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Calculation of solvation interaction energies for protein adsorption on polymer surfaces

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Abstract—Of the interactions that govern protein adsorption on polymer surfaces, solvation interactions (repulsive hydration and attractive hydrophobic interactions) are thought to be among the most important. The solvation interactions in protein adsorption, however, have not been dealt with in theoretical calculation of the adsorption energy owing to the difficulties in modelling such interactions. We have evaluated the solvation interaction energies using the fragment constant method of calculating the partition coefficients of amino acids. The fundamental assumption of this approach is that the partition coefficients of amino acids between water and organic solvent phases are related to the free energies of transfer from bulk water to the polymer surface. The X-ray crystallographic protein structures of lysozyme, trypsin, immunoglobulin F_{ab}, and hemoglobin from the Brookhaven Protein Data Bank were used. The model polymer surfaces were polystyrene, polypropylene, polyethylene, poly(hydroxyethyl methacrylate) [poly(HEMA)], and poly(vinyl alcohol). All possible adsorption orientations of the proteins were simulated to study the effect of protein orientation on the solvation interactions. Protein adsorption on either hydrophobic or hydrophilic polymer surfaces was examined by considering the sum of solvation and other interaction energies. The results showed that the contribution of the solvation interaction to the total protein adsorption energy was significant. The average solvation interaction energy ranged from −259.1 to −74.1 kJ/mol for the four proteins on the hydrophobic polymer surfaces, such as polystyrene, polypropylene, and polyethylene. On the other hand, the average solvation interaction energies on hydrophilic surfaces such as poly(HEMA) and poly(vinyl alcohol) were larger than zero. This indicates that repulsive hydration interactions are in effect for protein adsorption on hydrophilic polymer surfaces. The total interaction energies of the proteins with hydrophobic surfaces were always lower than those with more hydrophilic surfaces. This trend is in agreement with the experimental observations in the literature. This study suggests that consideration of the solvation interaction energies is necessary for accurate calculation of the protein adsorption energies.

Key words: Protein adsorption; polymer surfaces; solvation energy; hydrophobic interaction; computer simulation; partition coefficient.

INTRODUCTION

Recent advances in the area of polymer chemistry have provided us with numerous new polymers for many applications. The progress made in the application of synthetic polymers as biomaterials, however, has been slow. Bioapplication is unique in the sense that the polymer surfaces always come in contact with biological fluids and the polymer surface properties are modified as a result of such contacts. For example, the biocompatibility of polymeric implants is known to be determined by the nature of the adsorbed blood proteins [1–3]. Despite the importance of protein adsorption in the bioapplications of polymers, the mechanisms of protein adsorption are not clearly understood. As a result, it is not easy to select the right polymers

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for particular applications. To date, the selection of the right polymers is almost on a trial-and-error basis. Clearly, a better understanding of the protein–surface interaction is necessary.

In an attempt to understand the mechanisms of protein adsorption at the solid–liquid interface, we previously examined the interaction energies for protein adsorption on polymer surfaces using a computer simulation approach [4]. The results obtained in that study suggested that proteins had a stronger affinity to hydrophilic polymer surfaces than to hydrophobic polymer surfaces. The results were contrary to the general belief that the affinity between a given protein and an adsorbent increases with the hydrophobicity of the surface [1, 2, 5–9]. This difference was most likely due to not taking into consideration the solvation interaction energies in our previous study. Solvation interactions arise as a result of the overlapping of structurally modified boundary layers of liquid. The overlapping of boundary layers of water on hydrophilic surfaces gives rise to a repulsive solvation interaction (hydration interaction) and on hydrophobic surfaces, to an attractive solvation interaction (hydrophobic interaction) [10]. Since solvation interactions cannot be accounted for by the classical theories of van der Waals-potential forces, they have to be examined independently [11]. Although the solvation forces have been measured experimentally using curved mica atomic force apparatus and their potential functions are available [12], their application to the modelling of protein–polymer interactions is difficult mainly because of the complex nature of the protein surface. This paper describes our recent attempt to calculate the solvation interaction energies for protein adsorption on polymer surfaces.

METHODS

Distribution of hydrophobic and hydrophilic residues on the protein surface

The three-dimensional protein structure has an energetic balance between the internal interactions and the protein–solvent interactions [13]. The distribution of hydrophobic and hydrophilic residues on the protein surface is related to the relative tendency of each amino acid residue to react with water molecules. Polar or charged residues tend to be on the surface and nonpolar residues in the interior. However, since each amino acid residue cannot be distributed independently owing to the primary structure of protein, the distributions vary from one protein to another. Thus, the hydrophobic amino acid residues are by no means all buried [14].

We examined the distribution of hydrophobic and hydrophilic residues on the protein surface using a rotation-cut off approach. The X-ray crystallographic structure of protein was obtained from the Brookhaven Protein Data Bank. Protein was rotated on a two-dimensional plane. The method of rotating a protein molecule to simulate different orientations has been described in detail previously [4], and is only briefly reviewed here. Two rotational angles were used for the three-dimensional rotation of a protein molecule. Angle θ_1 was for rotation on the Y-axis and angle θ_2 on the Z-axis. Both θ_1 and θ_2 were calculated from the geometrical center of the protein. The value of angle θ_2 depended on the value of angle θ_1. The value of θ_1 was increased at 5° intervals and as a result, 1633 different orientations were obtained. At each orientation, a protein molecule was brought to just contact the polymer surface. The closest contact distance between the atomic centers of the protein and the polymer was the sum of the van der Waals radii of the two

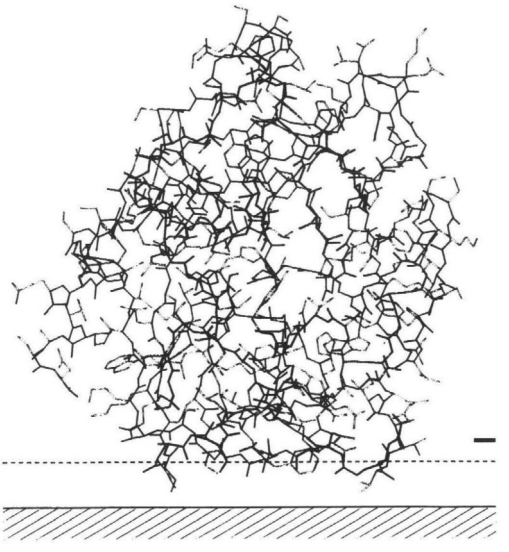

Figure 1. Example of a threshold (dotted line) which was set to identify the amino acid residues in contact with the polymer surface. The threshold value was set as 2.8 Å. Trypsin is used in this example and it was rotated with 1633 orientations.

contacting atoms. The van der Waals radii used in our calculation were 1.00, 1.35, 1.35, 1.60 and 1.85 Å for H, N, O, C, and S, respectively [15].

