

Master copy

2005 CP2, 3

3.2 PROTEINS: STRUCTURE, PROPERTIES, AND ADSORPTION TO SURFACES

Thomas A. Horbett

The importance of proteins in biomaterials science stems primarily from their inherent tendency to deposit on surfaces as a tightly bound adsorbate, and the strong influence these deposits have on subsequent cellular interactions with the surfaces. It is thought that the particular properties of surfaces, as well as the specific properties of individual proteins, together determine the organization of the adsorbed protein layer, and that the nature of this layer in turn determines the cellular response to the adsorbed surfaces. Since the cellular responses largely determine the degree of biocompatibility of the material, the properties of proteins and their behavior at interfaces need to be understood by those interested in biomaterials. Figure 1 illustrates the interaction of a cell with an adsorbed protein layer on a solid substrate.

It is also worth noting that this subject has other important aspects, including the fact that the interfacial behavior of proteins is a fundamental, general property of proteins and enzymes that needs to be understood to fully appreciate protein behavior. In addition, phenomena at the air-water interface (e.g., interfacial coagulation and foaming), at the oil-water interface (e.g., the "receptor" proteins located in cell membranes that serve many important signalling functions), and at the solid-liquid interface in nonbiomaterial settings (e.g.,

Biomaterials Science

Copyright © 1996 by Academic Press, Inc.
All rights of reproduction in any form reserved.

133

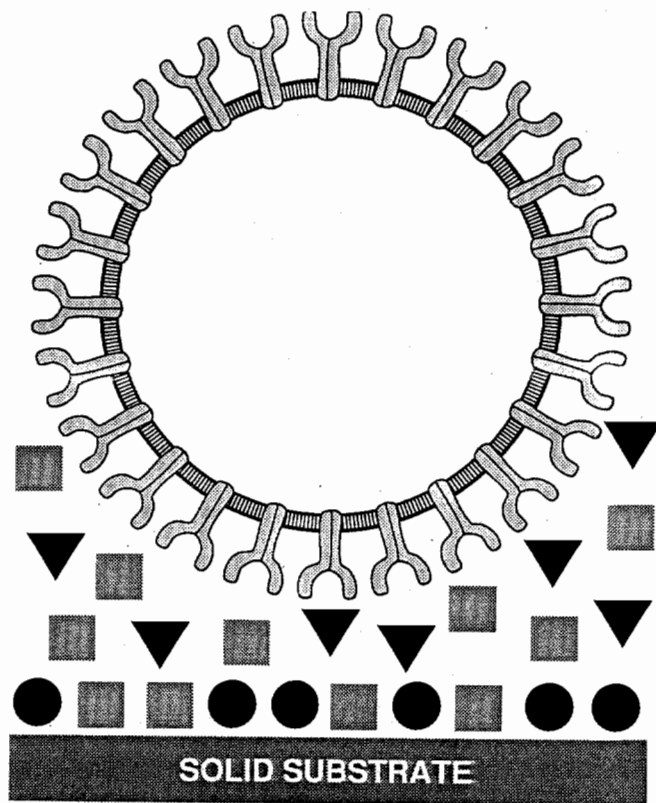


FIG. 1. Cell interaction with an adsorbed protein layer on a solid substrate. The cell is shown as a circular space with a bilayer membrane in which the adhesion receptor protein molecules (the slingshot-shaped objects) are partly embedded. The proteins in the extracellular fluid are represented by squares and triangles. The receptor proteins recognize and cause the cell to adhere to only the surface-bound form of one protein, the one represented by a solid circle. The bulk phase of this same adhesion protein is represented by a triangle, indicating that the solution and solid-phase forms of this same protein have a different biological activity. The figure is schematic and not to scale.

marine fouling, bacterial adhesion, and cell growth on surfaces in culture), are all strongly influenced by the behavior of proteins at interfaces.

A brief historical sketch of proteins at interfaces reminds us that the current biomaterials interest in this subject is just the latest in a long series of studies of this phenomenon. Thus, for example the fact that shaking of protein solutions causes the proteins to undergo "interfacial coagulation," in which the proteins are actually separated from the solution phase into the surface phase owing to their denaturation and insolubilization, was reported in 1851. Actual experimentation on proteins at interfaces began as early as 1873, when the viscosity at the air interface of protein solutions was shown to be considerably greater than in the bulk phase of the solution by observing the resistance to a magnetic field of a small magnetic needle floating at the solution interface. The behavior of proteins at the air-water interface was heavily studied in the 1920s through the early 1940s by Langmuir and others, with an emphasis on the structural changes in the proteins as judged from the rather large areas occupied per molecule when a monolayer was

formed. The subject has been revived again in more recent times owing to the interest of biomaterials scientists and those immobilizing enzymes, mostly focused on the solid-liquid interface. Most recently, the very modern technique of making variant or mutant proteins via single amino acid substitutions at a specific place in the protein chain, called "protein engineering," has been profitably applied to the further study of protein behavior at the air-water and the solid-liquid interfaces. The protein variant studies have given new insights into the molecular mechanisms of protein behavior at interfaces, since a correlation between changes in thermodynamic stability of the protein variants and the changes in surface activity was found (Horbett, 1993).

