Journal of Biomaterials Science, Polymer Edition

ISSN: 0920-5063 (Print) 1568-5624 (Online) Journal homepage: http://www.tandfonline.com/loi/tbsp20

Protein adsorption on polymer surfaces: calculation of adsorption energies

Donghao R. Lu & Kinam Park

To cite this article: Donghao R. Lu & Kinam Park (1989) Protein adsorption on polymer surfaces: calculation of adsorption energies, Journal of Biomaterials Science, Polymer Edition, 1:4, 243-260, DOI: 10.1163/156856289X00136

To link to this article: https://doi.org/10.1163/156856289X00136

Published online: 02 Apr 2012.

J. Biomater. Sci. Polymer Edn, Vol. 1, No. 4, pp. 243-260 (1990)

Protein adsorption on polymer surfaces: calculation of adsorption energies

DONGHAO R. LU and KINAM PARK*
Purdue University, School of Pharmacy, West Lafayette, IN 47907, USA

Received 20 October 1989; accepted 5 January 1990

Abstract—In an attempt to understand the mechanisms of protein adsorption at the solid-liquid interface, we have calculated the interaction potential energy between the protein and the polymer surface by a computer simulation approach. The adsorption of four proteins—lysozyme, trypsin, immunoglobulin F_{ab}, and hemoglobin—on five polymer surfaces was examined. The model polymers used for the calculation were polystyrene, polyethylene, polypropylene, poly(hydroxyethyl methacrylate), and poly(vinyl alcohol). All possible orientations of the protein on the polymer surfaces were simulated and the corresponding interaction energies for the initial contact stage of protein adsorption were calculated. In the calculation of interaction energies, the hydrophobic interaction was not treated explicitly owing to the difficulty in the theoretical treatment. The results showed that the interaction energy was dependent on the orientation of the protein on the polymer surfaces. The energy varied from -850 to $+600$ kJ/mol with an average of about -155 kJ/mol. The interaction energy was also dependent on the type of polymer. The average interaction energies of the four proteins with poly(vinyl alcohol) were always lower than those with the other polymers. The interaction energy was not dependent on the protein size. It was found that the dispersion attraction played the major role in protein adsorption on neutral polymer surfaces.

Key words: Protein adsorption; polymer surfaces; orientation of adsorbed protein; interaction energy; computer simulation.

INTRODUCTION

Proteins at solid-liquid interfaces play important roles in a variety of biological processes. For example, the nature of blood proteins adsorbed onto biomaterials is known to determine the biocompatibility of the implants [1-3]. Other processes involving protein adsorption are solid phase immunoassays [4], contact lens fouling [5,6], loss of protein drugs on container surfaces [7], protein interaction with chromatography materials during separation and purification [8], and impairment of equipment in the food-processing industry [4].

Many aspects on protein adsorption at solid-liquid interfaces have been studied and several good reviews are available [4,7,9-14]. The mechanisms of protein adsorption and the structures of the adsorbed protein molecules, however, have not been clearly understood. Although much has been studied on the amount of protein adsorbed under various conditions, little is known about the conformation or the functional properties of the adsorbed proteins. It is necessary to have new approaches which can examine the structure or orientation of the adsorbed proteins at the interface.

Several kinetic models for protein adsorption have been presented. Lundstrom *et al.* assumed that adsorbed protein molecules had two forms, an original one and one

* To whom correspondence should be addressed.

with changed conformation after adsorption [12]. The two forms competed for the same area at the interface. From the rate equations the steady-state amounts of proteins in the two different forms could be calculated. The main interest of their work was in the shape of the adsorption isotherms. Aptel *et al.* discussed the adsorption rate and relaxation times in their protein adsorption model, which included (1) transport of protein molecules to the interface, (2) adsorption/desorption at the interface, and (3) structural changes of the adsorbed protein at the interface [15].

Although these models described some kinetic features of protein adsorption, they did not describe other important aspects of protein adsorption, such as the magnitude of the protein–surface interaction energies and the conformation of the proteins at the interface.

Of the various aspects of protein adsorption at the solid–liquid interface, the nature of the interactions between proteins and the adsorbent surface is thought to be most important. Although adsorption theories for small molecules have been well established [16,17], the theoretical work for protein adsorption at solid–liquid interfaces is still in its infancy.

THEORETICAL MODEL FOR STUDYING PROTEIN ADSORPTION

We have examined the nature of the interaction potential energy, which is believed to be the major factor in adsorbing proteins onto the solid surface. Protein adsorption at the solid–liquid interface involves at least two stages: an initial contact of a protein with the solid surface and subsequent conformational changes. As a first step, our aim was to calculate the interaction potential energy for protein adsorption at the initial contact stage. All possible orientations of the protein against the surface were simulated at the molecular level and the corresponding interaction energies were calculated. Calculation was only for the initial contact stage and conformational changes after protein adsorption were not considered, simply because information on such changes is not yet available.

Although the interaction potential energies can be calculated by a quantum-chemical approach, it is impractical for macromolecules [18]. As an alternative, an approach based on classical mechanics can be employed. It is assumed that the total interaction potential energy for protein adsorption is equal to the sum of the individual pair potentials between atoms in a protein and atoms of the solid surface. The interaction potential energy between any two atoms can be expressed as the sum of the following contributions: the electrostatic interaction, the electrostatic-induced dipole attraction, the dispersive attraction, and the overlap repulsion. Therefore, the interaction potential energy between a protein molecule and a model polymer surface can be evaluated by calculating the sum of those contributions using the following equations:

$$\Delta E = \sum_i \sum_j \Delta E_{ij}$$

$$= \sum_i \sum_j \left\{ 1389.4168 q_i q_j \times R_{ij}^{-1} - 694.7084(f_i \alpha_i q_i^2 + f_j \alpha_j q_j^2) \times R_{ij}^{-4} \right.$$

$$\left. - \left[\frac{1516.0732 f_i \alpha_i f_j \alpha_j}{(f_i \alpha_i / n_i)^{1/2} + (f_j \alpha_j / n_j)^{1/2}} + D_{ij} \right] \times R_{ij}^{-6} + 4.184 c_i c_j \times R_{ij}^{-12} \right\} \quad (1)$$

where i represents the ith atom in a protein molecule and j represents the jth atom of the solid surface. ΔE_{ij} is the interaction potential energy of the ith atom in the protein molecule reacting with the jth atom of the solid surface at a distance R_{ij}. q_i, α_i, and n_i denote the effective charge, the dipole polarizability, and the effective number of electrons of the ith atom, respectively. c_i is a coefficient determined by the fitting of accurate self-consistent-field molecular orbital approximation results [19,20]. The coefficient c_i has considered the environmental effects of the atoms, as reflected in the definition of classes of atoms (see Table 1). The optimization coefficient f_i is introduced to improve the fitting for more accurate calculation [19]. D_{ij} is a correction coefficient for the R_{ij}^{-6} term, since the calculation is for a macromolecular system [21,22]. The value of the coefficient D_{ij} is related to the nuclear charges of two interacting atoms and the values are listed in Table 2. The interaction energy is given in kJ/mol, α in Å^3, c in kcal$^{1/2}$ Å^6/mol$^{1/2}$, D_{ij} in kJ Å^6/mol, and R_{ij} in Å.