The diameter of water was given as 2.8 Å [16]. A distance of 2.8 Å from the edge of the polymer atom was set as the threshold to examine the distribution of surface amino acid residues. The value of the threshold was chosen for the reason that if the distance between any atom and the polymer surface was less than 2.8 Å, there would be no space to accommodate water molecules. The surface amino acid residues were distinguished as follows. If half of the total number of atoms of an amino acid residue was present below the threshold, the amino acid residue was counted as a surface amino acid residue. Figure 1 illustrates the rotation-cut off approach to examine the distribution of hydrophobic and hydrophilic amino acid residues on the surface of a trypsin molecule. It should be noted that these surface amino acid residues were the ones that contacted the polymer surface upon adsorption. The surface amino acid residues located in the valleys of the protein surface were not considered, since the protein molecules were treated as hard balls. The distribution of surface amino acid residues was calculated as the ratio of the number of molecules of a particular amino acid occurring on the protein surface to the total number of molecules of the amino acid in a protein. The four proteins examined were lysozyme, trypsin, immunoglobulin F_{ab}, and hemoglobin.

Modelling of the solvation interactions for protein adsorption

When a protein molecule adsorbs to a polymer surface, water molecules between the protein and the polymer need to be displaced. Figure 2 describes the removal of water molecules as a result of contacts between the amino acid residues and the polymer surface. A repulsive solvation interaction (hydration interaction) arises whenever water molecules are associated with surfaces containing hydrophilic

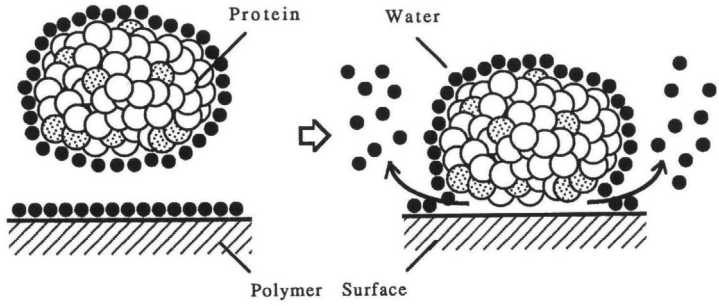

Figure 2. Schematic description of protein adsorption on the polymer surface. Water molecules are removed from the sites of contacts between amino acid residues and the polymer surface. Some of the water molecules may remain trapped in certain spaces between the protein and the surface if the sizes of the spaces are larger than the size of water molecules. The dotted circles on the protein represent hydrophobic amino acid residues. The solvation energy depends on the distribution of hydrophobic amino acid residues on the protein surface and the hydrophobicity of the polymer surface.

groups, and its strength depends on the energy necessary to disrupt the ordered water structure and ultimately dehydrate the surface [17]. If water molecules are associated with hydrophobic surfaces, an attractive solvation interaction (hydrophobic interaction) occurs and its strength depends on the hydrophobicity of the surface or surface groups [12, 18, 19]. Thus, the model for computer calculation should consider both attractive hydrophobic and repulsive hydration interactions.

Our model assumes that the solvation energy in the interaction between amino acid residues and the polymer surface is the same as the free energy of transferring both amino acid residues and the polymer surface from water to apolar solvents. This is a reasonable assumption since the adsorption of amino acids residues of a protein on the polymer surface eliminates water molecules between them and the resulting environment would be similar to the apolar solvent. The solvent transfer model has been used extensively for the problem of protein stability associated with the hydrophobic interaction [20–22]. Thus, our model is as good as the model used to calculate the hydrophobic interactions for protein stability.

The free energy of transferring amino acid residues from water to the organic solvent phase is calculated from the partition coefficient between the two phases [16, 23]. Thus, the hydrophobic interaction energy for protein adsorption on the polymer surfaces can be calculated by considering the partition coefficients of amino acid residues and the repeating units of polymers. The type of organic solvent used in the solvent transfer model varies; however, nonpolar hydrogen-bonding solvents such as octanol, ethanol, and dioxane have been used most widely and successfully [24].

For amino acid residues and their side chains, the free energies of transfer have been termed 'hydrophobicities', and the magnitude of the hydrophobicity is a measure of how much the amino acid residue or the side chain seeks or avoids association with water [25]. Although there are a number of hydrophobicity scales available, there are mainly two studies which assign thermodynamic parameters to individual atoms or groups of atoms in protein side-chains. One is the approach established by Eisenberg and McLachlan [16]. They used the atomic solvation parameters (ASPs) of each atom which describe the strength of the water–solvent interaction. The values of the ASPs were determined according to the free energy of

transfer from the organic solvent to water. The free energy of transfer of each amino acid was calculated using the following equation:

$$\Delta G = \sum_{\text{atoms } i} \Delta\sigma_i A_i, \tag{1}$$

where A_i is the accessible surface area of each atom and $\Delta\sigma_i$ is the atomic solvation parameter. The differences among various carbon units, however, were not explicitly considered in this approach.

The other approach was developed by Abraham and Leo [26]. They used the fundamental fragment constants to evaluate the hydrophobicity of amino acids or amino acid residues in protein [27, 28]. Since the fragment constants are defined as nonambiguous entities that can be utilized in computer programs, this method allows estimation of the solvation energy upon protein adsorption on polymer surfaces. The distinction of fragment constants for various carbon units also gives a more accurate calculation of the solvation energies upon protein adsorption. For these reasons, the fundamental fragment constant method was used in our study.

The partition coefficients ($P_{\text{octanol/water}}$) of amino acid residues, which address the hydrophobicity scale, were calculated based on the fragment constants, f. The following equation was used to calculate log P of each amino acid residue and log P of repeating units of each polymer surface:

$$\log P = \sum_{n=1}^{N} a_n f_n + \sum_{m=1}^{M} b_m F_m, \tag{2}$$

where a is the number of occurrences of fragment f of structural type n and b is the number of occurrences of factor F of structural type m. The F factors, which were derived empirically, consider the change of hydrophobicity due to the branching of chains or groups (F_{cBr} or F_{gBr}), the bond types (F_{b}), and the proximity effects of polar groups with n carbon separation ($F_{\text{p}n}$). The values of f and F for each fragment in amino acid residues can be found in the literature [26, 28]. These values were rearranged and tabulated for the input into the computer as listed in Table A1 of the Appendix. Examples of the calculation of the log P value of an amino acid residue and the calculation of log P of repeating units of polymers using the fragment method are given in Table A2 of the Appendix. The log P values of the polymers used in our study are listed in Table 1. If log P of a polymer is larger than zero, the polymer is considered hydrophobic. If log P of a polymer is equal to or less than zero, the polymer is considered hydrophilic. It is seen in Table 1 that polystyrene, polypropylene, and polyethylene are hydrophobic, while poly(HEMA) and poly(vinyl alcohol) are hydrophilic.