STRUCTURE AND PROPERTIES OF PROTEINS RELEVANT TO ADSORPTION

The soluble proteins present in biological fluids such as blood plasma and peritoneal exudate are the type of proteins that are primarily involved in adsorption to implanted materials. Insoluble proteins, such as collagen, which form the structural basis of tissue, are not normally free to diffuse to the implant surface, although they may be deposited in fibrous form adjacent to or actually on the implant by cells as part of the foreign body capsule formation. The soluble proteins differ from the insoluble proteins in many ways, including the fact that they are less regular in their amino acid composition and three-dimensional structure. The soluble proteins are therefore difficult to describe except in certain general terms. Fundamentally, this diversity originates in the linear sequence of amino acids that uniquely characterizes each protein. This sequence is the same for all molecules of a particular type of protein, yet both the length and sequence differ dramatically from protein to protein. For example, all albumin molecules have the same sequence, while all fibrinogen molecules have another sequence that is much longer and very different than the albumin sequence.

The role of the sequence of amino acids in generating diversity can best be appreciated by a brief review of the properties of the amino acid side chains, since these vary greatly (see Table 1). Some of the amino acids have side chains that carry no charge at any pH yet exhibit considerable polar character (serine, threonine). The ionizable side chains vary from fairly acidic ones (aspartic and glutamic acid are fully negatively charged at the physiological pH of 7.4) to more basic amino acids such as the imidazole group in histidine (which carries a partial positive charge at pH 7.4) and the still more basic amino groups in lysine and arginine that carry full charges at pH 7.4.

Another group of amino acids have no acid, base, or polar character in their side chains, instead being somewhat hydrocarbonlike in character, as attested by their generally much lower solubility in water. However, these so-called hydrophobic or "water-hating" amino acids vary considerably in this respect, depending on their specific structure. Thus, for example, the single methyl group side chain in alanine contributes only 0.5 kcal per mole to the free energy of

TABLE 1 Structure and Properties of Amino Acid Side Chains

Amino acid	Structure	Group and pK	Charge	Hydrophobicity ^a	Surface tension ^b
Isoleucine	$\begin{array}{c} \text{CH}_3 \\ \\ -\text{CH}-\text{CH}_2-\text{CH}_3 \end{array}$		Neutral	0.73	-15.2
Phenylalanine	$-\text{CH}_2-\text{C}_6\text{H}_5$		Neutral	0.61	-17.3
Valine	$\begin{array}{c} -\text{CH}-\text{CH}_3 \\ \\ \text{CH}_3 \end{array}$		Neutral	0.54	-3.74
Leucine	$\begin{array}{c} -\text{CH}_2-\text{CH}-\text{CH}_3 \\ \\ \text{CH}_3 \end{array}$		Neutral	0.53	-21.9
Tryptophane	$-\text{CH}_2-\text{C} \begin{array}{l} \diagup \text{C}_5\text{H}_4\text{N} \\ \diagdown \text{H} \end{array}$		Neutral	0.37	-9.6
Methionine	$-\text{CH}_2-\text{CH}_2-\text{S}-\text{CH}_3$		Neutral	0.26	-3.01
Alanine	$-\text{CH}_3$		Neutral	0.25	0.96
Glycine	$-\text{H}$		Neutral	0.16	1.12
Cysteine	$-\text{CH}_2\text{SH}$	$-\text{SH}: 8.3$	0 to -1	0.04	0.69
Tyrosine	$-\text{CH}_2-\text{C}_6\text{H}_4-\text{OH}$	$-\text{OH}: 10.9$	0 to -1	0.02	-15.1
Proline	$\begin{array}{c} \quad \\ \text{H}_2\text{C} \quad \text{CH}_2 \\ \diagdown \quad / \\ \text{C} \\ / \quad \backslash \\ \text{H}_2 \end{array}$		Neutral	-0.07	-0.49
Threonine	$\begin{array}{c} -\text{CH}-\text{OH} \\ \\ \text{CH}_3 \end{array}$		Neutral	-0.18	0.59
Serine	$-\text{CH}_2-\text{OH}$		Neutral	-0.26	0.76
Histidine	$-\text{CH}_2-\text{C}_4\text{H}_3\text{N}_2$	$-\text{NH}-: 6.0$	0 to +1	-0.40	1.03
Glutamic acid	$-\text{CH}_2-\text{CH}_2-\text{CO}_2\text{H}$	$-\text{CO}_2\text{H}: 4.3$	0 to -1	-0.62	0.86
Asparagine	$-\text{CH}_2-\text{C}(=\text{O})-\text{NH}_2$		Neutral	-0.64	1.17
Glutamine	$-\text{CH}_2-\text{CH}_2-\text{C}(=\text{O})-\text{NH}_2$		Neutral	-0.69	1.21
Aspartic acid	$-\text{CH}_2-\text{CO}_2\text{H}$	$-\text{CO}_2\text{H}: 3.9$	0 to -1	-0.72	0.96
Lysine	$-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NH}_3$	$-\text{NH}_2: 10.8$	0 to +1	-1.1	0.92
Arginine	$-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NH}-\text{C}(=\text{NH}_2)-\text{NH}_2$	$-\text{NH}_2: 12.5$	0 to +1	-1.8	1.03

