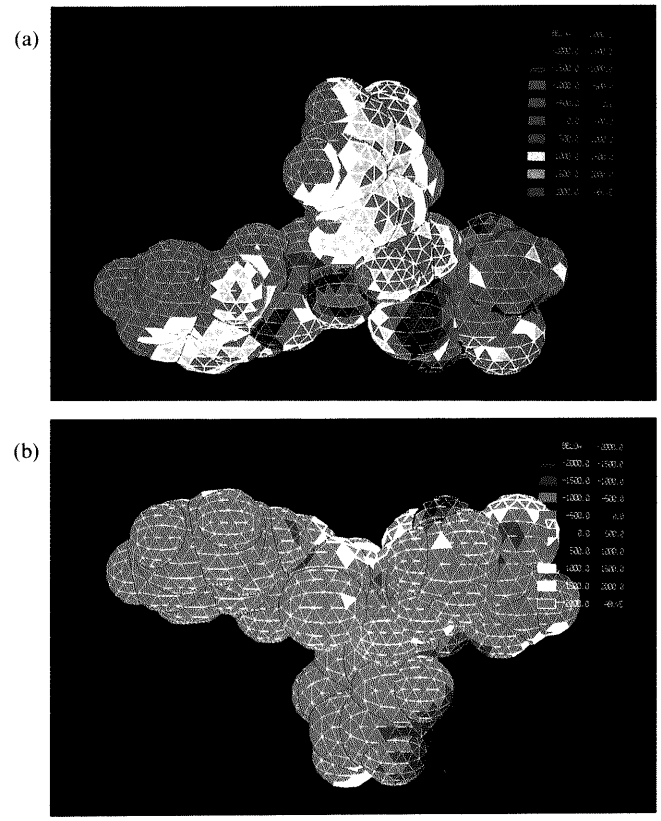


Fig. 12. Hc on the van der Waals Surface of ZFA

For each patch the value is calculated from Eq. 17 (see the legend to Fig. 9).

11(b)) are due to Asp 158 and His 159 of the enzyme. Figure 11 also indicates that the benzene ring of the P3 benzyloxycarbonyl group is located in a hydrophobic environment. As shown in Fig. 10, half of this benzene ring interacts with solvents, so that the Hf indices of the patches on this side are zero.

The Hc, an index of the correspondency from the viewpoint of hydrophobicity between the guest molecule (ZFA) and its binding site of the host molecule (papain), is represented in Fig. 12 by using a color code. The peptide backbone of ZFA exhibits positive values (red color), indicating that this moiety forms hydrogen bonds to the backbone of Gly 65—Gly 66. Most of the benzene ring of P2 Phe shows highly positive values; the exceptional negative values (blue color) are due to the interaction with Asp 158 and His 159. The benzene ring of the P3 benzyloxycarbonyl group, except for the part interacting with solvents, also shows highly positive values.

The Hf index for each group (Hf^{group}) is listed in the second column of Table III. The position of the binding site in a protein, defined as a region interacting with a group in a ligand, is changeable with the orientation and size of the group. When the atom in the protein is far from the ligand, the effect of the atom on the Hf index is reduced by ϕ (see Eq. 15). Furthermore, the Hf^{group} index is dependent on the contact surface area of the group that comes into contact with the binding site by association. As a result, this index is influenced by the orientation and the size of the group. Both of the binding sites interacting with the benzene rings of P2 Phe and P3 benzyloxycarbonyl are more hydrophobic than other binding sites, and the binding site interacting with the carbonyl group of P1 Ala is more hydrophilic. The P1 Ala side chain (group 9) seems to interact with a hydrophilic binding site. This side chain also interacts with solvent water molecules.[22] The second column of Table III clearly indicates that half of the inhibitor (group 1—6) interacts with relatively hydrophobic binding sites while the remainder interacts with hydrophilic binding sites.

The third column (Hc) of Table III shows that the benzene ring and the methylene group of P2 Phe, and the benzene ring of the P3 Z-group, have highly positive values. Accordingly, these moieties (and the corresponding binding sites of the enzyme) seem to stabilize the papain–ZFA complex in view of the hydrophobic effect. On the contrary, hydrophilic groups having highly positive values such as the carbonyl group of P1 Ala and the amide group between P1 Ala and P2 Phe would contribute to the stabilization through electrostatic interaction; these hydrophilic groups were found to have highly positive values of the guest–host electrostatic correlation potentials.[22]

