

Plasma Protein Adsorption: The Big Twelve^a

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INTRODUCTION

FIGURE 1 presents a protein adsorption complexity spectrum and in schematic form suggests that proteins vary greatly in complexity. The simplest include the insulin dimer, lysozyme, and myoglobin, whose X-ray crystallographic, three-dimensional structures are well-known and whose function is generally well understood. The structures of such proteins can be imaged and manipulated using modern computer graphics algorithms and equipment. Through careful consideration of the surface chemistry of such proteins and by appreciation that the surface chemistry is far from uniform on a protein particle (there are different "faces," each with different interaction potentials with specific surfaces or interfaces), one can develop estimates or hypotheses as to optimum interaction orientations for such model proteins at model interfaces. This approach has been very fruitful in the study of the adsorption of chicken and human lysozyme at model surfaces,^{1,2} as well as in studies of the adsorption of myoglobin on model surfaces and the interaction of the adsorbed protein with specific monoclonal antibodies.³

Most of the plasma proteins, however, are considerably more complex. Their detailed three-dimensional structures are not known. For example, the plasma immunoglobulins vary greatly, particularly their Fab portions and variable regions, because of their wide and varied specific antigen binding characteristics. Although the structure of fibrinogen is fairly well-known,⁴ the detailed three-dimensional structure of even the major domains is not yet available.

The problem increases in complexity if one considers multicomponent solutions, particularly multicomponent solutions of complex proteins, such as serum or plasma and tears, because one now has a variety of proteins all interacting with the surface in a competitive fashion, and interacting with each other, both on the surface and in the bulk solution.

FIGURE 1 also includes a complexity axis for the surface or interface. One can consider that the simplest interface available is probably the air-water interface. Much of what we know of protein adsorption and protein behavior at interfaces is taken from Langmuir Blodgett monolayer studies.^{5,6} Studies at liquid-liquid, particularly oil-water, interfaces have also provided much information and such interfaces are

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extremely useful model systems.^{7,8} One can directly monitor the kinetics of adsorption, the spreading characteristics of the adsorbed proteins, conformational changes, and two-dimensional ordering-structuring by Langmuir Blodgett and interfacial tension methods.^{7,8}

The surface of an amorphous, homogenous homopolymer, such as poly(methyl methacrylate) (PMMA) or poly(dimethyl siloxane) (PDMSO), could also be considered relatively simple. One must remember, however, that such surfaces may not be truly rigid, particularly the surfaces of elastomers, such as PDMSO, because of the

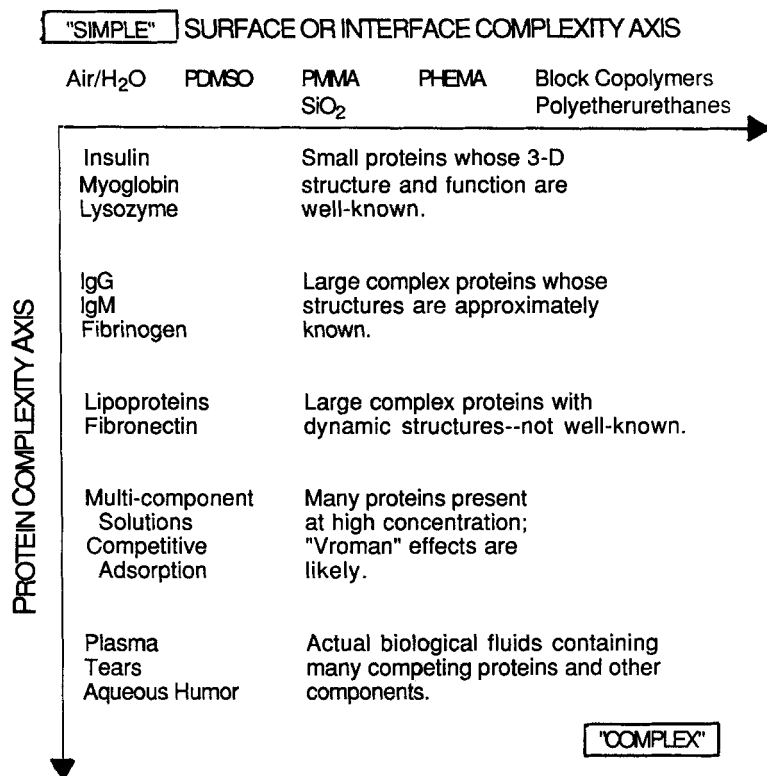


FIGURE 1. A protein adsorption complexity matrix. The upper left represents "simple" systems; the lower right shows highly complex systems. A solid surface complexity axis runs from left to right; the protein complexity axis runs from top to bottom. See text for abbreviations and details.

mobility of polymer chains and segments at room and body temperature. In addition, the intrinsic mobility and dynamics of proteins themselves suggest that very complex interactions and even interpenetrations may occur. Even for a rigid surface, such as PMMA, the amphiphilic nature of the polymer's hydrophilic ester group and the hydrophobic backbone can result in slight water uptake, and even side-chain orientation or reorientation at the polymer-water interface.⁹ Virtually all polymer surfaces are dynamic. Methods are available for at least qualitatively characterizing the nature of

the surface in air and underwater and even the dynamics of surface restructuring in going from one environment to another.⁹