^aA "consensus value" for the hydrophobicity of the amino acid side chains is given (Eisenberg, 1984). More positive values are more hydrophobic. According to Eisenberg, the magnitude of the values "may be considered roughly in kcal/mole for transfer from a hydrophobic to a hydrophilic phase" (e.g., from ethanol to water).

^bThe values are the surface tension lowering of water solutions of the amino acids in units of ergs/cm²/mole per liter (Bull and Breese, 1974).

transfer from water to an organic phase, whereas the double-ringed indole group in tryptophan contributes 3.4 kcal. The diverse character of the amino acid side chains, together with the variations in the proportions of the amino acids present in each particular protein, means that such physicochemical properties as their solubility and ability to interact with surfaces are also diverse.

An important consequence of the primary sequence and its inherent diversity in chemical nature is the fact that, unlike most synthetic polymers, each protein has a single, distinct, three-dimensional structure that it will assume under physiological conditions (see Table 2). These shapes are dictated by the formation of multiple noncovalent bonds formed throughout the protein's three-dimensional structure that leads to a metastable state. With soluble proteins, these shapes are often roughly spherical or globular in outline, although the important plasma protein fibrinogen violates this somewhat because it is elongated into a "three bead on a string" structure. More important here than the particular structure assumed by a protein is the fact that a unique arrangement of the amino acid sequence in three-dimensional space exists for each protein. Furthermore, the spatial arrangement results in the hydrophobic residues preferentially located "inside" the protein where they are shielded from water, while the ionized and polar residues are usually on the outside of the protein and in contact with the aqueous phase.

This spatial arrangement of the amino acids in proteins has a direct bearing on the interaction of proteins with surfaces because it means that the many residues "buried" inside the protein may not be able to participate in bond formation with the surface. For these interior residues to interact with the surface, the protein would have to unfold. Furthermore, it means that the type of residues that initially contact the interface have essentially been narrowed to the largely polar residues at the protein surface. The extremely wide range of chemical interactions theoretically possible because of the many different types of amino acid residues in the protein sequence does not necessarily come into play because there is a strong, overriding structural influence that tends to prevent the full array of possible interactions from being expressed.

The folded protein structures have densities of about 1.4 g/cm³. In comparison with water's density of 1.0, or the density of most synthetic polymers of about 1.1, this basic fact about proteins reflects their tightly folded structure. It is therefore convenient to think of proteins in a physicochemical sense as quite compact, externally charged wax droplets in water, in which the interior hydrophobic core is analogous to wax and the surface amino acids are the charged species. It is in this way that the polyelectrolyte behavior of proteins is expressed. That is, owing to their large size and corresponding large number of charged amino acid side chains of varying acidity or basicity, proteins have on them a large number of charges, both positive and negative, and these are distributed around the exterior of the protein. Therefore, depending on the pH and ionic strength of the media, a large range of charge interactions can be expected between the protein and a surface. The polyelectrolyte behavior is expressed most clearly in the degree of adsorption. Many proteins exhibit a maximum in the amount adsorbed to

neutral or slightly charged surfaces at a pH at which the net charge on the protein is minimal, i.e., near the isoelectric pH. On surfaces which themselves carry a large net charge, however, the interactions are dominated by the degree of opposition of charge on the protein and the surface, so that negatively charged proteins adsorb preferentially to positively charged surfaces and positively charged proteins adsorb preferentially to negatively charged surfaces.