The effective charge, q, represents the gain or loss in the electronic population of an atom in a molecule relative to its electronic population in an isolated atom. When two atoms form a covalent bond, the more electronegative atom is said to have a

Table 1.
Classes and expansion coefficients of atoms in amino acids[a]

Atom	Class	Functional group	q	α	f	c
H	1	Amino	0.266	0.33	0.35	1.28
	2	Methyne, methylene	0.204	0.36	0.10	162
	3	Methyl	0.205	0.36	0.10	22.3
	4	Carboxyl	0.404	0.29	0.95	5.53
	16	Ring C	0.253	0.34	0.10	131
	23	Sulfhydryl	0.060	0.42	0.85	69.3
C	5	Carboxyl	0.511	1.10	0.10	667
	6	Methyl	−0.608	3.60	0.10	536
	7	Methylene	−0.383	2.75	0.10	508
	8	Methyne	−0.135	2.00	0.10	362
	14	Ring, fully substituted or next to N	−0.078	1.90	0.10	234
	17	Ring	−0.218	2.20	0.10	948
	18	Ring, alcoholic, phenolic	0.008	1.70	0.10	186
	19	Ring, junction, geminal to C	−0.032	1.80	0.10	13.4
	20	Ring, junction, geminal to N	0.187	1.45	1.00	1646
	21	Methylene, α to S	−0.503	3.20	0.10	21.2
	24	Ring, α to amino group	0.310	1.30	2.60	982
	25	Ring, geminal to N	0.012	1.70	0.10	439
	26	Carbonyl, ring	0.423	1.20	0.10	385
	28	Ring, α to methyl group	−0.090	1.90	0.10	72
N	11	Amino	−0.554	2.10	0.15	245
	12	Ring, without H	−0.317	1.50	0.10	362
	13	Amido	−0.630	2.35	0.10	1111
	15	Ring, with H	−0.473	1.90	0.95	998
O	9	Hydroxyl in carboxyl group	−0.539	1.45	0.21	555
	10	Carbonyl in carboxyl group	−0.409	1.25	0.10	476
	27	Carbonyl, attached to ring	−0.380	1.20	0.80	268
S	22	Sulfhydryl	0.123	3.15	0.35	1035

[a] The values were taken from refs [19] and [20].

Table 2.
The correction coefficient D_{ij} for the R^{-6} term[a]

Interacting atom pair	D_{ij} (kJ Å^6/mol)
Hydrogen-hydrogen	103.76
Hydrogen-carbon	483.60
Hydrogen-nitrogen	395.87
Hydrogen-oxygen	302.51
Hydrogen-sulfur	1120.75
Carbon-carbon	2253.93
Carbon-nitrogen	1845.03
Carbon-oxygen	1409.93
Carbon-sulfur	5223.51
Nitrogen-nitrogen	1510.31
Nitrogen-oxygen	1154.15
Nitrogen-sulfur	4275.87
Oxygen-oxygen	881.97
Oxygen-sulfur	3267.53
Sulfur-sulfur	12 105.52

[a]The values were taken from ref. [20].

δ^- charge and the less electronegative atom a δ^+ charge. This gives the values for the effective charges of all atoms in our calculation. Polarizability, α, is defined conceptually as the propensity for a given distribution of electrons to be spatially distorted. The property is inversely related to the electronegativity of a given atom. The effective number of electrons, n, is defined as the nuclear charge minus the effective charge.

Equation (1) has been used to calculate the interaction energies between two molecules [19] and the interaction energy inside proteins or peptides [21]. The theoretical background of the equation has been described [23,24]. The equation covers the long-range electrostatic interaction (first term), the medium-range electrostatic-induced dipole attraction (second term), the short-range dispersive attraction (third term), and a correction for the overlap repulsion (fourth term).

The atoms in the protein and the atoms of the solid surfaces were assigned to a set of classes based on their interaction abilities and reacting domains to other atoms. The classification follows the work of Clementi *et al.* [20]. From the classification, the values for the effective charges were obtained. The classes of atoms and their expansion coefficients are listed in Table 1. It should be noted that the coefficients f and c have already considered hydrogen bonding and other interactions (e.g. π-bonding) as possible domains of their applicabilities [25].

It should be noted that the hydrophobic interaction term is not included in Eq. (1). The hydrophobic amino acids are typically buried in the interior of the native conformation of water-soluble proteins [26], but sometimes they are grouped together on the protein surface to form hydrophobic patches [4,27]. The hydrophobic interaction is often considered to be one of the major driving forces for protein adsorption onto hydrophobic surfaces [28,29]. At the present stage of the theoretical treatment, however, it is extremely difficult to model such an interaction. There are no simple theories of the hydrophobic interaction, although several promising approaches have been attempted [30]. Since the hydrophobic interaction term is absent in Eq. (1), the results on the calculation of interaction energies in this study should be taken with caution.

CALCULATION AND SIMULATION PROCEDURES FOR PROTEIN ADSORPTION

Since we only considered the initial contact stage, proteins were treated as having their original structures during contact with the surface. The X-ray crystallographic structures of the proteins were used for the calculation. It has been found by a two-dimensional NMR study that the backbone and the interior side chains of polypeptide structures in solution are nearly the same as those in the crystals [31]. The coordinates of the protein structure are readily available in a computer-readable format from the Brookhaven Protein Data Bank [32]. Since the coordinates of the hydrogen atoms in the protein were not available in the data from the Brookhaven Protein Data Bank, a computer program was written to calculate them based on the crystallographic structure of the protein and a set of coordinate data for the standard amino acids. Protein adsorption was simulated under the physiological condition of pH 7. Thus, the side chains of arginine, aspartic acid, glutamic acid, and lysine had unit electronic charges. These ionizable groups are mostly found on the exterior of a protein and are exposed to water molecules. When these groups exist in the interior of the protein, oppositely-charged ionizable groups are usually found to form an ion pair or salt bridge [33]. Therefore, regardless of the positions of these amino acid residues in the protein, the ionized state was used for the calculation of the coordinates of the hydrogen atoms.

The effective charges, q, in Table 1 are the ones for the atoms of individual amino acids. During the calculation some modifications were carried out to balance the total charges of the protein chain, because the effective charges for individual atoms in each amino acid residue were changed owing to the formation of peptide bonds. After all the effective charges had been assigned to the corresponding atoms, they were normalized to make the sum of the effective charges of all the neutral amino acids in the protein chain equal to zero. For the ionized amino acid residues of the protein chain, the excess charges due to the ionization were removed before the normalization and added back afterwards. Thus, the total charge for the whole chain became equal to the sum of the unit charges of the ionized residues.

The total interaction energy was calculated by summing all the pair potentials in the system. The proteins used in our study were human lysozyme (EC 3.2.1.17), bovine β-trypsin (EC 3.4.21.4), immunoglobulin F_{ab} from human kol serum and human (deoxy) hemoglobin. The coordinate data were obtained from the Brookhaven Protein Data Bank and the coordinates of the hydrogen atoms were added. Table 3 shows the molecular weights, numbers of amino acid residues, net charges of the proteins at pH 7, and references for the proteins.