Table 1.
The log P values of the repeating units of the polymer calculated using the fragment constant method

Polymer surface	Log P
Polystyrene	2.50
Polypropylene	1.49
Polyethylene	1.08
Poly(HEMA)	−0.19
Poly(vinyl alcohol)	−0.44

Once the partition coefficients were calculated, the free energy change (in kJ/mol) corresponding to the transfer of the molecule from water to the organic solvent was calculated by the following equation [26]:

$$\begin{aligned}\Delta G &= -2.303\ R\ T \log P \\ &= -2.303 \times 0.0083 \times 298 \times \log P. \end{aligned} \quad (3)$$

The solvation energies of protein adsorption were calculated using Eqns (2) and (3). This apporach considered free energy changes due to the environmental adjustments of the amino acid residues (or the fragments of the amino acid residues) and the polymer repeating units on the site of contacts.

The amino acid residues (or fragments of the amino acid residues) which come into contact with the polymer surface were distinguished using the same threshold method described above. A given protein was rotated with 1633 orientations on the polymer surface to examine the effects of adsorption orientation on the solvation energy. The polymer surface was modelled as a flat surface composed of repeating units of a polymer chain as described previously [4]. The repeating units were arranged in a two-dimensional grid. The number of polymer repeating units which were covered with the amino acid residues of the adsorbed protein was determined. The total solvation energy of protein adsorption was calculated by combining the solvation energy for surface amino acid residues (or surface fragments) and that for the polymer surface.

At this time, we only consider the initial contact stage of protein adsorption. Therefore, proteins were treated as having their original structures during contact with the surface. The X-ray crystallographic structures of four proteins (lysozyme, trypsin, immunoglobulin F_{ab}, and hemoglobin) were used for the calculation. The initial threshold of 2.8 Å was set to find the surface amino acid residues (or surface fragments) of the protein. The threshold value was varied to examine the effect of such a variation on the solvation energy. Since the protein surfaces were composed of a number of valleys and hills, only the portion of the protein surface that contacted the polymer surfaces upon protein adsorption was examined by the threshold approach. The solvation energy for the surface amino acid residues or the fragments was then evaluated using the fragment constant method.

In addition to the solvation energy, the van der Waals potential (electrostatic interaction, dispersion force, electrostatic-induced dipole interaction, and electron shell repulsion) was also calculated as described previously [4]. It is noted that in our previous publication [4] the closest contact distance between the atomic centers of the protein and the surface was set to be 90% of the sum of the van der Waals radii [15]. That was why positive van der Waals interaction energies resulted in certain protein orientations [29]. In this study the interaction energies were calculated using the full van der Waals radii. The total protein adsorption energy was calculated by combining the solvation energy and the van der Waals interaction energy [30].

A computer program was written for the studies of (a) the distribution of hydrophobic and hydrophilic residues on the protein surface, and (b) the simulation of different adsorption orientations and the evaluation of the solvation energy upon protein adsorption. The program in FORTRAN was executed on an IBM 3090-180E mainframe computer system at the Purdue University Computing Center.

RESULTS

The distributions of hydrophobic and hydrophilic residues on lysozyme, trypsin, immunoglobulin F_{ab} and hemoglobin surfaces were examined. The results are listed in Tables 2, 3, 4 and 5, respectively. It can be seen that the hydrophilic amino acid residues, rather than the hydrophobic amino acid residues, tend to be on the protein surface. The distribution of the hydrophobic amino acid residues on the protein surfaces ranged from 9.6% (for immunoglobulin F_{ab}) to 28.0% (for hemoglobin). Tables 2–5 show that the most abundant hydrophobic amino acid residues on the protein surface are alanine and proline. Isoleucine, leucine, and valine were observed on the protein surface only occasionally. It is interesting to note that phenylalanine and tryptophan did not appear at all on the surface of the four proteins.

The free energy changes associated with the solvation interactions upon protein adsorption on polymer surfaces were calculated. The threshold for the surface amino acid residues (or the surface fragments) was varied from 2 to 6 Å for the studies of the four proteins on the polyethylene surface. A plot of the solvation interaction energy vs. the threshold value is shown in Fig. 3. In the plot, the energies corresponding to the treshold of 2.8 Å, which represents the diameter of a water molecule, are also shown. The solvation interaction energy has a minimum at the

Table 2.
Distribution of hydrophobic and hydrophilic amino acid residues on the surface of lysozyme

Amino acid residue	On surface	(Total)	% on surface
Ala	2	(14)	14.3
Ile	0	(5)	0.0
Leu	0	(8)	0.0
Met	0	(2)	0.0
Phe	0	(2)	0.0
Pro	2	(2)	100.0
Trp	0	(5)	0.0
Val	2	(9)	22.2
	6	(47)	12.8
Arg	8	(14)	57.1
Asn	5	(10)	50.0
Asp	4	(8)	50.0
Cys	0	(8)	0.0
Glu	2	(3)	66.7
Gln	4	(6)	66.7
Gly	9	(11)	81.8
His	1	(1)	100.0
Lys	3	(5)	60.0
Ser	2	(6)	33.3
Thr	0	(5)	0.0
Tyr	2	(6)	33.3
	40	(83)	48.2
Total	46	(130)	35.4

Table 3.
Distribution of hydrophobic and hydrophilic amino acid residues on the surface of trypsin

Amino acid residue	On surface	(Total)	% on surface
Ala	4	(14)	28.6
Ile	0	(15)	0.0
Leu	1	(14)	7.1
Met	0	(2)	0.0
Phe	0	(3)	0.0
Pro	2	(8)	25.0
Trp	0	(4)	0.0
Val	2	(17)	11.8
	9	(77)	11.7
Arg	1	(2)	50.0
Asn	5	(16)	31.3
Asp	0	(6)	0.0
Cys	1	(12)	8.3
Glu	2	(4)	50.0
Gln	2	(10)	20.0
Gly	7	(25)	28.0
His	0	(3)	0.0
Lys	6	(14)	42.9
Ser	19	(34)	55.9
Thr	4	(10)	40.0
Tyr	2	(10)	20.0
	49	(146)	33.6
Total	58	(223)	26.0

Table 4.
Distribution of hydrophobic and hydrophilic amino acid residues on the surface of immunoglobulin F_{ab}

Amino acid residue	On surface	(Total)	% on surface
Ala	2	(28)	7.1
Ile	1	(11)	9.1
Leu	0	(28)	0.0
Met	1	(4)	25.5
Phe	0	(12)	0.0
Pro	3	(30)	10.0
Trp	0	(9)	0.0
Val	0	(38)	0.0
	7	(160)	4.4
Arg	4	(13)	30.8
Asn	2	(13)	15.4
Asp	3	(17)	17.6
Cys	2	(11)	18.2
Glu	2	(5)	13.3
Gln	4	(19)	21.1
Gly	13	(38)	34.2
His	0	(5)	0.0
Lys	4	(22)	18.2
Ser	24	(68)	35.3
Thr	8	(37)	21.6
Tyr	0	(17)	0.0
	66	(265)	24.9
Total	73	(435)	16.8

Table 5.
Distribution of hydrophobic and hydrophilic amino acid residues on the surface of hemoglobin