Hydrophobic energies (HE) obtained for the groups of ZFA are listed in Table III. Those values were calculated according to the previous method.[7-9] The calculation was performed based upon the assumption that no conformational change occurred during the complexation. ZFA–papain crystals are usually obtained from reactive Z–Phe–Ala chloromethyl ketone.[14] However, in this calculation the methylene group (group 11) of the C-terminal is assumed to be not in a chloromethyl form, but a "methylene" radical in the state free from the enzyme. Therefore, the value for this group (−1.37 kcal/mol) is somewhat over-estimated. As can be seen from the fourth column (HE) of Table III, the benzene rings of P2 Phe and P3 Z gain hydrophobic stabilization energies by forming a complex. The latter shows less stabilizing energy because of less desolvation; the contact surface area of this group is smaller than the former group (see Fig. 10). Hf^{group}, Hc^{group} and HE in Table III indicate different aspects of the hydrophobic interaction between ZFA and papain, and no clear correlation was found between these quantities. HE is the hydrophobic energy obtained by group in ZFA. Hf^{group} is the indication of the hydrophobicity of the corresponding binding site of papain. The binding site corresponding to such a group having highly negative Hf^{group} would gain large hydrophobic energy by the association. Hc^{group} is the indication of the hydrophobic correspondency beween the group and the binding site. For a hydrophobic group with a large Hc^{group} index, the hydrophobic stabilization is expected to occur by association, accompanied with hydrophobic interaction on the corresponding binding site.

Although the substrate specificity of papain is not so high, this enzyme is thought to prefer a hydrophobic amino acid residue in the P2 position of its substrate.[25] Our results indicate that the hydrophobicity of the binding site for this position is relatively high. This site is situated on the surface of the enzyme molecule, and hence contacts many water molecules in the native state. As a result of formation of the complex with ZFA, this binding site would undergo an effective desolvation accompanied with desolvation on the side chain of the P2 residue, and the complex would gain substantial hydrophobic stabilization energy. The superiority of a hydrophobic residue over a hydrophilic residue in the P2 position of substrate can be ascribed in part to these effects.

The interacting mode between a ligand and a protein can be qualitatively understood through other representations,

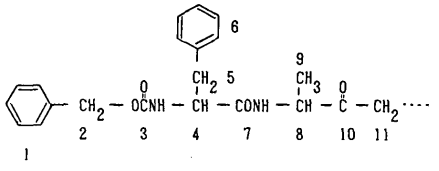
TABLE III. Hf^{group} Index and Hc^{group} Index

Group	Hf^{group} index ($\times 10^{-3}$)	Hc^{group} index ($\times 10^{-4}$)	HE[a] (kcal/mol)
1	−1.07	2.44	−1.61
2	−0.40	0.97	−0.65
3	−0.10	−0.41	1.33
4	0.07	−0.15	−0.03
5	−0.89	1.87	−0.48
6	−4.07	5.24	−1.75
7	0.45	1.32	0.88
8	0.79	−1.65	−0.29
9	0.55	−1.14	−0.66
10	1.06	3.33	1.18
11	0.17	−0.42	−1.37

a) The hydrophobic energy (HE) for an *i*-th group was calculated by means of the following equation.[7-9]

$$HE = \sum_{j}^{i\text{-th group}} \{(\phi \cdot f_i \cdot ASA_i \cdot S_j)/SA_i\}$$

where f_i is the transfer free energy per unit surface area of the *i*-th group, S_j is the surface area of the *j*-th spherical triangle defined on the van der Waals surface of the group, and ϕ is defined by Eq. 16 (see the text). ASA_i and SA_i are solvent accessible surface area and van der Waals area interacting with solvent water molecules in the state free from protein, respectively, for the *i*-th group.



such as a ball and stick illustration, *etc.* However, the Hf and Hc indices proposed here make it possible to evaluate the hydrophobic nature of binding sites of a host molecule and the hydrophobic correspondency between a host molecule and a guest molecule in a quantitative manner; these aspects of protein-ligand interaction had been difficult to quantify. These indices are strictly empirical, but they are simple to use and also suitable for graphical representation. Therefore, they are useful for drug design. These indices can be used as parameters in quantitative structure–activity relationship (QSAR) analysis. An analysis using these indices is in progress.

We also described the use of the spherical triangle to obtain an accurate and uniform molecular surface. It is particularly effective to represent the physical properties of a host molecule on the surface of an interacting guest molecule (such as the Hf and Hc indices presented here and host-on-guest electrostatic potential[2]). The ensemble of centers of spherical triangles also provide a "dot surface" model of a molecules, in which each of the dots has the same weight from an areal point of view.

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