The polymer surface was treated as a semi-infinite continuum with a statistically arranged polymer lattice. The surface density of this continuum was taken to be equal

Table 3.
Properties of the proteins used in the study

Protein	No. of amino acids	Mw	Net charge at pH 7	Ref.
Lysozyme	130	14 000	+8	34
Trypsin	223	23 200	+6	35
Ig F_{ab}	445	50 000	+3	36
Hemoglobin	574	64 500	+2	37

to the number of atoms per repeating unit area of the polymer chain. The unit area was taken to be 2.5×2.5 Å^2. The atoms of the repeating unit were statistically arranged to hold in the geometrical center of the unit. Only the first lattice layer was considered, since it was found during the preliminary study that the other layers made a very small contribution to the interaction energy. As a consequence of using the continuum model, the interaction potential energy of a protein molecule with the solid surface was independent of the horizontal location on the surface. When a protein molecule with a certain orientation is adsorbed on a homogeneous solid surface, the total potential energy is not expected to be dependent on the relative lateral position over the surface. A similar treatment was used for the study of the adsorption of small molecules [38]. We chose five model polymer surfaces for the calculation: polystyrene, polyethylene, polypropylene, poly(hydroxyethyl methacrylate) (poly(HEMA)), and poly(vinyl alcohol) surfaces. These polymers were chosen because they consisted of atoms for which the expansion coefficients were available. Since the number of atoms per unit area is different on different polymer surfaces, the pair potential energy was normalized based on the number of atoms per unit area of the polyethylene surface.

To calculate the interaction energies of a protein molecule with different orientations against the solid surface, we used a scan approach which allowed us to simulate all the possible orientations. A protein molecule was rotated and the protein surface was then brought to just contact the solid surface. Figure 1 shows the approach used. The protein molecule was rotated by an angle θ_1 on the Y-axis (A in Fig. 1) and the interaction energy was calculated. After the calculation, the protein molecule was brought back to its reference orientation (B in Fig. 1). The reference orientation of the protein was obtained from the Brookhaven Protein Data Bank. The protein molecule was then rotated by an angle θ_2 on the Z-axis (C and D in Fig. 1) and the calculation process was repeated. Both θ_1 and θ_2 were calculated from the geometrical center of the protein. The value of θ_2 was determined in such a way that the distance between a and a′ on the protein surface (D in Fig. 1) remained the same throughout the calculation. Thus, the value of angle θ_2 depended on the value of angle θ_1. In our study, the value of θ_1 was increased at 5° intervals, resulting in 1633 different orientations. For each orientation, the interaction energy between the protein and the polymer surface was calculated.

As described in Eq. (1), the interaction energy is actually the sum of the four interaction contributions. To find the contribution which plays a major role in protein

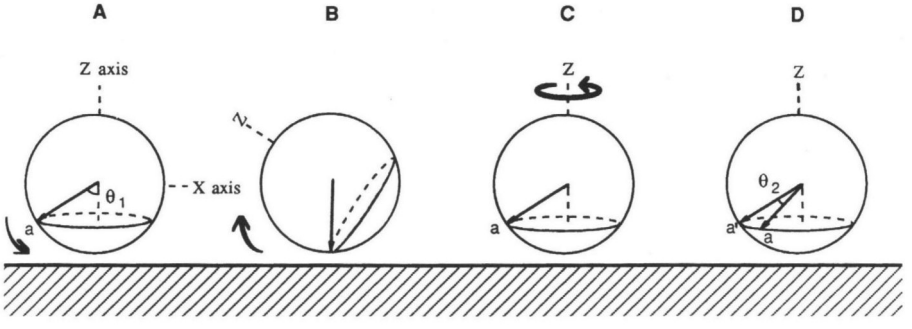

Figure 1. Procedure to obtain different orientations of a protein on the polymer surface. Steps A–D are repeated after the protein is rotated by angle θ_2, whose value is determined by angle θ_1.

adsorption, each of the contributions (electrostatic interaction, electrostatic-induced dipole attraction, dispersive attraction, and overlap repulsion) was calculated separately using the orientation for the lowest interaction energy on polyethylene.

Owing to the enormous calculation and the need for a large central memory, the operation is practical only with a high-speed computer. We used an IBM 3090-180E computer system for the calculation. The machine is one of IBM's current flagship series of mainframe computers and has 64 MBytes of main storage, 30 GBytes of disk storage, and 16 data channels. It also contains an IBM 3090 Vector Facility as an integral component of the IBM 3090 central processor. The standard FORTRAN 77 computer language was used for the programming.

RESULTS

The interaction energies for the adsorption of lysozyme, trypsin, immunoglobulin F_{ab}, and hemoglobin on the five polymer surfaces where calculated. Each protein was rotated with 1633 orientations on the five polymer surfaces and the histograms of the energy distribution were generated. Figure 2 shows the histograms of the interaction energies for the adsorption of four proteins on polyethylene. A negative value of the interaction energy indicates that there is an attraction between the protein and the polymer surface. Thus, the lower (more negative) the value, the stronger the attraction. The interaction energies of the four proteins with polyethylene ranged from − 690.3 kJ/mol (immunoglobulin F_{ab}) to 281.7 kJ/mol (lysozyme) depending on the type and orientation of the protein. Most of the interaction values in Fig. 2 are negative. This means that the proteins are attracted to the surface for most of the

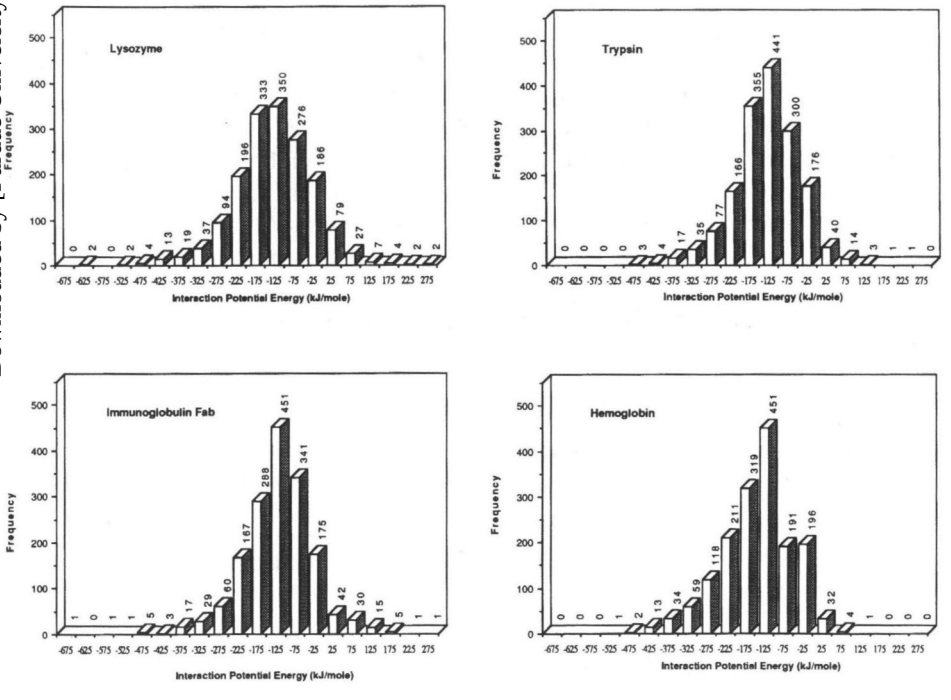

Figure 2. Interaction energy distributions for 1633 orientations of four proteins in contact with the polyethylene surface.

orientations. The fraction of the positive interaction energies ranges from 2.3% for hemoglobin to 7.4% for lysozyme. The presence of both negative and positive interaction energies implies that the same protein can be either adsorbed onto or repelled from the surface simply based on the orientation against the surface. This clearly indicates that not all, but most, protein molecules reaching the surface are adsorbed after the first collision.