The structural singularity of a particular protein generally applies only under conditions that are at least approximately physiological, i.e., in the range of 0 to 45°C, pH 5 to 8, and in aqueous solutions of about 0.15 M ionic strength. Beyond these conditions, proteins are subject to the phenomena of denaturation, a word which is meant to indicate that they lose their normal nature or structure. The natural or "native" protein can be made to undergo a transition to the denatured form simply by heating it, for example. The denatured protein is quite different than the native protein, and, in particular, generally loses the "inside/outside" nature reflected in the preferential location of polar residues on the surface and nonpolar residues on the inside of the molecule. In addition, the protein also loses the singularity of its structure when it is denatured. Instead, it will tend to become much more like a random coil that is characteristic of synthetic polymers. Denatured proteins typically lose their solubility, become much less dense, and lose biological functions such as enzyme activity. The stability of selected proteins listed in Table 2 is seen to vary greatly.

The retention of a protein's native structure upon adsorption, even under physiologic conditions, is one of the more interesting aspects of protein behavior at interfaces, for the unfolded protein, with many more exposed hydrophobic amino acid residues, is clearly capable of forming many more bonds per molecule with a surface than the native protein. The multiple bonding involved in adsorption to surfaces is a major feature that distinguishes the adsorption of proteins from the adsorption of small molecules. Generally, proteins adsorbed at the solid interface are not denatured (see the following discussion).

ADSORPTION BEHAVIOR OF PROTEINS AT SOLID-LIQUID INTERFACES

The adsorption of proteins to solid surfaces qualifies for one of the traditional definitions of adsorption in that it represents a preferential accumulation of the protein in the surface phase. The protein adsorbed to the surface does not merely reflect the retention of a thin layer of the adjacent protein solution; it is *not* sorption as might occur on a porous, absorptive matrix such as filter paper. This can be seen from the fact that the concentration of protein in the surface phase is much higher than the bulk phase from which it came. Thus, for example, typical values for adsorption of proteins are in the range of 1 μg/cm², a plateau or monolayer value typically reached at higher bulk concentrations (see Fig. 2). To convert this two-dimensional value into an equivalent volumetric concentration unit, we can assume a monolayer of a typical protein for which a 100-Å (or 10⁻⁶ cm) diameter is a good approximation. Then,

TABLE 2 Properties of Selected Proteins

Protein	Function	Location	Size	Shape ^a	pI	Stability ^a	Surface activity
Albumin	Carrier	Blood	65 kDa	42 × 141 Å (1)	4.8	Denatures at 60°C (8)	Low on PE
Fibrinogen	Clotting	Blood	340 kDa	460 × 60 Å (2) trifolular string	5.8	Denatures at 56°C (9)	High on PE
IgG	Antibody	Blood	165 kDa	T-Shaped	6.5		Low on PE
Lysozyme	Bacterial lysis	Tear; hen egg	14.6 kDa	45 × 30 × Å (3)	11	$\Delta G_n = -14$ kcal/mol (10)	High on negatively charged surfaces
Hemoglobin	Oxygen carrier	Red cells	65 kDa	Globular 55 Å (4)	6.87	Normal form	Very high on PE
Hemoglobin S	Oxygen carrier	Sickle red cells	65 kDa	Spherical A2B2 tetramer	7.09	Lower than A form	Oxy form of HbS has much higher air-water activity than normal Hb
Myoglobin	Oxygen carrier	Muscle	16.7 kDa	45 × 35 × 25 Å (5) Spherical monomer	7.0	$\Delta G_n = -12$ kcal/mol (10)	Unknown
Collagen	Matrix factor	Tissue	285 kDa	3000 × 15 Å (6) Triple helical rod		Melts at 39°C (11)	
Bacterio-rhodopsin	Membrane protein		26 kDa	30–40 Å long (7) Seven-rod structure that self-localizes in membranes			High at cell membrane
Tryptophan synthase alpha subunit ("wild type")	Enzyme		27 kDa		5.3	$\Delta G_n = -8.8$ kcal/mole; denatures at 55°C (12)	High air-water activity compared with ovalbumin
Tryptophan synthase (glu → ileu) variant alpha subunit	Enzyme		27 kDa			$\Delta G_n = -16.8$ kcal/mole (12)	Much less active at air-water interface than wild type

^aThe numbers in parentheses refer to these references: (1) Peters, 1985, p. 176; (2) Stryer, 1981, p. 172; (3) Stryer, 1981, p. 138; (4) Stryer, 1981, p. 59; (5) Stryer, 1981, p. 49; (6) Stryer, 1981, p. 188; (7) Eisenberg, 1984, p. 599; (8) Peters, 1985, p. 186; (9) Loeb and Mackey, 1972; (10) Norde and Lyklema, 1991, p. 14; (11) Stryer, 1981, p. 191; (12) Yutani *et al.*, 1987.

a 1-cm² area containing 1 μg corresponds to a local protein concentration of $1/10^{-6} = 10^6$ μg/cm³ or 1 g/cm³. Given that the density of a pure protein is 1.4 g/cm³, this layer is indeed tightly packed. Furthermore, the 1 g/cm³ is equivalent to 1000 mg/cm³, which is far higher than the bulk protein concentration of solutions from which such adsorbates form (typically 1 mg/cm³). Thus, the surface phase is often 1000 times more concentrated than the bulk phase, corresponding to high local concentration indicative of the existence of a very different state of matter and thus truly deserving of a special term, namely, the adsorbed state.