As one continues along the material surface complexity axis (FIGURE 1), one eventually gets to such complex surfaces as block copolyetherurethanes, the most widely used materials for cardiac assist devices.¹⁰ Such materials may have two or more phases and interphases present in the bulk structure and at the surface. Bulk and surface morphology are dependent on processing history, as well as on specific polymer chemistry. In addition, there is some evidence that the more hydrophobic phase tends to dominate the surface in air or vacuum, while the more hydrophilic phase tends to dominate it in an aqueous environment.¹⁰ There is also concern that because of the dynamics of such elastomers, particularly due to the mobility of the soft segment phase, the water-equilibrated surface may restructure in order to optimally equilibrate with an adsorbed protein film—that is, the surface in water may change during the protein adsorption process as a result of the protein microenvironment with which it is now trying to equilibrate.

Although the situation in FIGURE 1, particularly at the bottom right, would appear to be quite complex, the situation at the top left is indeed relatively simple and is beginning to become qualitatively, even quantitatively, understood.

Most of what we know about protein adsorption is summarized in FIGURE 2, a general kinetic model for the process, assuming a one component protein solution for simplicity. A protein of bulk concentration, C_o , diffuses to and collides with the interface. At time zero initial contact occurs. If the interaction forces are sufficient, the protein stays on the surface for a certain residence time, probably in the range of milliseconds to seconds. Air-water interface studies have shown that a minimum contact area is required, which probably relates to the magnitude of the hydrophobic

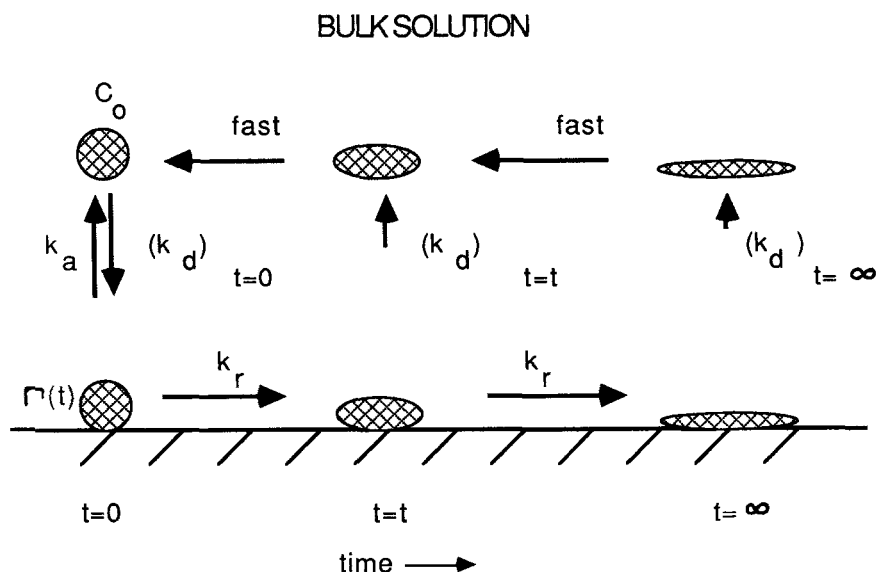


FIGURE 2. A kinetic protein adsorption model based in part on the ideas of Lundström, Walton, and Jennissen.^{15,21-24} C_o is the bulk protein concentration; Γ is the surface concentration, which is a function of time t ; k_a is the on-rate constant and $k_d(t)$ are the off-rate constants, which are a function of contact time (residence time).