The histograms for the four proteins on the polystyrene, polypropylene, poly(HEMA), and poly(vinyl alcohol) surfaces have similar features to those on polyethylene, although the values of the interaction energies are different. The average interaction energies and the standard deviations, σ, are listed in Table 4 to show implicitly the corresponding histograms. The average interaction energies range from -103.5 to -209.3 kJ/mol. For the four proteins examined, the average interaction energies with poly(vinyl alcohol) were always smaller than those with the other hydrophobic polymers. This indicates that the protein-surface interaction at the initial contact stage becomes more attractive as the surface becomes more hydrophilic.

Table 5 lists the lowest interaction energies for each protein on a particular polymer. The values in the table cluster around -650 kJ/mol. The lowest interaction energies of the four proteins with poly(vinyl alcohol) are smaller than any other values. Table 6 lists the highest interaction energies that can be obtained from each protein-polymer pair. Unlike the lowest interaction energies in Table 5, the highest interaction energies vary widely from 35.2 to 619.2 kJ/mol. The highest interaction energies of the proteins with poly(vinyl alcohol) are smaller than those with other polymers. The data in Tables 4, 5, and 6 indicate that the interaction energy appears to depend on the nature of the polymer surface and that the proteins tend to have stronger interactions with hydrophilic polymer surfaces than with hydrophobic polymer surfaces at the initial contact stage. This result, however, should be taken with caution, since we have not considered the hydrophobic effect in the calculation of the interaction energy. The result could be reversed if the hydrophobic interaction is accounted for. As shown in Table 3, the molecular weights of the proteins range from 14 000 to 64 500. In tables 4, 5, and 6, no apparent trend in the interaction energies is seen as the protein size varies. This suggests that the protein-surface affinity at the initial contact stage is independent of the protein size.

Since the interaction energy with the surface depends on the orientation of the protein, we have identified two particular orientations of each protein which result

Table 4
Average interaction energies and standard deviations, σ (in kJ/mol) 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	−108.5	140.3	−112.6	115.7	−103.5	125.8	−142.0	118.0
Polyethylene	−134.5	99.6	−135.5	83.2	−126.8	91.1	−151.9	90.5
Polypropylene	−160.2	98.3	−164.1	79.6	−165.2	89.0	−195.4	90.5
Poly(HEMA)	−160.4	107.6	−159.0	88.4	−150.6	97.0	−174.5	98.9
Poly(vinyl alcohol)	−192.4	110.4	−191.6	92.7	−183.9	101.0	−209.3	104.7

Table 5.
The lowest interaction energies (in kJ/mol) for the proteins in contact with the polymer surfaces

	Lysozyme	Trypsin	Ig F_{ab}	Hemoglobin
Polystyrene	−685.9	−523.6	−753.5	−589.8
Polyethylene	−632.5	−466.9	−690.3	−538.9
Polypropylene	−679.2	−487.9	−716.1	−557.2
Poly(HEMA)	−741.9	−538.5	−780.5	−598.1
Poly(vinyl alcohol)	−797.6	−600.1	−855.7	−654.0

Table 6.
The highest interaction energies (in kJ/mol) for the proteins in contact with the polymer surfaces

	Lysozyme	Trypsin	Ig F_{ab}	Hemoglobin
Polystyrene	619.2	511.2	487.2	350.3
Polyethylene	281.7	223.7	257.2	147.0
Polypropylene	162.8	98.5	162.8	35.2
Poly(HEMA)	223.8	175.2	220.4	112.9
Poly(vinyl alcohol)	153.1	97.2	151.1	34.1

in the lowest and the highest interaction energies. The three-dimensional rotation angles for the two orientations are listed in Tables 7 and 8. Figure 1 can be referred to to understand how the proteins were rotated with the angles. It was interesting to observe that a particular orientation resulted in the lowest (or the highest) interaction energy on all the polymer surfaces tested for immunoglobulin F_{ab} and hemoglobin. For lysozyme and trypsin, however, the orientation with the lowest (or the highest) interaction energy was dependent on the nature of the polymer surface. For example,

Table 7.
Rotation angles (angle θ_1, angle θ_2) in radians for the orientation with the lowest interaction energy[a]

	Lysozyme	Trypsin	Ig F_{ab}	Hemoglobin
Polystyrene	0.611, 2.452	1.571, 2.705	1.396, 2.334	0.349, 4.974
Polyethylene	1.396, 4.129	1.571, 2.705	1.396, 2.334	0.349, 4.974
Polypropylene	0.611, 2.452	1.047, 0.304	1.396, 2.334	0.349, 4.974
Poly(HEMA)	1.396, 4.129	1.047, 0.304	1.396, 2.334	0.349, 4.974
Poly(vinyl alcohol)	1.396, 4.129	1.047, 0.304	1.396, 2.334	0.349, 4.974

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

Table 8.
Rotation angles (angle θ_1, angle θ_2) in radians for the orientation with the highest interaction energy[a]

	Lysozyme	Trypsin	Ig F_{ab}	Hemoglobin
Polystyrene	2.531, 4.597	0.960, 2.167	1.047, 1.723	1.047, 0.811
Polyethylene	1.920, 3.001	0.960, 2.167	1.047, 1.723	1.047, 0.811
Polypropylene	1.920, 3.001	0.960, 2.167	1.047, 1.723	1.047, 0.811
Poly(HEMA)	1.920, 3.001	0.960, 2.167	1.047, 1.723	1.047, 0.811
Poly(vinyl alcohol)	1.920, 3.001	0.611, 4.904	1.047, 1.723	1.047, 0.811

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

the orientation which resulted in the lowest interaction energy of lysozyme on both polystyrene and polypropylene was different from that on the other three polymers. It should be noted, however, that the two orientations resulted in about the same interaction energy values. For lysozyme on polypropylene, the energy with the rotation angles of 0.611 (angle θ_1) and 2.452 radians (angle θ_2) was -679.2 kJ/mol, while the energy with the rotation angles of 1.396 and 4.129 radians was -674.4 kJ/mol. These two values are practically the same. The same phenomenon was found for trypsin. For trypsin on polypropylene the energy with the rotation angles of 1.047 and 0.304 radians was -487.9 kJ/mol, while the energy with the rotation angles of 1.571 and 2.705 radians was -477.8 kJ/mol. Thus, it may be said that all the proteins possess a certain orientation which is energetically most favorable for adsorption onto the surface. Not all the protein molecules, however, are expected to adsorb with the orientation, since it is only one of the 1633 orientations. It is possible, however, that once a protein molecule adsorbs to the surface in any orientation, it may undergo rotation on the surface to achieve the most favorable orientation before conformational changes.

Figure 3 shows the interaction energy of the four proteins on polyethylene as a function of the rotation angle θ_2 in the angular domain near the lowest energy orientation. The rotation angle θ_1 for the lowest energy orientation can be found in Table 7 and the average interaction energies of the four proteins are listed in Table 4. For hemoglobin, orientations in the angular domain between 3.93 and 5.50 radians (which is equal to 90°) have an interaction energy lower than the average value. For the other three proteins, orientations in the angular domain of 40° are energetically more favorable for adsorption than other orientations with the average interaction energy. Thus, it appears that the interaction energy is not changed drastically by small perturbations of the protein molecule from its lowest energy orientation.