A second major feature of the adsorbed phase is the selectivity of the process that leads to enrichment of the surface phase in one protein versus another. Here, we are speaking about adsorption as it typically occurs to biomaterial surfaces, namely from a complex mixture of proteins in the bulk phase. Since there is a limited amount of space on the surface of the solid and it can only accommodate a small fraction of the total protein typically present in the bulk phase, there is competition for the available surface sites. The monolayer of adsorbed protein is the limiting amount that can be adsorbed, resulting in competition for sites on the surface (see Fig. 2). Second, as has been discussed earlier, the proteins vary a great deal in

their fundamental amino acid sequences and three-dimensional structures and therefore have very different abilities to adsorb to surfaces; their "surface activity" differs (see Table 2).

Therefore, depending on the two major driving forces for adsorption, namely, the relative bulk concentration of each protein and its intrinsic surface activity, the outcome of the competitive process of adsorption is an adsorbed layer that is richer in some proteins than others; the surface composition differs from the bulk composition. Furthermore, because the proteins have different affinities for each type of surface, the outcome of the competition is different on each surface. Our concept of the adsorbed layer formed on solid surfaces exposed to mixtures of proteins, then, is one in which variable degrees of enrichment of each of the proteins occur, so that, for example, some surfaces may be richer in albumin while others have more fibrinogen. Table 3 contains some surface enrichment data for the adsorption of plasma proteins to several surfaces.

The adsorption of proteins to solid surfaces is largely irreversible and therefore leads to the immobilization of the protein species in the surface phase since they are no longer free to diffuse away. This is somewhat different from the idea one has with gas adsorption, because there the molecules can often