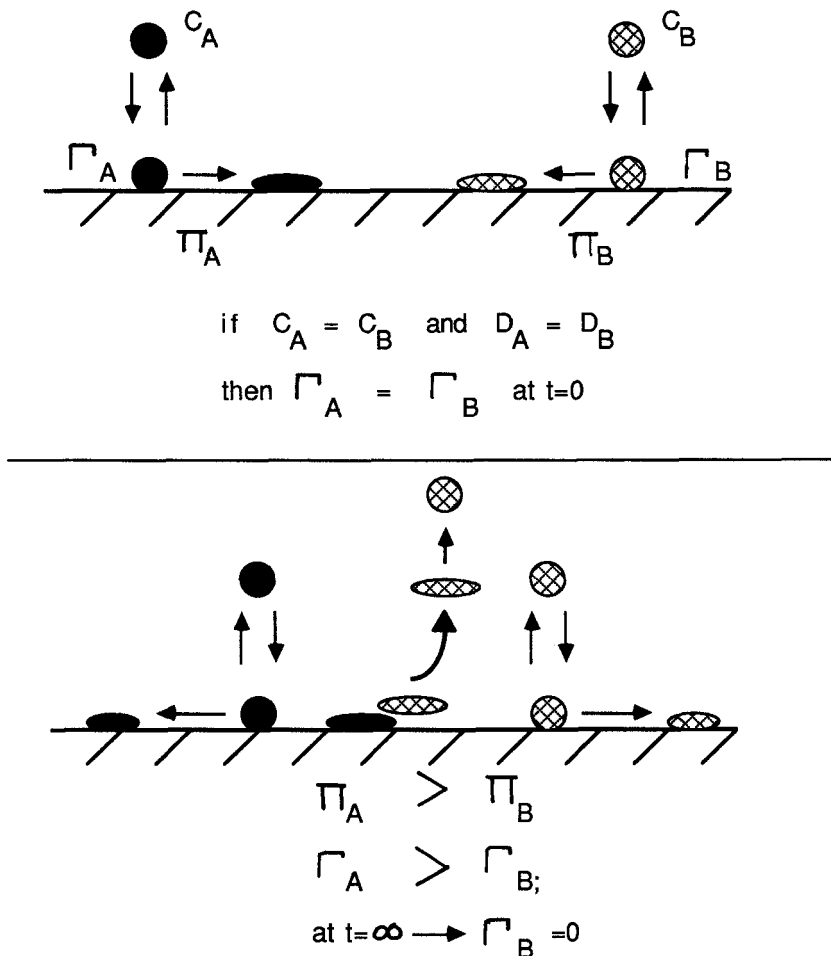


FIGURE 3. The competitive surface spreading hypothesis (from Bagnall.⁸) Top: Two proteins, *A* and *B*, have the same bulk concentration and diffusion coefficients and therefore have the same surface concentration (Γ) at $t = 0$ (initial contact). If the spreading pressure (π) of one is greater than the other, then eventually the protein with the larger π will dominate the interface. Here we show that *A* displaces *B*, since $\pi_A > \pi_B$.

interaction required for initial stabilization of the protein-surface complex.^{5,11} The protein can desorb at this stage; an appropriate desorption rate constant is indicated in FIGURE 2.

While on the surface, the protein may begin a surface denaturation process, which is probably related to its intrinsic conformational lability. This is also related to the fact that globular proteins are only marginally stable,¹² and an energy of 5–15 kcal/mole is sufficient to denature them in normal buffer solutions.^{11–13} Therefore, the interactions with the surface, particularly interactions of a hydrophobic nature, significantly affect the solution equilibrium of the protein.¹¹

There may be a strong configurational entropy driving force in going from the

globule to a more extended state, particularly if the extended state can be accommodated by maintaining a degree of hydrophobic interaction comparable to that provided by the globular state. Hydrophobic interaction can, of course, be provided, in part, by interactions with a partially hydrophobic surface. Dill has modeled and considered the configurational entropy aspects of globular protein structure,¹² as well as of hydrophobic surfaces containing alkyl chains—such as those commonly used in chromatography.¹⁴

A distorted or extended protein is generally less soluble in solution; thus, one expects the desorption tendency to decrease, allowing for increased binding and/or surface denaturation. Desorption of the protein is therefore a function of its residence time on the surface. With increasing contact times the probability for desorption decreases, as indicated in FIGURE 2. Assuming that the adsorption process does not result in any covalent bond changes in the protein molecule, the model then also assumes that if a denatured or partially denatured protein does desorb, it rapidly renatures to the equilibrium globular state. This portion of the model is controversial, as there is evidence in the literature that some desorbed proteins are permanently changed^{15,16} due to covalent bond changes during the process.

Competitive adsorption in two component systems has been modeled by Cuypers *et al.*¹⁷ His results suggest that the "Vroman effect"^{18,19} is predicted by a competitive adsorption model which allows an exponential decrease in the affinity constant with increasing occupancy of the surface, similar to the classical Langmuir treatments of adsorption which incorporate a lateral interaction and variable surface site energy term.²⁰

The effect of a surface-induced conformational change has been treated in a preliminary way by the models developed by Lundstrom *et al.*,²¹⁻²³ in which two states are considered: the initial state at time = 0, and the "equilibrium" state at long contact times, $t = \infty$. Lundstrom's models, which to date have been published only for single component solutions, nicely predict and explain adsorption behavior of labile globular proteins in single component systems.

FIGURE 3 begins to ask the question of what happens during a competitive adsorption process and is based on the ideas of Bagnall⁸ and Jennissen,²⁴ who showed that the surface denaturation or accommodation process is dependent on the number and type of neighbors. If two different proteins have adsorbed next to each other, one is generally more labile or conformationally adaptable to the interface than the other. We can say that one "spreads" at the interface more effectively than the other. We can consider a spreading constant for such a protein, analogous to the solid-vapor and solid-liquid spreading constants so commonly used in classical surface chemistry.²⁰ One would expect that the spreading characteristics would be related to the solution denaturability¹³ of the protein, and particularly to its behavior at water-air and water-oil interfaces.⁵⁻⁸ Clearly, the more "surface active" protein would spread more effectively and may displace the other protein from the interface. This is another mechanism by which the Vroman effect can be explained. Clearly, the next step is to develop a model which incorporates the ideas of Cuypers,¹⁷ Lundstrom,²¹⁻²³ and Bagnall,⁸ but generalized for complex multicomponent systems.