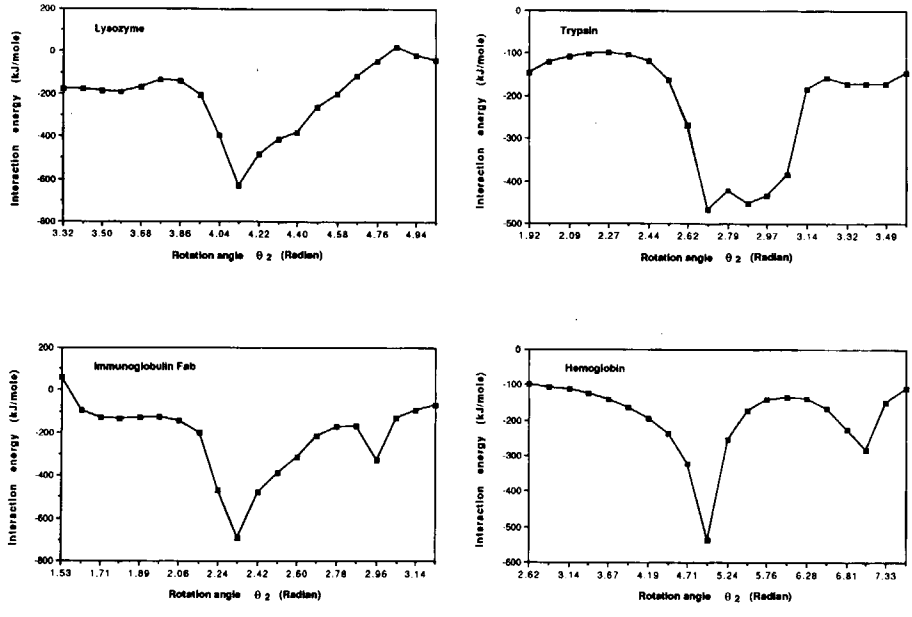

Figure 3. Interaction energy changes in the angular domain near the lowest energy orientation on polyethylene.

For the adsorption of lysozyme, trypsin, immunoglobulin F_{ab}, and hemoglobin on polyethylene, the electrostatic interaction, the electrostatic-induced dipole attraction, the dispersion attraction, and the overlap repulsion were calculated separately for the orientations with the lowest interaction energies (Table 9). The model polyethylene surface is neutral at pH 7 and the sum of the effective charges of the surface is equal to zero. Therefore, the sum of the contributions by the electrostatic interaction of all atom pairs became zero. Among the other three contributions, the dispersion interaction was shown to be the major force holding the proteins on the neutral polymer surface. It is noted that the electrostatic-induced dipole attraction can easily compensate the overlap repulsion.

The proteins were rotated according to the angles listed in Tables 7 and 8, and the protein images were plotted using a protein structure display algorithm. Figures 4 and 5 show the three-dimensional vector images which simulate the four proteins adsorbed on polyethylene. The orientations in Fig. 4 are for the lowest interaction

Table 9.
Contributions of the interaction energy for the orientation having the lowest interaction energy (in kJ/mol) of each protein in contact with the polyethylene surface

	Lysozyme	Trypsin	Ig F_{ab}	Hemoglobin
Electrostatic	0.0	0.0	0.0	0.0
Dipole	−252.1	−206.4	−303.2	−198.5
Dispersion	−607.8	−322.1	−494.1	−416.4
Overlap repulsion	227.4	**61.6**	107.0	76.0
Total	−632.5	**−466.9**	−690.3	−538.9

Table 10.
Surface amino acid residues of the proteins for the lowest energy orientation. The sequence number of each amino acid residue is shown in parentheses

Lysozyme	Trypsin	Ig F_{ab}	Hemoglobin
(1) Lys	(5) Tyr	(229) Gly	(45) His
(4) Glu	(6) Thr	(230) Arg	(53) Ala
(7) Glu	(8) Gly	(231) Ser	(54) Gln
(10) Arg	(9) Ala	(233) Arg	(57) Gly
(14) Arg	(53) Asp	(268) Asp	(60) Lys
(41) Arg	(54) Asn	(269) Gly	(61) Lys
(87) Asp	(57) Val	(270) Ser	(90) Lys
(88) Asn	(58) Val	(271) Asp	(471) Glu
(129) Gly	(59) Glu	(272) Gln	(472) Ser
	(60) Gly	(280) Gly	(474) Gly
	(61) Asn	(281) Arg	(475) Asp
	(99) Arg	(283) Thr	(487) Lys
	(125) Lys	(285) Ser	
	(134) Val	(286) Arg	
	(136) Lys	(287) Asn	
		(289) Ser	
		(290) Lys	
		(296) Gln	
		(298) Asp	
		(299) Ser	

energies, while those in Fig. 5 are for the highest interaction energies. The plots show all atoms except hydrogen. In the figures the thickness of the bars depends on the position of the chemical bonds. The thicker the bars, the closer the chemical bonds to the front. For the orientations of proteins shown in Figs 4 and 5, we examined the number and sequence of amino acid residues which are in direct contact with the polymer surface. Those amino acid residues contacting the surface will be called 'the surface amino acid residues'. The cut-off threshold distance for the determination of the surface amino acid residues was set to be 4Å from the polymer surface. Tables 10 and 11 show the surface amino acid residues and their sequence numbers for the four proteins on polyethylene with the lowest and the highest energy orientations, respectively. The number of surface amino acid residues of each protein in Table 10 is much larger than that in Table 11. This indicates that the energy-favorable orientation has a larger number of surface amino acid residues than the orientation which is not favorable for adsorption. This is reasonable, since the most important contribution in the interaction energy was found to be the dispersion interaction, as shown in Table 9. Thus, it may be said that the larger the number of surface amino acid residues, the more favorable the orientation for adsorption. However, although the number of surface amino acid residues is an important factor in determining the energetically favorable orientation, it is not the only factor. As we examined 1633 orientations for each protein on polyethylene, we found that there were many orientations which had a larger number of surface amino acid residues than that of the orientation for the lowest energy. Lysozyme, trypsin, immunoglobulin F_{ab}, and hemoglobin had 173, 7, 3, and 37 such orientations, respectively. This indicates that the type and arrangement of amino acid residues on the exterior of the proteins are also important in determining the protein–surface interaction.