Amino acid residue	On surface	(Total)	% on surface
Ala	20	(72)	27.8
Ile	0	(0)	0.0
Leu	0	(72)	0.0
Met	0	(6)	0.0
Phe	0	(30)	0.0
Pro	6	(28)	21.4
Trp	0	(6)	0.0
Val	0	(62)	0.0
	26	(276)	9.4
Arg	0	(12)	0.0
Asn	6	(20)	30.0
Asp	13	(30)	43.3
Cys	0	(6)	0.0
Glu	2	(24)	8.3
Gln	2	(8)	25.0
Gly	14	(40)	35.0
His	4	(38)	10.5
Lys	8	(44)	18.2
Ser	10	(32)	31.3
Thr	8	(32)	25.0
Tyr	0	(12)	0.0
	67	(298)	22.5
Total	93	(574)	16.2

threshold of 2.8 Å and then increases as the threshold value becomes larger. The solvation energies by the polyethylene surface were always negative and the magnitude depended on the orientation of the adsorbed protein. The adsorption orientation which resulted in a larger number of amino acid residues or fragments contacting the polymer surface released more water molecules from repeating units of the polymer surface, and therefore the energy decreased. The solvation energy by the protein, however, was dependent on the adsorption orientation as well as on the threshold value used in the study. A higher threshold value resulted in more amino acid residues (or fragments) being calculated and this resulted in a higher (more positive) energy since the number of hydrophilic residues was always greater than the number of hydrophobic residues on the protein surface, as shown in Tables 2–5. Thus, the choice of the size of a water molecule (2.8 Å) as the threshold value appeared to be most reasonable, and this value was used for subsequent studies.

The solvation interactions of the four proteins on five polymer surfaces were examined. Each protein was rotated with 1633 orientations on the polymer surfaces. Figures 4 and 5 show the distributions of the solvation interaction energies of the four proteins on polyethylene and poly(vinyl alcohol) surfaces, respectively. The negative value of the solvation interaction energy indicates that the attractive hydrophobic interaction is greater than the repulsive hydration interaction. The

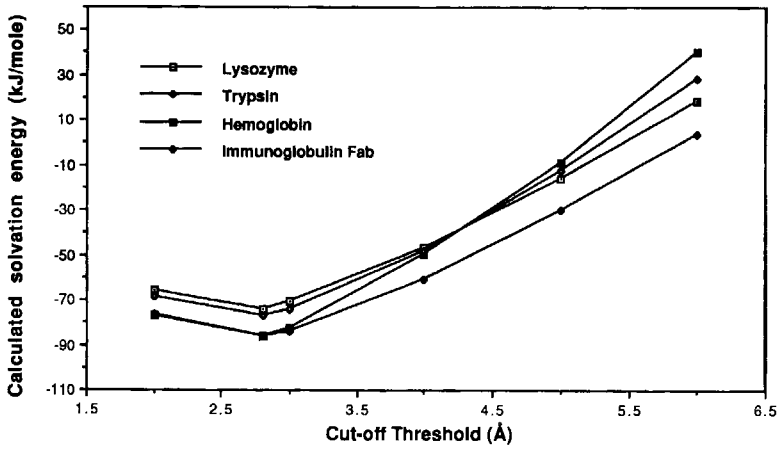

Figure 3. Calculation of the solvation interaction energy on polyethylene as a function of the threshold value.

lower (more negative) value means a stronger attraction. It can be seen from the figures that the solvation interaction was dependent on the orientation of the adsorbed protein. A different adsorption orientation may change the magnitude of the interaction totally, and may even change the sign of the interaction in the case of the polyethylene surface. The solvation interaction energy for the four proteins on polyethylene ranged from -359.0 kJ/mol (Ig F_{ab}) to 24.1 kJ/mol (lysozyme). The solvation interaction energy for the four proteins on the poly(vinyl alcohol) surface ranged from 3.9 kJ/mol (Ig F_{ab}) to 351.4 kJ/mol (Ig F_{ab}). It should be noted that the positive value of the solvation interaction energy means a repulsion between the protein and the polymer surface. Thus, it is clear that water molecules on poly(vinyl alcohol) are not displaced easily by the adsorbing proteins.

The histograms of the solvation interaction energies corresponding to protein adsorption on polystyrene and polypropylene were similar to those on polyethylene. The histograms on poly(HEMA) were similar to those on poly(vinyl alcohol). The average values of the solvation energy for the four proteins on the five polymer surfaces are listed in Table 6. The hydrophobicities of the polymer surfaces are in the

Table 6.
Average solvation interaction energies (in kJ/mol) and the standard deviations, σ, for the proteins in contact with the polymer surfaces with 1633 different orientations

	Lysozyme		Trypsin		Ig F_{ab}		Hemoglobin	
	Ave. E	σ	Ave. E	σ	Ave. E	σ	Ave. E	σ
Polystyrene	−232.1	128.3	−246.1	124.8	−230.1	123.5	−259.1	127.1
Polypropylene	−119.7	70.0	−132.4	69.0	−121.0	66.4	−136.3	69.1
Polyethylene	−74.1	47.2	−86.3	46.9	−76.7	44.0	−86.4	46.3
Poly(HEMA)	67.4	37.0	56.8	30.7	60.6	36.0	68.1	35.3
Poly(vinyl alcohol)	95.0	50.1	84.8	43.3	87.5	49.2	98.3	48.6