THE BIG TWELVE

Blood plasma, however, consists of far more than one or two proteins. High-resolution, two-dimensional electrophoresis shows over 150 bands from plasma. It has been estimated that a typical cell has over 5000 different proteins! We have chosen to

consider only those proteins in plasma that are expected to compete effectively for the interface in the initial collision or contact process, *i.e.*, those which are present at sufficient plasma concentration to be considered major constituents. We have chosen to consider only those proteins generally present in plasma at about 1 mg/ml or higher concentration. The twelve such proteins are shown in FIGURE 4 and described in TABLE 1. FIGURE 4 somewhat schematically separates the proteins by electrophoretic mobility and sedimentation coefficient, *i.e.*, by net charge and size, respectively. Albumin is clearly the major constituent, followed by IgG. The high- and low-density lipoproteins are also major components. The properties of these twelve proteins are summarized briefly and qualitatively in TABLE 1. TABLE 2, based on a simple diffusion limited mass transport view, asks the question, "When does the next protein arrive?"²⁵ or "Who gets there first?"

Clearly, protein collisions with the surface are dominated by albumin. Seven times as many albumin molecules as IgG molecules strike the surface, and a thousand times more albumin molecules than IgM molecules strike the surface. So it is certainly reasonable and predictable that the surface would be largely populated at or near time zero by albumin, assuming that there is sufficient interaction between albumin and the surface to lead to a reasonable residence time.²⁶ About one in seven of the collisions with the surface involves IgG. If IgG has a greater affinity or greater spreading pressure at the surface than does albumin, then the spreading IgG molecule may tend to displace some of the preadsorbed albumin from the surface. Thus, although the surface is rich in albumin initially, one may find that the concentration shifts to IgG with increasing time. One could also argue that, although fibrinogen may occupy 1 in 150 or so of the sites at time zero, the sites it does occupy are particularly stable and perhaps it is "irreversibly" adsorbed. Assuming that the affinity constant for albumin is not high, occasionally an albumin molecule will desorb. Although the now vacant surface site is seeing a collision frequency continually dominated by albumin, if a molecule of fibrinogen collides with that site, the adsorptive interaction, with its much higher affinity constant, will be longer lived and more stable than if albumin had collided with the vacant site.

Thus, there are two approaches to the Vroman effect. One can assume no conformational change, but essentially a surface occupancy lateral interaction model, such as that developed by Cuypers,¹⁷ or one can assume a surface denaturation model, such as that being developed by Lundstrom.²¹⁻²³ Clearly, both are important, and both are occurring, not only for the Big Twelve discussed in TABLES 1 and 2, but for the other 150 or more protein constituents in plasma.

Nevertheless, we now know enough to begin to develop models, at least for two and three component systems. When those match the excellent, competitive adsorption data now becoming available,¹⁹ we can, using more sophisticated mathematical and computer techniques, extend them to many components and parameters. A key problem is obtaining parameters which reflect or at least relate to the interaction energy at time 0, the surface denaturability, and perhaps the interaction energy at very long times.

We must know the nature of the solid interface. Polyurethanes are probably too complex at this stage for truly fundamental understanding. The best models are the column chromatography supports used for protein separation. Here the highly hydrophilic, nonionic nature of agarose and related matrices provide only very low energy interactions with proteins. Basically, such interactions prove to be weak and highly reversible, and therefore protein mixtures pass through such columns with little or no retention. By derivatizing agarose supports with low-density ionic, hydrophobic, or charge-transfer groups, one now imparts an interaction mechanism. The protein, depending on its structure and repertoire of surface functional groups, interacts with

TABLE 1. Properties of the "Big 12"—Plasma Proteins Whose Concentration is >1 mg/ml^a

Protein	Plasma Concentration		Molecular Weight	Diffusion Coefficient (10 ⁻⁷ cm ² /s)	Electrophoretic Mobility and Net Charge	CD ^{1/2}	Carbohydrate (%)	Sialic Acid (%)
	g/l = mg/ml	μmol						
Albumin	40	600	66,000	6.1	α ₁ , -19	1500	0	0
IgG	8-17	53-113	150,000	4.0	α ₂ -γ ₃	200	2.9	0.3
LDL	4.0	100*						1.6 M/M
HDL	3	2	2,000,000	2.0	β	3	—	—
α-Macroglobulin	3	18	170,000	4.6	α ₁	39	—	—
	2.7	3.3	725,000	2.4	α ₂	5	8.4	1.8
Fibrinogen	2-3	6-9	340,000	2	β ₂ , -26	11	2.5	48 M/M
Transferrin	2.3	7.5*						0.6
		30	77,000	5.0	β ₁	67	5.9	4-8 M/M
α-Antitrypsin	2	40	(51,000)				(2 chains) biantennary	1.4
			54,000	5.2	α ₁	91	14	variable 4 M/M
Haptoglobins			(45,000)				content variable, biantennary	3.6
	2.0	20	100,000	1-1				6 M/M
	1.6-3.0	8-15	200,000	4.7 (monomer)	α ₂	43	20	(variable) 5.3
	1.2-2.6	3-6.6	400,000	1-2 (2-2)			(in β-chain) bi- & triantennary	
C3	1.6	9	180,000	4.5	β ₂	19	2.7	0.01
IgA	1-4	7-27	150,000	4	α ₂ -γ ₃	30	7.5	1.8
		15*						9 M/M
IgM	0.05-2	0.06-2	900,000	2.6	β ₂ -γ ₃	1.6	11.8	1.3
		1*						42 M/M