DISCUSSION

Recently, computer simulation has been used to investigate various aspects of protein structures and functions. Computer simulation allows a detailed theoretical approach to investigate the internal dynamics, ligand–protein interactions, structural flexibility, and internal interactions of proteins [21, 39, 40]. Several proteins, such as bovine pancreatic trypsin inhibitor [41], ferrocytochrome, myoglobin, and lysozyme [42], were studied to determine the mean square fluctuations in the atomic positions. The results were compared with a number of experimental data and it was

Table 11.
Surface amino acid residues of the proteins for the highest energy orientation. The sequence number of each amino acid residue is shown in parentheses

Lysozyme	Trypsin	Ig F_{ab}	Hemoglobin
(50) Arg	(113) Gly	(287) Asn	(146) Pro
(66) Asn	(115) Gln	(289) Ser	(147) Glu
(67) Asp	(139) Lys	(290) Lys	(150) Ser
(68) Gly	(165) Tyr		
(69) Lys	(167) Glu		
	(170) Lys		

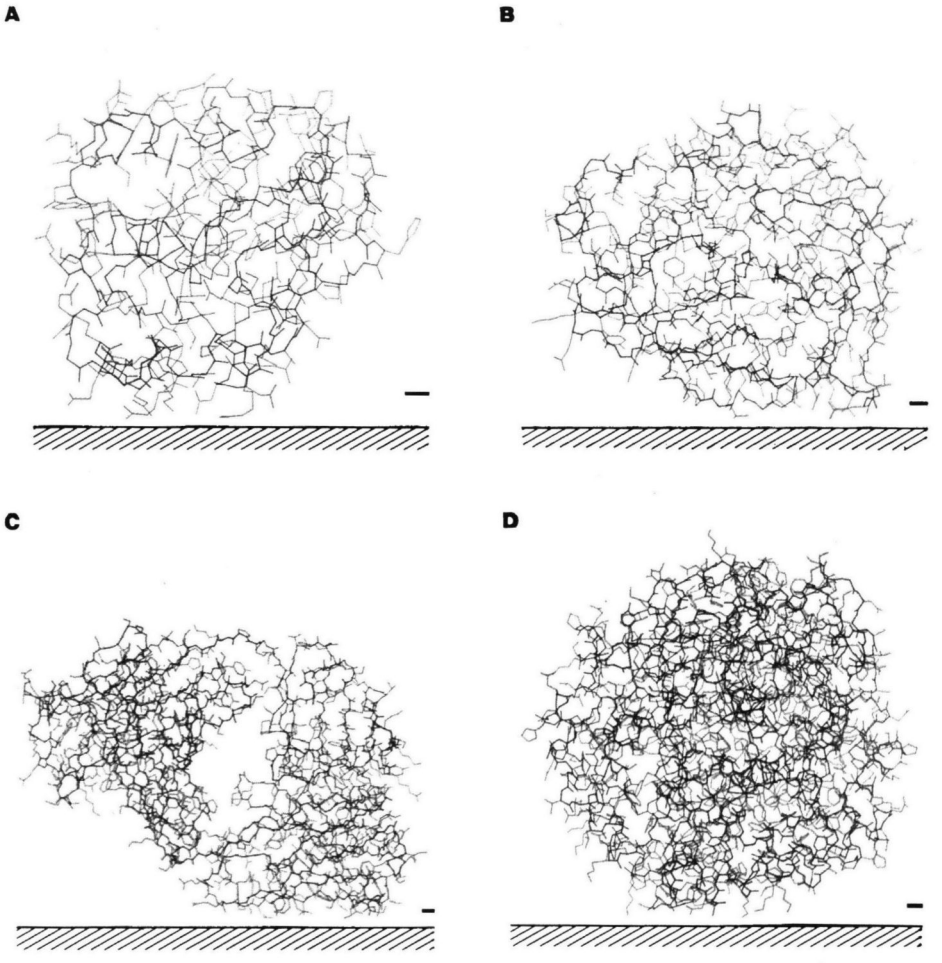

Figure 4. Structures of the four proteins on polyethylene with orientations having the lowest interaction energies. Bar = 2 Å. (A) Lysozyme; (B) trypsin; (C) immunoglobulin F_{ab}; (D) hemoglobin.

positions. The results were compared with a number of experimental data and it was found that the relative mobility of the atoms and regions within the proteins was accurately reflected in the molecular dynamics simulations [39]. The computer simulation method was also used to understand how polypeptide chains fold into the three-dimensional conformations of native proteins [43].

Although computer simulation has been successfully used in many areas dealing with proteins, the number of applications on protein adsorption has been limited. Horsley *et al.* used a graphics workstation to study the adsorption characteristics of hen and human lysozymes [27]. The distribution of hydrophobic, polar, and charged atoms on the protein surfaces was studied by calculating Corey–Pauling–Kaltun surfaces. The ionized state of each residue was simulated based on the Feldmann scheme and the scheme of the Eisenberg atomic solvation parameter. After postulating a kinetic model for the protein adsorption process, they suggested that the early states of protein adsorption were related to the physical chemistry of the solid surface and the structure and orientation of the protein [44].

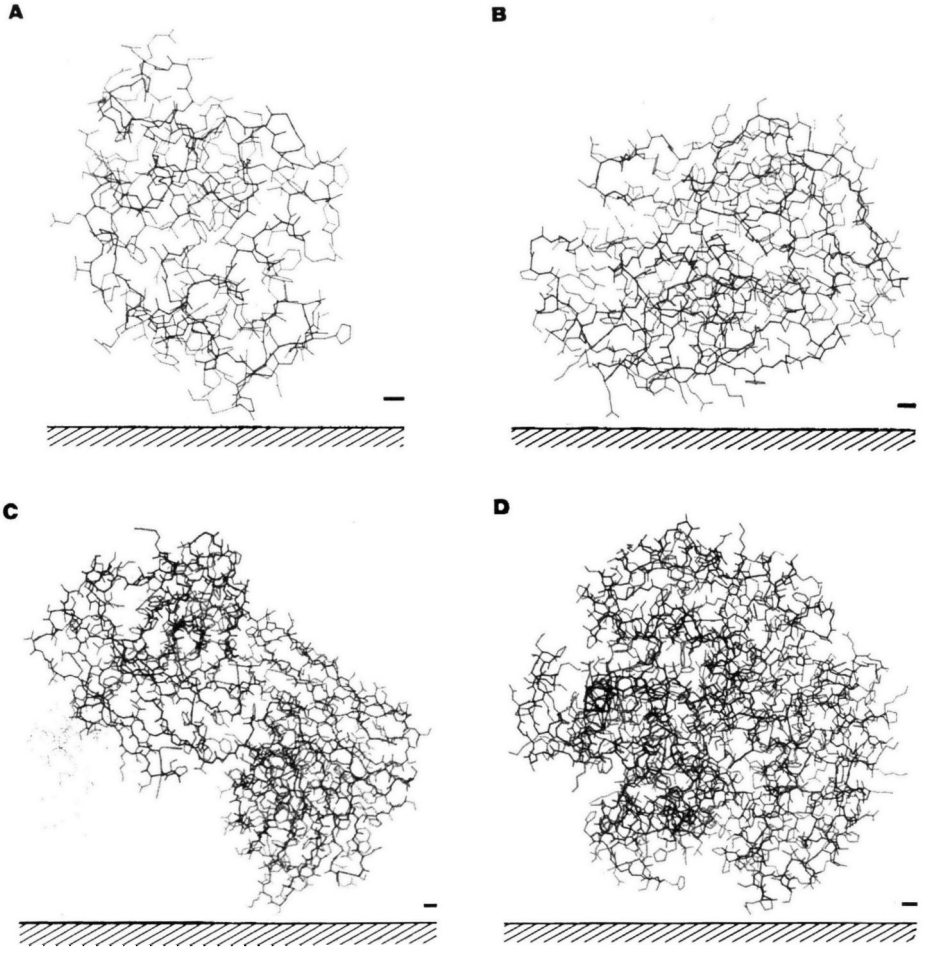

Figure 5. Structures of the four proteins on polyethylene with orientations having the highest interaction energies. Bar = 2 Å. (A) Lysozyme; (B) trypsin; (C) immunoglobulin F_{ab}; (D) hemoglobin.