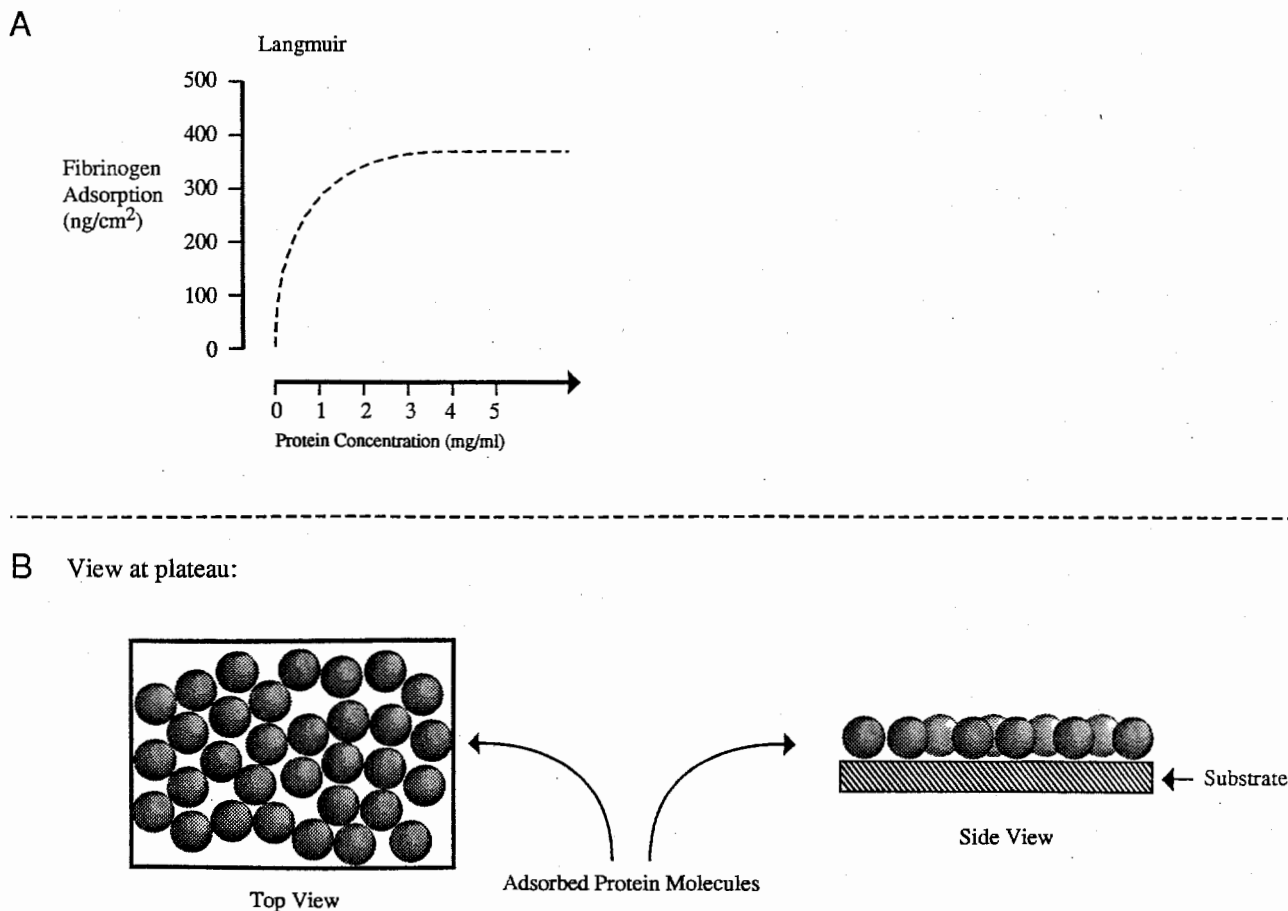


FIG. 2. Adsorption isotherms and the monolayer concept.

TABLE 3 Enrichment of Proteins Adsorbed on Polyethylene Exposed to Blood Plasma

Protein	Enrichment ^a
Fibrinogen	1.3
γ -globulin	0.53
Albumin	0.88
Hemoglobin ^b	79

^aEnrichment was calculated as the ratio of the surface fraction of the protein compared with the bulk fraction. The fraction in each case was calculated as the amount of each protein divided by the total amount of all of the proteins listed in the table. The data were measured in the author's laboratory using radiolabeled proteins to measure their adsorption from blood plasma.

^bHemoglobin is not considered a "normal" plasma protein, but is nonetheless present (typically at 0.01 mg/ml or less) in most plasma and serum preparations as a result of leakage from the red cells during preparation of the protein fraction of blood. *In vivo*, it would normally be present only in trace amounts unless a disease that caused hemolysis existed.

desorb from the surface if the pressure is lowered. When the solution phase variable corresponding to pressure is lowered—that is, when protein concentration is lowered by rinsing the surface with a protein-free medium—the protein does not readily come off of many surfaces, even after days of further rinsing. Effectively or operationally, the adsorption is irreversible unless some drastic change in the solvent is made, such as the introduction of a detergent that binds strongly to the adsorbed protein as well as the underlying substrate. The fact that the proteins are effectively immobilized by the adsorption process means that any of the processes they are normally exposed to in the bulk phase will proceed very differently in the surface phase, if only because the diffusion of one of the species is now halted. Furthermore, since transport is generally hindered near a solid interface, the approach and departure of species with which the protein may interact is greatly altered. Perhaps the most relevant consequence of the immobilization of a protein in the field of biomaterials is the often-noted phenomenon by which an adsorbed protein is able to cause a cell for which it has a receptor to adhere to the adsorbed protein–solid interface, whereas the same protein in the bulk phase is not bound by the cell (illustrated in Fig. 1). This is the case for fibrinogen and platelets; i.e., platelets adhere to adsorbed fibrinogen, but do not bind dissolved fibrinogen.¹ Table 4 contains a list of the platelet adhesion proteins present

in blood plasma (line 3c) and the receptors affected by the protein (line 3a).

The orientation of proteins in the adsorbed phase must also be considered because the proteins are not uniform in properties or structure across their surface. Indeed, the existence of regions that are enriched in one of the categories of side chains discussed earlier, e.g., a "patch" of acidic residues, is a feature that influences the functions of proteins. Similarly, particular sequences of amino acids in the protein are well known to bind specifically to a variety of agents, especially to cell-membrane bound receptors. As far as is known, proteins are not very free to rotate once adsorbed, owing to multiple bonding, and therefore a fixed portion of the surface of the protein is exposed to the bulk phase. The degree of uniformity of such orientation is, however, not known. For example, it is not known whether all the proteins are oriented the same way in order to optimize the bonding of certain favorable regions with the surface.

The reactions of proteins in the adsorbed phase may be broadly classed into noncovalent reactions represented by structural transitions, and covalent reactions, such as those that occur with protein complement C3 on hemodialyzer membranes. In the latter case, the adsorption renders this protein subject to proteolytic cleavage by other proteins in the complement system; this cleavage proceeds at only a very low rate for C3 in the bulk phase. The role of surface adsorption in this case is believed to be the formation of a covalent bond between the C3 and hydroxyl groups on the surface that somehow prevents an inhibitor that normally is present, and normally prevents further activation, from doing its job.