^aData are from refs. 43 and 44. Numbers used for calculation of CD^{1/2} are indicated by asterisks.

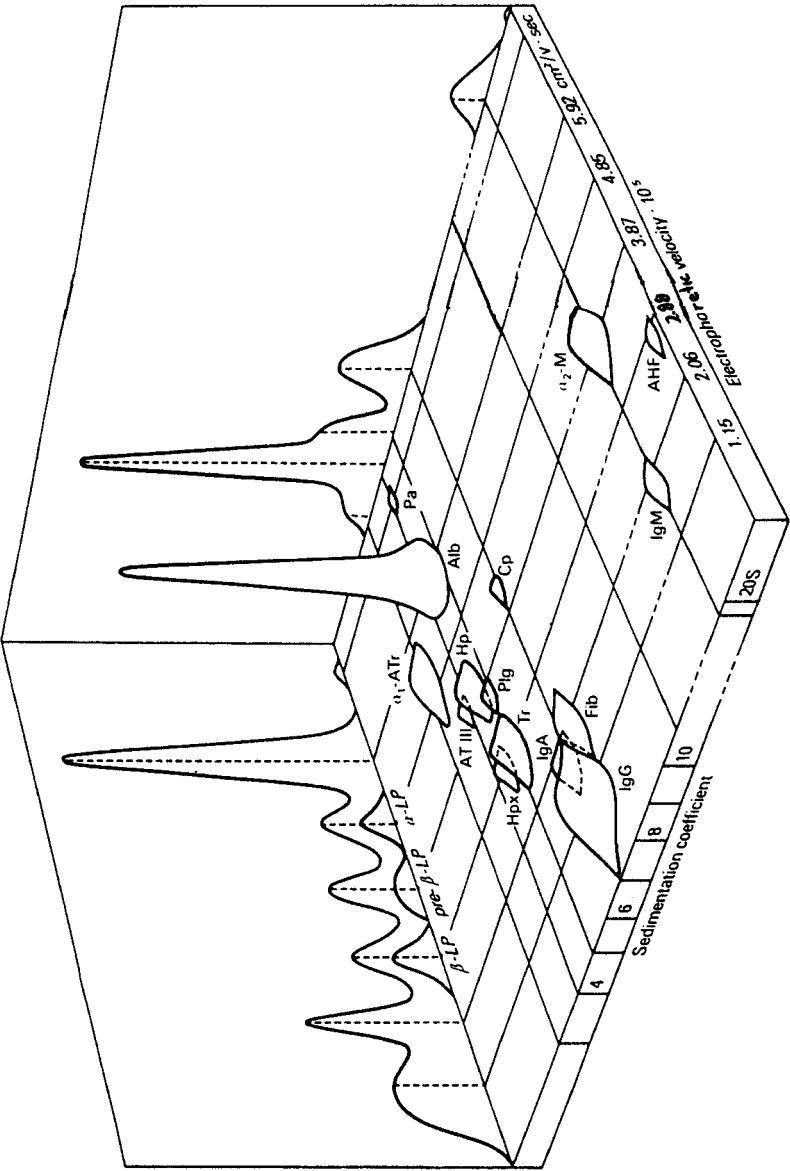


FIGURE 4. The major plasma protein constituents (based on ref. 27).

TABLE 2. Plasma Concentrations (*C*) and Diffusion Coefficients (*D*) for the Big 12 in Plasma (Data from TABLE 1)^a

Protein	<i>C</i> (μmol/l)	<i>D</i> (10 ⁻⁷ cm ² /s)	<i>C</i> √ <i>D</i>
Albumin	~600	6.1	1500
IgG	~100	4.0	200
α-Antitrypsin	~40	5.2	91
Transferrin	~30	5.0	67
Haptoglobin 1-1	~20	4.7	43
HDL	~18	~4.6	39
IgA	~15	4.0	30
Complement 3	~9	4.5	19
Fibrinogen	~7.5	2.0	11
α-Macroglobulin	~3.3	2.4	5
LDL	~2	~2.0	3
IgM	~1	~2.6	1.6

^aRate of arrival of protein molecules at the interface $dn/dt = C(D/\pi t)^{1/2}$.

the immobilized ligand on the agarose particle and is "retained." The adsorption is reversible if the interaction energy is not too high and if the number of interaction points per protein molecule is not greater than one. After the appropriate residence time the protein molecule is desorbed and goes on down the column, where it interacts with another ligand and another particle, stays for a moment, is released, and goes on. This process continues, and for a column of particular length, packing or support, and protein concentration and mixture, leads to a residence time or retention time in the column. This retention time is an empirical measure of the interaction energy or the affinity constant and the residence time of each adsorption event.