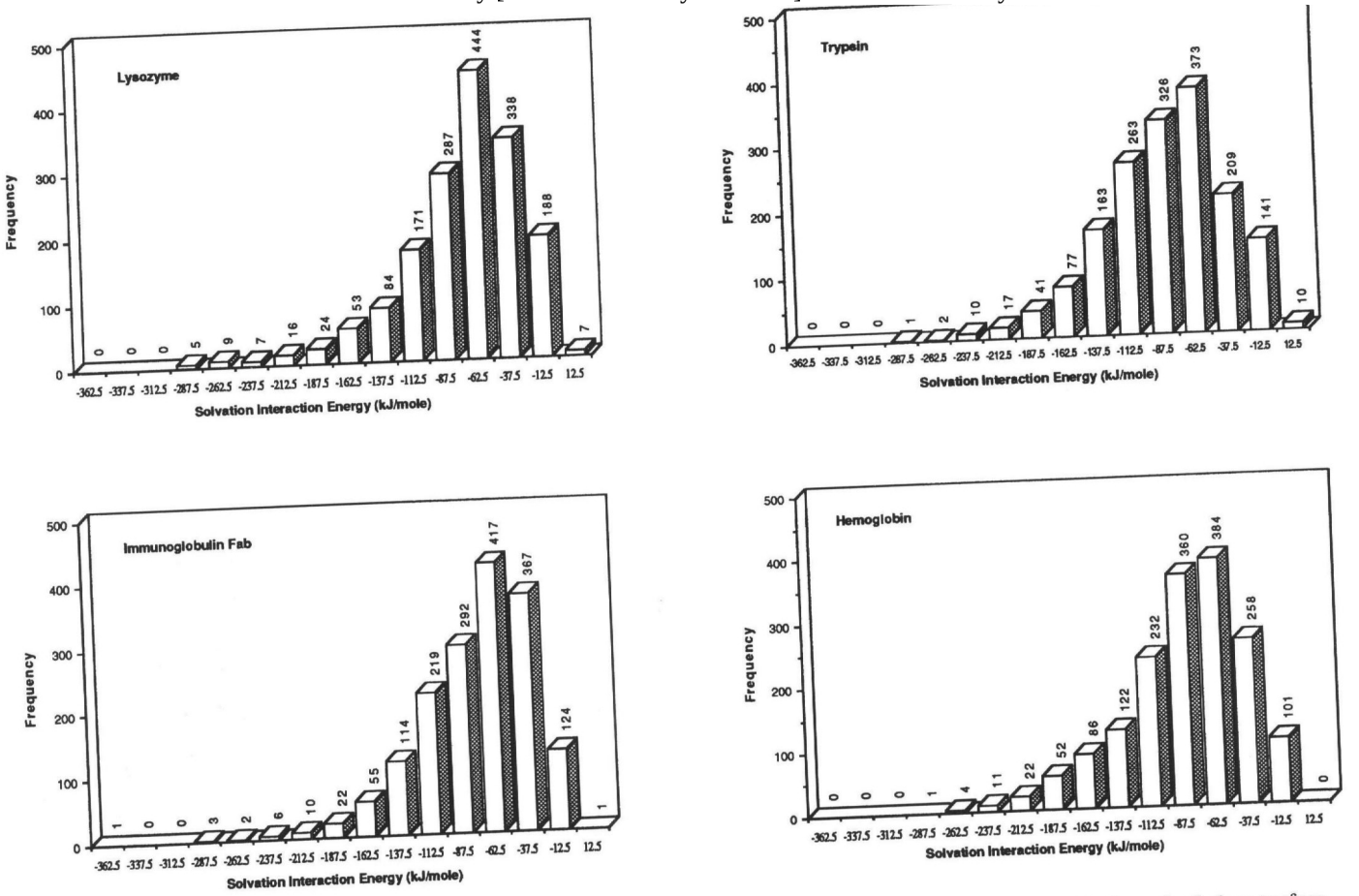

Figure 4. Distributions of the solvation interaction energies for 1633 orientations of the four proteins in contact with the polyethylene surface.

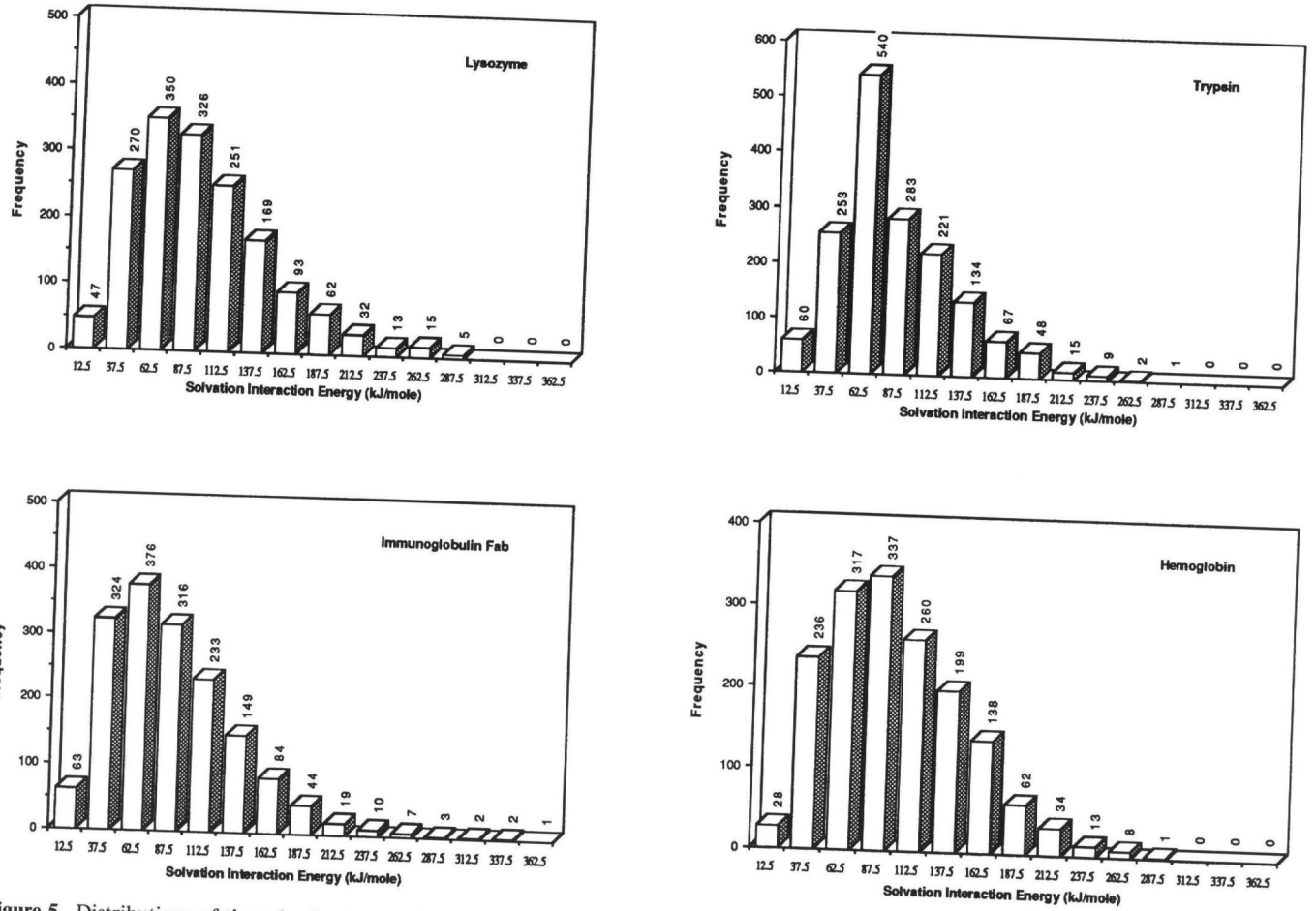

Figure 5. Distributions of the solvation interaction energies for 1633 orientations of the four proteins in contact with the poly(vinyl alcohol) surface.

Table 7.
Rotational angles (angle θ_1, angle θ_2; in radians) for the orientation with the lowest solvation interaction energy[a] (with a cut-off threshold of 2.8 Å)

	Lysozyme	Trypsin	Ig F_{ab}	Hemoglobin
Polystyrene	0.611, 2.452	1.222, 4.501	1.396, 2.334	1.658, 3.982
Polypropylene	0.611, 2.452	1.222, 4.501	1.396, 2.334	1.658, 3.982
Polyethylene	1.745, 0.987	1.222, 4.501	1.396, 2.334	1.658, 3.982
Poly(HEMA)	2.531, 0.613	1.658, 4.602	0.873, 0.800	2.007, 1.160
Poly(vinyl alcohol)	2.531, 0.613	1.658, 4.602	0.873, 0.800	2.007, 1.160

[a]The reference orientation for the rotation is the one obtained from the Brookhaven Protein Data Bank.