TABLE 4 Principles Underlying the Influence of Adsorbed Plasma Proteins on Platelet Interactions with Biomaterials

1. Synthetic foreign materials acquire bioreactivity only after first interacting with dissolved proteins. The principal means by which the transformation from an inert, nonthrombogenic polymer to a biologically active surface takes place is the interaction of the proteins with the surface, which then mediates cell adhesion.
2. Platelets are a major example of why and how adsorbed proteins are influential in cell–biomaterials interactions.
3. Sensitivity of platelets to adsorbed proteins is due to:
 - a. Receptors (IIb/IIIa and Ib/IX) bind specifically to a few of the adsorbed plasma proteins, mediating adhesion.
 - b. Concentrating, localizing, immobilizing effects of adsorbing proteins at the interface accentuate the receptor–adhesion protein interaction.
 - c. Adhesion proteins in plasma for platelets: fibrinogen, fibronectin, vitronectin, and von Willebrand factor.
4. Principles of protein adsorption to biomaterials:
 - a. Monolayer adsorption and consequent competition for available adsorption sites means that not all proteins in the plasma phase can be equally represented.
 - b. Driving forces: intrinsic surface activity and bulk phase concentration.
 - c. Surfaces vary in selectivity of adsorption.
 - d. Biological activity of the adsorbed protein also varies on different surfaces.

¹This applies to platelets that have not been previously activated by agonists such as ADP or thrombin. Platelets exposed to agonists do bind bulk-phase fibrinogen. See last section of 3.2 for further discussion.

TABLE 5 Enthalpy of Adsorption of Proteins to Surfaces

Protein	Surface ^a	Enthalpy of adsorption	Reference
HSA	α -Fe ₂ O ₃	+1.9 mJ·m ⁻² at pH 5 +7.0 mJ·m ⁻² at pH 7	Norde, p. 275 in Andrade (1985)
RNase	PS-H	+4 mJ·m ⁻² at pH 5 -2 mJ·m ⁻² at pH 11	Norde, p. 278 in Andrade (1985)

^aThe α -Fe₂O₃ surface referred to is hematite. PS-H is an abbreviation for negatively charged polystyrene latex particles of high surface charge density.

Clotting factor XII also requires a surface to become active in the clotting cascade. It also undergoes cleavage, but in this case the role of the surface is thought to be to perturb the three-dimensional structure of the protein to render it active as a protease. The activation of factor XII by a conformational change induced by the adsorption process is one of the better-known cases in which a noncovalent change induced by adsorption occurs, but in general it is thought that all proteins that adsorb to solid surfaces may undergo limited conformational change. Given the metastable condition of the protein molecule, and the driving force for better adsorption through further bond formation that would arise from partial exposure of residues in the protein interior, a change in the protein's structure might seem to be likely. However, many proteins and enzymes retain at least some of their biological activity in the adsorbed state, and the solid phase immunoassay technique relies on the use of adsorbed antibody or antigen whose structure cannot be totally altered for this method to work in the binding of antigens or antibodies. Therefore, conformational changes upon adsorption seem to be limited in nature; adsorption to solid surfaces does not seem to result in a fully denatured protein.

The kinetics of adsorption of proteins to solid surfaces generally consist of a very rapid initial phase that is diffusion limited, followed by a slower phase upon approach to the steady-state value. In the initial phase, the proteins typically adsorb as quickly as they arrive at the relatively empty surface, so that plots of amount adsorbed versus time^{1/2} in this regime have the linearity characteristic of a diffusion-controlled process. In the later, slower phase, it is presumably more difficult for the arriving proteins to find and fit into an empty spot on the surface.

The thermodynamics of protein adsorption are not easily characterized because the process appears to be essentially irreversible. Thus, when one exposes solid surfaces to a series of increasing concentrations of protein solutions, and then rinses away the bulk protein solution, an increasing amount of protein is retained on the surface until a plateau is reached at higher concentrations, as shown in Fig. 2, where typical adsorption isotherms are shown. Because the adsorption is irreversible, the calculation of an equilibrium binding constant from a plot of adsorption versus bulk concentration, and its conversion to a free energy value in the usual way, is not a valid method to obtain thermodynamic

information. However, direct measurements of the heat of adsorption have been made for several proteins on a variety of surfaces and under various conditions of pH and temperature (see Table 5). The enthalpy of the adsorption process has been observed to vary a great deal, even being positive in some cases. The observation of positive enthalpies upon spontaneous adsorption to certain surfaces must mean that the process is entropically driven in these cases. The net negative free energy characteristic of a spontaneous process means that $T \Delta S$ is greater than the positive ΔH term in the formula $\Delta G = \Delta H - T \Delta S$. More generally, all protein adsorption processes are thought to be strongly driven by entropic changes. The importance of entropic factors in this process can easily be envisioned to arise from changes in water binding to the surface and the protein as well as limited unfolding of the protein on the surface.