It is relatively straightforward to rank proteins on the basis of their interaction with hydrophobically derivatized agarose, so-called hydrophobic chromatography supports. This was done a decade ago by Hofstee.²⁸ One can do the same with cationic and anionic derivatized columns to get a measure of electrostatic interaction. As the hydrophobic and electrostatic interactions are probably the two dominant ones, because most of the hydrogen bonding possibilities are swamped by the 55-M concentration of water in aqueous solutions and even in blood plasma, one can characterize the intermolecular interaction potential for a protein interacting with chromatographic supports.

One must also apply what is known about the structure of the protein. If its three-dimensional structure is known, as discussed earlier for lysozyme, insulin, and myoglobin, then one should do graphics modeling and attempt to predict the optimum orientation of the protein for interaction with certain model surfaces. Structural information is available, even for the big plasma proteins, particularly fibrinogen,⁴ where the charge distribution and the different domains and fragments are well-known, the four carbohydrate residues are precisely located, and where one can begin to guess as to what might be the orientation of the molecule on certain types of surfaces.

One possibly important feature of plasma proteins, which has been largely ignored in consideration of their interfacial properties, is the fact that virtually all of them are glycoproteins and contain carbohydrate chains, generally terminated with negatively charged sialic acid residues. Although Kim and coworkers²⁹ studied the adsorption of desialylated immunoglobulins and fibrinogen many years ago, there has been little consideration of the role of carbohydrates. This is probably because the major

carbohydrate-containing proteins in plasma, which make up half or so of the Big Twelve (TABLE 1), have been largely ignored by the blood compatibility and surface chemistry community.

TABLE 3 briefly summarizes the glycoprotein and sialic acid composition of plasma proteins. The haptoglobins, for which very little adsorption data is available,³² contain 20% carbohydrate. Unfortunately, the structure of the haptoglobins is not well-known and indeed they constitute a family of proteins rather than being a distinct, homogenous molecule, such as albumin. Two important protease inactivators, α -antitrypsin and α -macroglobulin, also have high glycoprotein contents. Transferrin, an iron-binding protein, is also fairly rich in carbohydrate. The adsorption characteristics of α -macroglobulin and transferrin have been studied by Young and Cooper.³³ There has been virtually no work on haptoglobin adsorption³² or on antitrypsin, IgM, or complement C3. The extensive study by Young did show that the adsorption of macroglobulin and transferrin is apparently not of very high affinity. Although there could be many reasons for this, it does lead one to suggest that perhaps proteins with high carbohydrate contents, particularly if that carbohydrate is more or less uniformly distributed on the surface of the molecule, might enhance the solubility of the molecule and mask hydrophobic amino acid residues on its surface, thereby decreasing its adsorption at hydrophobic interfaces. Kim's study showed that desialylated fibrinogen did not influence fibrinogen adsorption,²⁹ probably because the carbohydrate in fibrinogen is located in the coiled-coiled region near the disulfide knot and on the β -chain in the terminal domains. There is apparently no carbohydrate on the γ -chain or the more extended, and probably more surface-active, α -chain. Also, the total carbohydrate in fibrinogen is relatively low.⁴

The role of carbohydrate in adsorption, which we call the "glycoprotein hypothesis," is probably only important for haptoglobin and antitrypsin. The carbohydrate in IgM is localized and not uniformly distributed around the molecule. Macroglobulin has such a unique dimeric structure that its adsorption will probably prove to be dependent on its unique structure and/or shape in solution.

FIGURE 5 illustrates the structure and composition of the plasma lipoproteins. We have included HDL, particularly HDL 3, and LDL 1 and 2 in the Big Twelve previously discussed (TABLE 1). There is evidence from at least two groups that the lipoproteins have unique and important adsorption properties.³⁵⁻³⁷ Breemhaar *et al.* have shown preferential adsorption of high-density lipoprotein onto hydrophobic supports, including a demonstration of the Vroman effect with LDL, HDL, and fibrinogen mixtures.³⁵ We have shown that LDL adsorption from single component solutions increases significantly when going from 20° to 37°C on elastomers, but not on

TABLE 3. Carbohydrate and Sialic Acid Composition of Major Plasma Proteins (from Refs. 30, 31)