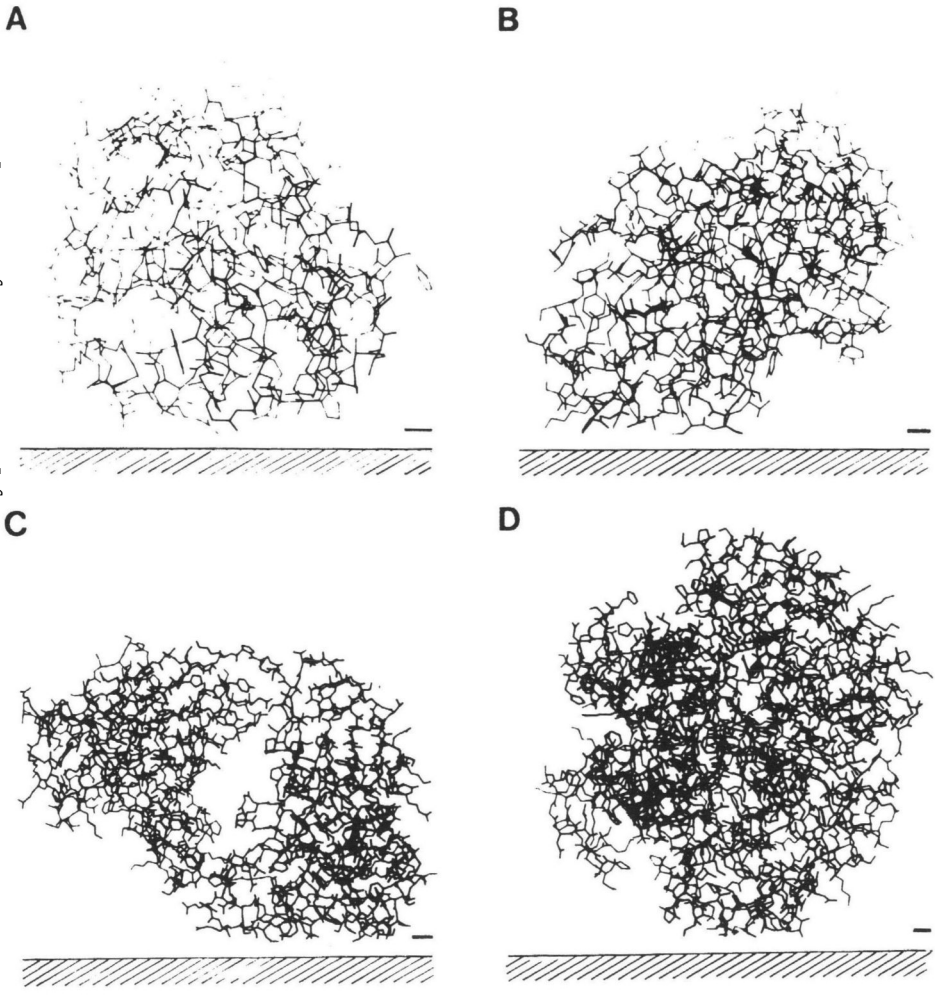

Figure 6. Structures of the four proteins on the polyethylene surface with orientations having the lowest solvation energies. Bar = 2 Å. (A) Lysozyme; (B) trypsin; (C) immunoglobulin F_{ab}; (D) hemoglobin.

order polystyrene > polypropylene > polyethylene > poly(HEMA) > poly(vinyl alcohol), according to the log P values calculated for the repeating units (Table 1). The solvation interaction energy upon protein adsorption is also in the same order, and this indicates that the hydrophobicity of the polymer surface dictates the magnitude of the solvation interaction energy for protein adsorption.

The orientations with the lowest solvation energies for the four proteins on five polymer surfaces were also examined. Table 7 lists the rotational angles which represent the orientations with the lowest solvation interaction energies. Figure 6 (see p.139) shows the three-dimensional vector images of the proteins with the lowest solvation energy orientations on the polyethylene surface. It can be seen from Table 7 that each protein has a specific orientation which always results in the lowest solvation energy on hydrophobic polymer surfaces. This orientation changes if the protein adsorbs onto hydrophilic polymer surfaces. With the exception of Ig F_{ab} on three hydrophobic surfaces, the orientations with the lowest solvation interaction energy are different from those with the lowest van der Waals interaction energy [4]. The values of the solvation interaction energy corresponding to those orientations are listed in Table 8. A clear trend found in Table 8 is that the attractive hydrophobic interaction dominates on hydrophobic polymer surfaces, such as polystyrene, polypropylene, and polyethylene. The positive values on poly(HEMA), with the exception of Ig F_{ab}, and on poly(vinyl alcohol) indicate that a repulsive hydration interaction is in effect between the proteins and those surfaces. This is reasonable, since water molecules are expected to be tightly bound to hydrophilic polymer surfaces and hydrophilic amino acid residues on the protein surfaces.

The total average interaction energies considering both the solvation energy and the van der Waals potential for the four proteins adsorbed on the five polymer surfaces are listed in Table 9. The total average interaction energy decreases, i.e. the

Table 8.
The lowest solvation interaction energies (in kJ/mol) for the four proteins in contact with the polymer surfaces

	Lysosyme	Trypsin	Ig F_{ab}	Hemoglobin
Polystyrene	−816.2	−720.6	−1007.2	−785.4
Polypropylene	−441.6	−403.7	−546.2	−433.9
Polyethylene	−293.9	−275.0	−359.0	−291.2
Poly(HEMA)	0.9	10.3	−4.6	3.7
Poly(vinyl alcohol)	10.8	18.8	3.9	12.2

Table 9.
Total average interaction energies (sum of solvation energy and the van der Waals potential; in kJ/mol) for the four proteins adsorbed on the polymer surfaces with 1633 different orientations

	Lysozyme Ave. E	Trypsin Ave. E	Ig F_{ab} Ave. E	Hemoglobin Ave. E
Polystyrene	−419.3	−431.0	−411.2	−464.8
Polypropylene	−337.9	−352.3	−343.0	−384.3
Polyethylene	−276.4	−286.0	−270.2	−301.0
Poly(HEMA)	−178.1	−184.9	−174.2	−188.9
Poly(vinyl alcohol)	−167.7	−174.7	−165.4	−179.7

Table 10.
Rotational angles (angle θ_1, angle θ_2; in radians) for the orientation with the lowest total interaction energy[a] (sum of solvation energy and the van der Waals potential)

	Lysozyme	Trypsin	Ig F_{ab}	Hemoglobin
Polystyrene	2.443, 4.781	1.047, 0.304	1.396, 2.334	1.571, 4.712
Polypropylene	2.443, 4.781	2.007, 3.867	1.396, 2.334	1.571, 4.712
Polyethylene	2.443, 4,781	1.047, 0.304	1.396, 2.334	1.571, 4.712
Poly(HEMA)	2.443, 4.781	2.007, 3.867	1.309, 2.459	1.571, 4.712
Poly(vinyl alcohol)	2.443, 4.781	2.007, 3.867	1.309, 2.459	1.571, 4.712