THE IMPORTANCE OF ADSORBED PROTEINS IN BIOMATERIALS

Table 4 summarizes the principles underlying the influence of adsorbed proteins in biomaterials used in contact with the blood. All of the principles listed also apply in other environments such as the extravascular spaces, albeit with other proteins and other cell types (e.g., macrophages in the peritoneum adhere via other receptors and other adhesion proteins). The platelets therefore provide a "case study," and we close this chapter by considering this case.

The sensitivity of platelet-surface interactions to adsorbed proteins is fundamentally due to the presence of adhesion receptors in the platelet membrane that bind to certain plasma proteins. There are only a few types of proteins in plasma that are bound by the adhesion receptors. The selective adsorption of these proteins to synthetic surfaces, in competition with the many nonadhesive proteins that also tend to adsorb, is thought to mediate platelet adhesion to these surfaces. However, since the dissolved, plasma-phase adhesion proteins do not bind to adhesion receptors unless the platelets are appropriately stimulated, while unstimulated platelets can adhere to adsorbed adhesion proteins, it appears that adsorption of proteins to surfaces accentuates and modulates the adhesion receptor-adhesion protein interaction. The type of surface to which the adhesion protein is adsorbed affects the ability of the protein to support platelet adhesion (Horbett, 1993). The principles that determine protein adsorption to biomaterials include monolayer adsorption, the intrinsic surface activity and bulk concentration of the protein, and the effect of different surfaces on the selectivity of adsorption.

More generally, all proteins are known to have an inherent tendency to deposit very rapidly on surfaces as a tightly bound adsorbate that strongly influences subsequent interactions of many different types of cells with the surfaces. It is therefore thought that the particular properties of surfaces, as well as the specific properties of individual proteins, together determine the organization of the adsorbed protein layer, and that the nature of this layer in turn determines the cellular response to the adsorbed surfaces.

Bibliography

- Andrade, J. D. (1985). Principles of protein adsorption. in *Surface and Interfacial Aspects of Biomedical Polymers*, J. Andrade, ed. Plenum Publ., New York, pp. 1-80.
- Bull, H. B., and Breese, K. (1974). Surface tension of amino acid solutions: A hydrophobicity scale of the amino acid residues. *Arch. Biochem. Biophys.* 161: 665-670.
- Eisenberg, D. (1984). Three-dimensional structure of membrane and surface proteins. *Ann. Rev. Biochem.* 53: 595-623.
- Horbett, T. A. (1982). Protein adsorption on biomaterials. in *Biomaterials: Interfacial Phenomena and Applications*, S. L. Cooper and N. A. Peppas, eds. ACS Advances in Chemistry Series, American Chemical Society, Washington, DC, Vol. 199, pp. 233-244.
- Horbett, T. A. (1986). Techniques for protein adsorption studies. in *Techniques of Biocompatibility Testing*, D. F. Williams, ed., CRC Press, Boca Raton, FL, pp. 183-214.
- Horbett, T. A. (1993). Principles underlying the role of adsorbed plasma proteins in blood interactions with foreign materials. *Cardiovascular Pathology.* 2: 137S-148S.
- Horbett, T. A., and Brash, J. L. (1987). Proteins at interfaces: current issues and future prospects. in *Proteins at Interfaces: Physicochemical and Biochemical Studies*, T. A. Horbett and J. L. Brash, eds. ACS Symposium Series, 343, American Chemical Society, Washington, DC, Vol. 343, pp. 1-33.
- Loeb, W. F., and Mackey, W. F. (1972). A "Cuvette Method" for the determination of plasma fibrinogen. *Bull. Amer. Soc. Vet. Clin. Path.* 1: 5-8.
- Norde, W., and Lyklema, J. (1991). Why proteins prefer interfaces. *J. Biomater. Sci.: Polymer Ed.* 2: 183-202.
- Peters, T. (1985). Serum albumin. In *Advances in Protein Chemistry*, Vol. 37 (C. B. Anfinsen, J. T. Edsall, and F. M. Richards, eds.), pp. 161-245. Academic Press, New York.
- Stryer, L. (1981). *Biochemistry*, 2nd Ed. W. H. Freeman, San Francisco.
- Yutani, K., Ogasahara, K., Tsujita, T., and Sugino, Y. (1987). Dependence of conformational stability on hydrophobicity of the amino acid residue in a series of variant proteins substituted at a unique position of tryptophan synthase alpha subunit. *Proc. Natl. Acad. Sci. U.S.A.* 84: 4441-4444.