Protein	Percent CHO	Percent Sialic Acid
Haptoglobin	~20	5.3
α -Antitrypsin	~14	3.6
IgM	~12	1.3
α -Macroglobulin	~8.4	1.8
Transferrin	~5.9	1.4
IgG	~2.9	0.3
C3	~2.7	0.01
Fibrinogen	~2.5	0.6
Albumin	~0	0

rigid supports.³⁶ This leads to the hypothesis that the lipid phase transition in LDL, which occurs in the vicinity of 37°C, may be important in controlling the adsorption properties and perhaps the surface denaturability of such proteins. We have also shown that the adsorption of LDL and HDL from single component solutions onto rigid hydrophobic and hydrophilic supports suggests a reaction-limited process for both classes of lipoproteins adsorbed on hydrophobic supports, but adsorption on hydrophilic surfaces is diffusion limited.³⁷ One could draw the hypothesis that the lipoproteins may therefore be undergoing significant conformational alteration on the hydrophobic supports. Although there is not very much data on lipoproteins, their dynamic structure and their very high lipid content suggests a major role in the plasma adsorption process.

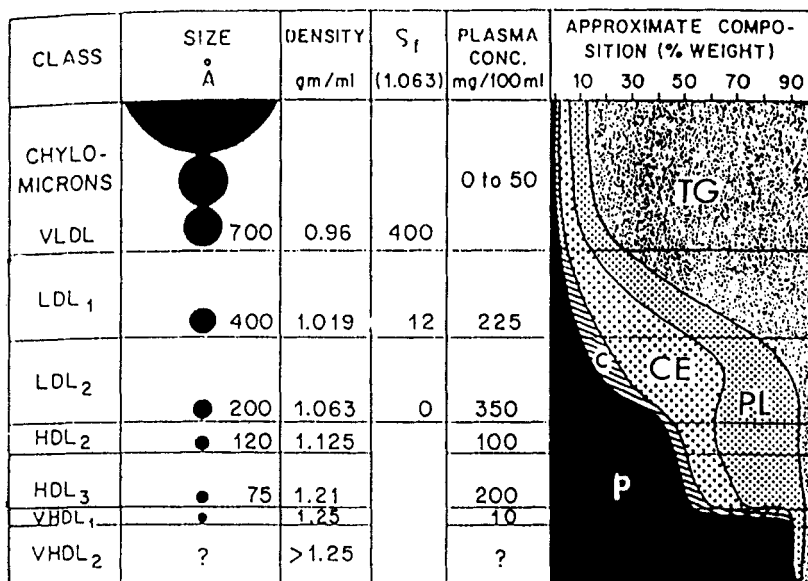


FIGURE 5. Schematic distribution of the major classes of plasma lipoproteins according to their flotation in the analytical ultracentrifuge. *TG* = triglycerides; *PL* = phospholipids; *CE* = cholesterol esters; *C* = cholesterol; *P* = apoprotein (from ref. 34).

SURFACE DENATURABILITY

In addition to the on- and off-rate constants, which can be derived as described above, one should have a measure of the ability of the protein molecule to denature at the interface as a function of time. Such information is very difficult to get at solid-liquid interfaces, although, in principle, some of the surface-sensitive spectroscopic techniques can provide some such information. For example, ATR-FTIR studies of the adsorption of a single component protein from dilute solutions can provide evidence of conformational change as a function of time at the interface.³⁸ Total internal reflection fluorescence also provides evidence for changes with time at the interface,³⁹ but such techniques are specialized, difficult to quantitate, and difficult to interpret in terms of actual structural changes. Also, they are substrate limited and

even substrate specific due to the optical properties required for the total internal reflection condition. Some such information is also available via ellipsometry in terms of changes in the refractive index and thickness of the adsorbed layer as a function of contact time (see refs. 17 and 21).

We feel that a more fruitful approach may be to study the behavior of proteins at water-air, water-oil, water-fluorocarbon liquid, and water-siloxane liquid interfaces, using the standard, proven, and inexpensive surface and interfacial tension techniques.^{6-8,20} With such techniques, one can measure the spreading pressure through the decrease in surface or interfacial tension of various individual proteins. One can also measure the temperature dependence of interfacial processes. Although this does not relate directly to the interface between a biomedical material and plasma, it helps characterize the interfacial activity of the various protein species of interest. One can develop an empirical parameter and use it as a coefficient or exponent in the appropriate terms in the equations. The problem is to get such data (chromatographic and interfacial activity data) for the proteins of interest. Although some such data is available in the literature, it is generally very protein specific, and there is no compilation of information on the interfacial activity of plasma proteins. If such data were available, the modeling and simulation of the Vroman effect and other complex interfacial processes could actually be straightforward.