[a]The reference orientation for the rotation is the one obtained from the Brookhaven Protein Data Bank.

interaction becomes stronger, as the polymer surface becomes more hydrophobic. For each protein on the polymer surfaces, the adsorption orientation with the lowest total interaction energy was examined and the corresponding rotational angles are listed in Table 10. These orientations are different from those in Table 7. The orientations in Table 10 are also different, with a few exceptions, from those calculated for the lowest van der Waals potential only [4]. Thus, it appears that the protein orientation with the lowest total interaction energy is determined by the relative contributions between the van der Waals potential and the solvation interaction energy. This clearly suggests that the solvation interaction energy is very important in determining an accurate total interaction energy. Only after consideration of the solvation energy does the calculation of the protein–surface interaction energy provide data which agree with the experimental observations. Solvation interactions are a useful supplement to conventional energetics in understanding protein adsorption.

DISCUSSION

It is well known that the attraction between hydrophobic molecules in water is unusually strong and that this strong interaction cannot be accounted for by the theories of van der Waals forces [31]. It was found in our study that the attractive hydrophobic interaction is important during protein adsorption, although the hydrophilic amino acid residues tend to be on the surface of proteins, as seen in Tables 2–5. This was partially due to the presence of some hydrophobic amino acid residues on the protein surface and mainly due to the elimination of water molecules from hydrophobic polymer surfaces.

The solvation energy calculated in this study is expected to be larger than the real solvation energy for the following reasons. First, the solvation energy was calculated for all atoms which are present below the threshold value of 2.8 Å. Since some of the atoms below the threshold value may not be exposed to water molecules, the contribution to the solvation energy by such atoms needs to be subtracted. Thus, a more accurate evaluation of the solvation energy can be made by identifying which atoms are exposed to water molecules. Second, the local environment of the amino acid residues has not been taken into account. The hydrophobic nature of some amino acids may change depending on the environment. Third, the polymer repeating units are not completely exposed to water. Since it is not clear exactly how

many repeating units are exposed to water, we did not make any attempt to estimate the fraction of repeating units shielded from water molecules. Although the real solvation energies may be smaller than those calculated in this study, our calculation clearly indicates that the solvation energy plays a significant role in protein adsorption.

The average solvation interaction energies for the four proteins on polystyrene, polypropylene, and polyethylene surfaces were about −241.9, −127.4, and −80.9, kJ/mol, respectively. These negative solvation energies imply that the hydrophobic interaction is overwhelming. The large difference in magnitude of the hydrophobic interaction energies among the three surfaces is due to the very diverse values of log P calculated for the repeating units of the polymer surfaces. It has been found that the interaction between two benzene molecules and the interaction between two cyclohexane molecules are about −8.4 and −113 kJ/mol, respectively [32]. Considering the very diverse values of the hydrophobic interactions for the small molecules, we can easily accept the diverse values calculated in our study for protein adsorption.

It can be seen from Table 9 that at the initial contact stage, the total adsorption energy (sum of the solvation energy and the van der Waals potential) for proteins on hydrophobic polymer surfaces is lower than that on hydrophilic polymer surfaces. This could be one of the reasons why protein adsorption is stronger on hydrophobic polymer surfaces than on hydrophilic polymer surfaces, as found in many experiments [1, 2, 5–9, 33–36]. It should be noted that the average total interaction energies for the proteins adsorbed on the poly(vinyl alcohol) surface were slightly lower than those on the poly(HEMA) surface. This is probably due to the strong van der Waals interaction energy for proteins adsorbed on the poly(vinyl alcohol) surface [4].

It has been mentioned that both the van der Waals potential and the solvation interaction energy were dependent on the adsorption orientation of the protein molecules. The adsorption orientations with the lowest van der Waals interaction energies (referred by rotational angles) have been described previously [4]. It was found that a protein appears to have a certain orientation which always possesses the lowest van der Waals interaction energy despite differences in the type of neutral polymer surface. The rotational angles in Table 7 indicate that this is also true in terms of the lowest solvation interaction energy, although the orientations on the hydrophobic surfaces were different from those on the hydrophilic surfaces. When we consider the sum of the van der Waals potential and the solvation interaction energy for each adsorption orientation (Table 10), we find that the orientations with the lowest total interaction energies for each protein on different polymer surfaces were the same, except for a few cases. It was found that the number of surface amino acid residues is an important factor in determining the orientations with the lowest van der Waals interaction energies [4]. The number of surface amino acid residues is also critical for the solvation interaction energy, since a larger number of amino acid residues (or fragments) contacting the polymer surface results in the release of more water molecules from the polymer surface, and thus a greater change in the energy value. Therefore, it seems reasonable to assume that the adsorption orientations with the lowest total interaction energies are determined largely by the number of contact sites between the protein and the polymer surface.

The ability to calculate the solvation interaction energy – mainly hydrophobic interaction energy – may be important in the understanding of conformational changes of the adsorbed proteins. It is generally believed that proteins after adsorption undergo conformational changes to expose the hydrophobic interior in order to maximize the hydrophobic interactions with the surface, and the conformational changes of adsorbed proteins occur to a larger extent on hydrophobic surfaces than on hydrophilic surfaces [34, 37, 38]. The hydrophobic interaction energy is usually obtained by subtracting the double-layer and van der Waals energies from the measured ones [39]. Apparently, it is not possible to obtain the hydrophobic interaction energy for protein conformational changes using such a method. Thus, calculation of the hydrophobic interaction using the method described in this paper may be very useful in understanding how the adsorbed protein molecules adapt themselves to a new environment.

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APPENDIX

Table A1.
Fragment constants, bond factors, branching factors, and polar proximity factors used for the calculation of log P of each fragment in the amino acid residues and repeating units of polymer surfaces (obtained from ref. 26).