Our study has shown that the computer simulation of protein adsorption on model polymer surfaces at the molecular level gives a new approach for investigating the theoretical aspects of the interaction between proteins and solid surfaces. By defining the classes of atoms for the interaction along with the f and c coefficients, the environmental effects for the atoms (e.g. the effect of water surrounding the atoms) and the possible interaction domains of the atoms have been considered [19, 20]. The interaction energy calculated using Eq. (1) depends on the values of the coefficients appearing in the equation. Thus, the calculated energy may not be considered absolute, even though the most reasonable values were chosen for the coefficients. The calculation using Eq. (1), however, presents the best theoretical approximation of the actual interaction energy.

It should be noted that the protein has been treated in this study as a relatively rigid molecule and conformational changes that may follow adsorption have not been considered. In other words, the potential energy calculated in this study is the interaction

is still useful in studying the mechanisms of protein adsorption. Protein conformational changes on polymers are known to occur during a period of hours to days, as examined by the elutability of adsorbed proteins by detergent [45]. Our approach is also useful in the study of competitive protein adsorption where more than one type of protein competes for the same surface sites. In the presence of high concentrations of various proteins in the bulk solution such as plasma, the surface will be covered with proteins almost immediately. In this situation, different proteins will compete for a finite number of adsorption sites and the adsorption will depend on the relative affinity to the surface [46]. The relative affinity may be largely determined by the orientation at the time of initial contact. One protein might have a greater fraction of favorable orientations per collision than another. The calculation of the orientation-dependent interaction energies shown in our study will provide valuable information on the nature of the competitive protein adsorption.

At present, the absolute value of the protein adsorption energy, especially for the initial contact stage, is difficult to obtain experimentally. Direct microcalorimetric measurement can provide the enthalpy changes resulting from the overall adsorption process. It has been shown by microcalorimetric study that when human γ-(7s)-globulin is adsorbed on glass, the mean net calorimetric heat of adsorption is about 7000 kJ/mol [47]. Since the enthalpy change was for the overall process of the adsorption including conformational changes, it was expected to be greater than the energy calculated for the initial contact stage. According to our calculation, as shown in Table 4, the average interaction potential energies for the initial contact stage ranged from about -100 to -200 kJ/mol for the four proteins considered on the five polymer surfaces. Tables 5 and 6 show that the lowest and the highest energies ranged from about -450 to -850 kJ/mol and from about $+35$ to $+600$ kJ/mol, respectively. The difference between the calorimetric data and the computer-calculated data may provide some information on the extent of the conformational changes of proteins after adsorption.

The average translational kinetic energy per mol of any colloidal particles undergoing Brownian motion in a gas or liquid is $(3/2)\,RT$, where R is the gas constant and T is the absolute temperature [48]. Thus, the kinetic energy at room temperature is less than 4 kJ/mol. As shown in Fig. 2, for the four proteins on polyethylene, the frequencies of orientations that result in interaction energies smaller than -4 kJ/mol are around 95% of the total orientations. This means that the majority of the protein molecules can remain on the surface after adsorption. This may explain the effective adsorption of protein molecules to the surface. Tables 4, 5, and 6 show that the same conclusions can be drawn for the polystyrene, polypropylene, poly(HEMA), and poly(vinyl alcohol) surfaces.

Chan and Brash have suggested from protein desorption studies that there are several populations of adsorbed states which presumably reflect heterogeneity with respect to both surface sites and protein sites [49]. Other investigators have also suggested that there is more than one adsorbed state for proteins on surfaces [50, 51]. Even though we assume homogeneous surface sites for protein adsorption, we can still expect a spectrum of different adsorbed states simply based on the orientation of adsorbed proteins. The orientation of a protein at the moment of initial contact may further affect the extent of the conformational changes and thus the overall adsorption energy.

From Tables 4, 5, and 6, it can be seen that the interaction energy at the initial contact stage is independent of the protein size. The size of immunoglobulin F_{ab} is about twice that of trypsin, but the average interaction energies are about the same. Horbett *et al* found that hemoglobin had a much stronger affinity for adsorption on polyethylene and other surfaces than did albumin and fibrinogen [52]. Since the size of hemoglobin is almost the same as that of albumin and much smaller than that of fibrinogen, the size of the proteins does not appear to be the determining factor for adsorption onto surfaces. The strong affinity of hemoglobin shown experimentally is probably due to the greater conformational changes after adsorption.

The calculation in this study shows that the interaction energy at the initial contact stage is related to the property of the polymer surface. Many experiments have also shown that protein adsorption is dependent on the nature of the polymer surface [4, 7, 9–14, 53, 54]. The amount of adsorbed protein is known to be related to the hydrophobicity (or hydrophilicity) of the surface [55–58]. In our study, the protein was assumed to have its original conformation, which has a closely packed hydrophobic interior and an exterior with the charged groups facing water molecules in an aqueous solution at the physiological pH. Attenuated total reflectance spectroscopy and detergent elutability studies have shown that the denaturation of proteins occurs as a function of their residence time on the surfaces [45, 59]. The infrared spectrum of albumin adsorbed to soft contact lenses changes gradually. These changes were interpreted to be due to the exposure of hydrophobic side chains, which caused loss of the α-helix and the formation of β-regions and a random conformation. When the conformational changes occur, the hydrophobic interior becomes exposed and interacts with the solid surface. Thus, the hydrophobic effect may be another major force for the overall adsorption. The prediction of protein structures after the conformational changes is a topic for future study.

From Table 7 it can be seen that the orientations with the lowest interaction energies are nearly the same for each protein adsorbed on different surfaces. Thus, it appears that a protein has a certain orientation which always possesses the lowest energy despite the differences in the type of neutral polymer surface. Table 8 shows that the same is true for the orientations with the highest interaction energies. It has been suggested that protein adsorption in certain orientations is more likely than in other orientations [4]. The results of our simulation studies confirm this suggestion and provide it with a theoretical basis. In our study, four proteins and five neutral polymer surfaces were examined. Since they may not represent all proteins and all polymer surfaces, a larger number of proteins and more diverse polymer surfaces need to be studied.

Acknowledgements

This study was supported by the National Heart, Lung and Blood Institute of the National Institutes of Health through Grant HL 39081 and in part by BRSG 2-507-RR05586-22.

REFERENCES

1. J. N. Mulvihill and J. P. Cazenave, *Colloids Surf.* **14**, 317 (1985).
2. L. Vroman and A. L. Adams, *J. Colloid Interface Sci.* **111**, 391 (1986).
3. K. Park, D. F. Mosher and S. L. Cooper, *J. Biomed. Mater. Res.* **20**, 589 (1986).
4. T. A. Horbett and J. L. Brash, in: *Proteins at Interfaces, Physicochemical and Biochemical Studies*, p. 1, J. L. Brash and T. A. Horbett (Eds). Am. Chem. Soc., Washington, DC (1987).