CONCLUSIONS

We offer the following conclusions:

1. FIGURE 6 suggests that the early stages of adsorption (very short contact times) are functions of the particular chemistry of the surface, the particular three-dimensional structure and orientation of the protein, and the number of species and their concentration. In addition, adsorption at short contact times is also a function of occupancy and lateral interactions, as modeled so nicely by Cuypers.¹⁷ We suggest that the time-dependent conformational adaptation to the surface, as modeled by Lundstrom,²¹⁻²³ is related to the bulk solution denaturation tendencies of the protein, including thermal denaturation, denaturation in urea or guanidinium chloride solutions, and denaturation in solutions of different pH. We further suggest that surface and interfacial tension measurements of proteins at water-air, water-oil, and other water-liquid interfaces will be useful measures of protein surface activity at the solid-water interface.
2. We suggest that lipoproteins are very important in the adsorption process and in the Vroman effect, due in part to their structural and compositional dynamics, particularly on hydrophobic elastomeric surfaces, where one might expect some interpenetration of protein and lipid components with the polymer chains.³⁶
3. It is known that the presence of carbohydrates in proteins both masks and protects the proteins from proteolytic attack and helps improve their solubility, as well as providing stabilization of conformation.^{30,31} It is therefore reasonable to suggest that proteins with high carbohydrate contents, particularly if the carbohydrate is more or less uniformly distributed over the protein, may show little adsorption or interaction with hydrophobic or with negatively charged supports.
4. At equilibrium adsorption will be dominated by proteins with the following characteristics: large molecular weight and large size, which suggests a large number of potential contact points with the surface; readily denaturable or

conformationally labile, permitting the protein to readily accommodate to the microenvironment of the interface; and low carbohydrate content, or at least significant regions of the molecule which are free of carbohydrate, because of the possible masking effect of carbohydrate at hydrophobic and/or negatively charged surfaces.

Although it is clear that fibrinogen is not necessarily the equilibrium species in plasma, it has the attributes described above. It is low in carbohydrate and what carbohydrate it does have is localized. It is conformationally quite labile and indeed has very high interfacial activity and spreading pressure. It is large and asymmetric and can have very large contact areas. It has a fairly asymmetric charge distribution,

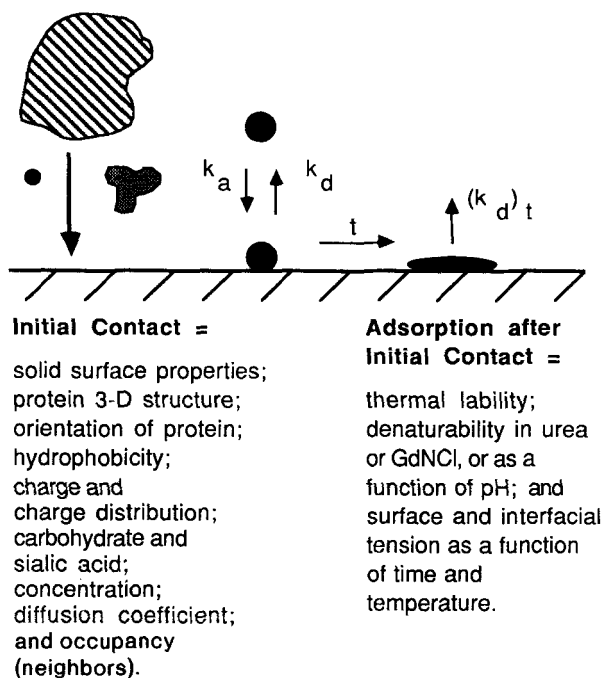


FIGURE 6. The key parameters involved in the initial contact phase of adsorption (left side) and in the time-dependent surface and protein "denaturation" processes (right side).

as well as significant hydrophobicity, so it can interact successfully with a wide variety of interfaces.

CAUTION

We have assumed throughout the discussion that there are no covalent changes imposed on the molecules prior to, during, or after the adsorption process. Clearly, the work of Brash and others demonstrates that covalent bond changes can indeed occur.¹⁶ Certainly plasma has a variety of active proteases and protease inhibitors, which

change in concentration depending on local needs and processes. Clearly the conformational adaptation to the surface which we have described may make a molecule more or less susceptible to proteolysis or to other chemical processes. Indeed, the very act of interacting with certain types of surfaces could, in part, direct covalent chemistries, such as possibly the interaction of C3 with nucleophilic surfaces.⁴⁰ The surface activation models, which are being developed by Sefton,⁴¹ Mann,⁴² and coworkers, coupled with the modeling and simulation suggested here, will be an important next step in attempting to treat truly practical blood-material interfaces.

SUMMARY

We have discussed the general principles of protein adsorption at solid-liquid interfaces from single component and multicomponent solutions, based on qualitative kinetic models that include mass transport considerations, initial interaction energies, surface-dependent conformational changes, and possible desorption processes. We have surveyed plasma protein components greater than one milligram per milliliter in concentration, which we call "The Big Twelve." We considered their size, concentration, diffusion coefficient, structure and function, and methods of estimating their "surface denaturability" by using bulk solution measures of denaturation and conformational change. We have suggested that the role of the carbohydrate moieties in plasma proteins may have some bearing on their adsorption properties. We further suggest that lipoproteins, because of their lipid phase transition and conformational lability at body temperature, may tend to dominate the adsorption process, particularly on mobile elastomeric polymer surfaces. We suggest that detailed consideration of the structure and characteristics of each of the proteins involved is necessary in order to begin to understand plasma adsorption processes. Detailed characterization and understanding of the solid surface in the aqueous and protein environments are also required.

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