Gly	H	f_H (0.23)		
Ala	CB	f_{CH_3} (0.89)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)
Val	CB	f_{CH} (0.43)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)
	CG1	f_{CH_3} (0.89)	F_b (−0.12)	0.5 $F_{c(Br)}$ (−0.065)
	CG2	f_{CH_3} (0.89)	F_b (−0.12)	0.5 $F_{c(Br)}$ (−0.065)
Leu	CB	f_{CH_2} (0.66)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)
	CG	f_{CH} (0.43)	F_b (−0.12)	
	CD1	f_{CH_3} (0.89)	F_b (−0.12)	0.5 $F_{c(Br)}$ (−0.065)
	CD2	f_{CH_3} (0.89)	F_b (−0.12)	0.5 $F_{c(Br)}$ (−0.065)

Table A1 - *continued*

Ile	CB	f_{CH} (0.43)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)	
	CG1	f_{CH_2} (0.66)	F_b (−0.12)	0.5 $F_{c(Br)}$ (−0.065)	
	CG2	f_{CH_3} (0.89)	F_b (−0.12)	0.5 $F_{c(Br)}$ (−0.065)	
	CD1	f_{CH_3} (0.89)	F_b (−0.12)		
Pro	CB	f_{CH_2} (0.66)	$F_b{}^a$ (−0.12)	$F_{g(Br)}$ (−0.22)	
	CG	f_{CH_2} (0.66)	$F_b{}^a$ (−0.12)		
	CD	f_{CH_2} (0.66)	$F_b{}^a$ (−0.12)	$F_{g(Br)}$ (−0.22)	
Phe	CB	f_{CH_2} (0.66)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)	
	Phenyl	$f_{C_6H_5}$ (1.90)	F_b (−0.12)		
Tyr	CB	f_{CH_2} (0.66)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)	
	HO phenyl	$f_{C_6H_4OH}$ (1.23)	F_b (−0.12)		
Trp	CB	f_{CH_2} (0.66)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)	
	Indole	$f_{C_8H_6N}$ (1.91)	F_b (−0.12)		
Ser	CB	f_{CH_2} (0.66)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)	
	OG	f_{OH} (−1.64)	F_b (−0.12)		F_{p2} (1.13)
Thr	CB	f_{CH} (0.43)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)	
	OG1	f_{OH} (−1.64)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)	F_{p2} (1.13)
	CG2	f_{CH_3} (0.89)	F_b (−0.12)		
Cys	CB	f_{CH_2} (0.66)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)	
	SG	f_{SH} (−0.23)	F_b (−0.12)		F_{p2} (0.76)
Met	CB	f_{CH_2} (0.66)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)	
	CG	f_{CH_2} (0.66)	F_b (−0.12)		
	SD	f_S (−0.79)	F_b (−0.12)		F_{p3} (0.35)
	CE	f_{CH_3} (0.89)	F_b (−0.12)		

Table A1 – *continued*

Gln	CB	f_{CH_2} (0.66)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)	
	CG	f_{CH_2} (0.66)	F_b (−0.12)		
	Carboxamide	f_{CONH_2} (−2.11)	F_b (−0.12)		F_{p3} (0.48)
Asn	CB	f_{CH_2} (0.66)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)	
	Carboxamide	f_{CONH_2} (−2.11)	F_b (−0.12)		F_{p2} (1.25)
Glu	**CB**	f_{CH_2} (0.66)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)	
	CG	f_{CH_2} (0.66)	F_b (−0.12)		
	CD	f_{COO^-} (−5.10)	F_b (−0.12)		F_{p3} (0.78)
Asp	**CB**	f_{CH_2} (0.66)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)	
	CG	f_{COO^-} (−5.10)	F_b (−0.12)		F_{p2} (2.03)
Lys	**CB**	f_{CH_2} (0.66)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)	
	CG	f_{CH_2} (0.66)	F_{b+4} (−0.19)		
	CD	f_{CH_2} (0.66)	F_{b+3} (−0.26)		
	CE	f_{CH_2} (0.66)	F_{b+2} (−0.40)		
	NZ	$f_{NH_3^+}$ (−3.40)	F_{b+1} (−0.78)		F_{p+5} (1.22)
Arg	**CB**	f_{CH_2} (0.66)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)	
	CG	f_{CH_2} (0.66)	F_b (−0.12)		
	CD	F_{CH_2} (0.66)	F_{b+4} (−0.19)		
	Guanide	$f_{NHCNH_2NH_2}$ (−5.65)	F_{b+3} (−0.26)		F_{p+4} (1.84)
His	CB	f_{CH_2} (0.66)	F_b (−0.12)	$F_{g(Br)}$ (−0.22)	
	Imidazole	$f_{C_3H_3N_2}$ (−0.31)	F_b (−0.12)		F_{p3} (0.38)
Amide backbone		2 (0.5 f_{NHCO}) (−2.71)	f_{CH} (0.43)	$F_b + F_{p1}$ [b] (−0.12 + 0.87)	

[a] F_b was calculated as (4 $F_{b\ ring}$)/3.
[b] $F_{p1} = -0.32\ [0.5 \times (-2.71) + 0.5 \times (-2.71)] = 0.87$.

Table A2.
Examples of the calculation of log P values of an amino acid residue and the repeating units of polymers using the fragment method.

(A) Calculation of log P of the threonine residue in protein

$$\begin{aligned}\log P &= 2(0.5\, f_{NHCO}) + f_{CH} + F_b + F_{p1} \\ &\quad + f_{CH} + f_{CH_3} + f_{OH} + 3F_b + F_{p2} + 2\, f_{g(Br)} \\ &= (-2.71) + 0.43 + (-0.12) + 0.87 + 0.43 + 0.89 \\ &\quad + (-1.64) + 3(-0.12) + (-0.26)(-2.71 - 1.64) + 2(-0.22) \\ &= -1.52\end{aligned}$$

(B) Calculation of log P of the repeating units of polymer surfaces

Polystyrene

$$\begin{aligned}\log P &= f_{CH_2} + f_{CH} + f_{C_6H_5} + 3F_b + F_{C(Br)} \\ &= 0.66 + 0.43 + 1.90 + 3 \times (-0.12) + (-0.13) \\ &= 2.50\end{aligned}$$

Polypropylene

$$\begin{aligned}\log P &= f_{CH_2} + f_{CH} + f_{CH_3} + 3F_b + F_{C(Br)} \\ &= 0.66 + 0.43 + 0.89 + 3 \times (-0.12) + (-0.13) \\ &= 1.49\end{aligned}$$

Polyethylene

$$\begin{aligned}\log P &= 2f_{CH_2} + 2\, F_b = 2 \times 0.66 + 2 \times (-0.12) \\ &= 1.08\end{aligned}$$

Poly(HEMA)

$$\begin{aligned}\log P &= 3\, f_{CH_2} + f_C + f_{CH_3} + f_{COO} + f_{OH} + 7\, F_b + F_{c(Br)} + F_{g(Br)} \\ &\quad + F_{p2}(f_{OH} + f_{COO}) + 0.5\, F_{p3}(f_{COO} + f_{COO}) \\ &= 3 \times 0.66 + 0.20 + 0.89 + (-1.34) + (-1.64) + 7 \times (-0.12) \\ &\quad + (-0.22) + (-0.13) + (-0.26)\,(-1.64 - 1.34) \\ &\quad + 0.5\,(-0.1)\,(-1.34 - 1.34) \\ &= 0.19\end{aligned}$$

Poly(vinyl alcohol)

$$\begin{aligned}\log P &= f_{CH_2} + f_{CH} + f_{OH} + 3\, F_b + 0.5\, F_{p1}\,(f_{OH} + f_{OH}) + f_{g(Br)} \\ &= 0.66 + 0.43 + (-1.64) + 3 \times (-0.12) \\ &\quad + 0.5\,(-0.42)\,(-1.64 - 1.64) + (-0.22) \\ &= -0.44\end{aligned}$$