5. M. R. Allansmith, D. R. Korb, J. V. Greiner, A. S. Henriquez, M. A. Simon and V. M. Finnemore, *Am. J. Ophthalmol.* **83**, 697 (1977).
6. F. H. Royce, B. D. Ratner and T. A. Horbett, in: *Biomaterials: Interfacial Phenomena and Applications*, Advances in Chemistry Series, Vol. 199. p. 453, S. L. Cooper and N. A. Peppas (Eds). Am. Chem. Soc., Washington, DC (1982).
7. J. D. Andrade, in: *Surface and Interfacial Aspects of Biomedical Polymers, Vol. 2: Protein Adsorption*, Plenum Press, New York (1985).
8. A. H. Nishikawa, *Chemtech* **5**, 564 (1975).
9. T. A. Horbett, in: *Biomaterials: Interfacial Phenomena and Applications*, Advances in Chemistry Series, Vol. 199, p. 233, S. L. Cooper and N. A. Peppas (Eds). Am. Chem. Soc., Washington, DC (1982).
10. J. L. Brash, in: *Interaction of the Blood with Natural and Artificial Surfaces*, p. 37, E. W. Salzman (Ed.). Marcel Dekker, New York (1981).
11. J. L. Brash, in: *Biocompatible Polymers*, p. 35, M. Szycher (Ed.). Technomic, Lancaster, PA (1983).
12. I. Lundstrom, B. Ivarsson, U. Jonsson and H. Elwing, in: *Polymer Surfaces and Interfaces*, p. 201, W. J. Feast and H. S. Munro (Eds). John Wiley, New York (1987).
13. W. Norde, *Adv. Colloids Interface Sci.* **25**, 267 (1986).
14. B. Ivarsson and I. Lundstrom, *CRC Crit. Rev. Biocompac.* **2**, 1 (1986).
15. J. D. Aptel, A. Carroy, P. Dejardin, E. Pefferkorn, P. Schaaf, A. Schmitt, R. Varoqui and J. C. Voegel, in: *Proteins at Interfaces, Physicochemical and Biochemical Studies*, p. 222, J. L. Brash and T. A. Horbett (Eds). Am. Chem. Soc., Washington, DC (1987).
16. A. W. Adamson, in: *Physical Chemistry of Surfaces*, p. 368, John Wiley, New York (1982).
17. J. Oscik and I. L. Cooper, in: *Adsorption*, p. 181, John Wiley, New York (1982).
18. M. Benzel, M. Savage and J. Andrews, *Sci. Comput. Automation* **2**, 27 (1989).
19. S. A. Fraga, *J. Comput. Chem.* **3**, 329 (1982).
20. E. Clementi, F. Cavallone and R. Scordamaglia, *J. Am. Chem. Soc.* **99**, 5531 (1977).
21. B. Coghlan and S. Fraga, *Comput. Phys. Commun.* **36**, 391 (1985).
22. P. Hobza and R. Zahradnik, *Chem. Rev.* **88**, 871 (1988).
23. S. Fraga, K. M. S. Saxena and M. Torres, in: *Biomolecular Information Theory*, p. 80, Elsevier, New York (1978).
24. W. P. Minicozzi and D. F. Bradley, *J. Comput. Phys.* **4**, 118 (1969).
25. S. Fraga, *Comput. Phys. Commun.* **29**, 351 (1983).
26. C. Tanford, *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, p. 141, John Wiley, New York (1980).
27. D. Horsley, J. Herron, V. Hlady and J. D. Andrade, in: *Proteins at Interfaces, Physicochemical and Biochemical Studies*, p. 290, J. L. Brash and T. A. Horbett (Eds). Am. Chem. Soc., Washington, DC (1987).
28. H. P. Jennissen, *J. Colloid Interface Sci.* **111**, 570 (1986).
29. S. I. Jeon, J. H. Lee, J. D. Andrade and P. G. de Gennes, *J. Colloid Interf. Sci.* (in press).
30. J. N. Israelachvili, *Intermolecular and Surface Forces*, p. 105, Academic Press, New York (1985).
31. K. Wuthrich, *Science* **243**, 45 (1989).
32. Protein Data Bank, Brookhaven National Laboratory, Brookhaven, New York.
33. S. K. Burley and G. A. Petsko, in: *Advances in Protein Chemistry*, Vol. 39, p. 125, C. B. Anfinsen *et al.* (Eds). Academic Press, San Diego (1988).
34. T. Imoto, L. H. Johnson, A. C. T. North, D. C. Phillips and J. A. Rupley, in: *The Enzymes*, Vol. VII, p. 666, P. D. Boyer (Ed.). Academic Press, New York (1972).
35. B. Keil, in: *The Enzymes*, Vol. III, p. 250, P. D. Boyer (Ed.). Academic Press, New York (1971).
36. M. Matsushima, M. Marquart, T. A. Jones, P. M. Colman, K. Bartels and R. Huber, *J. Mol. Biol.* **121**, 441 (1978).
37. A. L. Lehninger, *Principles of Biochemistry*, p. 126, Worth, New York (1982).
38. A. Clark, in: *The Theory of Adsorption and Catalysis*, p. 137, Academic Press, New York (1970).
39. M. Karplus, *Ann. NY Acad. Sci.* **439**, 107 (1985).
40. R. M. Levy and J. W. Keepers, *Comments Mol. Cell. Biophys.* **3**, 273 (1986).
41. J. A. McCammon, B. R. Gelin and M. Karplus, *Nature* **267**, 585 (1977).
42. M. Karplus, *Biomol. Stereodyn., Proc. Symp.* **2**, 211 (1981).
43. H. A. Scheraga, *Ann. NY Acad. Sci.* **439**, 170 (1985).
44. J. D. Andrade, J. Herron, V. Hlady and D. Horsley, *Croat. Chem. Acta, CCACAA* **60**, 495 (1987).

45. J. L. Bohnert and T. A. Horbett, *J. Colloid Interface Sci.* **111**, 363 (1986).
46. S. M. Slack and T. A. Horbett, *J. Colloid Interface Sci* **124**, 535 (1988).
47. E. Nyilas, T-H. Chiu and D. M. Lederman, in: *Biocolloids, Polymers, Monolayers, Membranes, and General Papers, Colloid and Interface Science*, Vol. V. p. 77, M. Kerker (Ed.). Academic Press, New York (1976).
48. D. H. Everett, *Basic Principles of Colloid Science*, p. 25, Royal Society of Chemistry (1988).
49. B. M. C. Chan and J. L. Brash, *J. Colloid Interface Sci.* **82**, 217 (1981).
50. W. J. Dillman and I. F. Miller, *J. Colloid Interface Sci.* **44**, 221 (1973).
51. D. F. Watkins and C. R. A. Robertson, *J. Biomed. Mater. Res.* **11**, 915 (1977).
52. T. A. Horbett, P. K. Weathersby and A. S. Hoffman, *J. Bioeng.* **1**, 61 (1977).
53. K. Park and S. L. Cooper, *Trans. Am. Soc. Artif. Intern. Organs* **16**, 483 (1985).
54. B. R. Young, W. G. Pitt and S. L. Cooper, *J. Colloid Interface Sci.* **125**, 246 (1988).
55. D. R. Absolom, W. Z. Zingg and A. W. Neumann, in: *Proteins at Interfaces, Physicochemical and Biochemical Studies,* p. 401, J. L. Brash and T. A. Horbett (Eds). Am. Chem. Soc., Washington, DC (1987).
56. H. Y. K. Chuang, W. F. King and R. G. Mason, *J. Lab. Clin. Med.* **92**, 483 (1978).
57. A. Baszkin and D. J. Lyman, *J. Biomed. Mater. Res.* **14**, 393 (1980).
58. K. Park, F. W. Mao and H. Park, *Biomaterials* **11**, 24 (1990).
59. E. J. Castillo, J. L. Koenig and J. M. Anderson, *Biomaterials* **5**, 319 (1984).