CHAPTER II.1.2

Adsorbed Proteins on Biomaterials

PROBLEMS

1. What are the three main driving forces that affect the competitive adsorption of proteins to biomaterials from complex mixtures such as blood plasma?

2. Calculate the amount of adsorbed protein in a close packed monolayer for an average protein of molecular weight 100,000. Assume the protein is spherical and has a partial specific volume of proteins being 0.73 ml/g.

3. When various biomaterials are implanted in the body, the responses often depend on the chemistry of the implanted material. Yet we know that the surface is covered with adsorbed protein, and thus the biological response is not due to direct interaction with the surface. How then are the differences in responses explained?

4. What are three methods that show the specific effect of adsorbed proteins on cellular interactions with biomaterials?

5. As illustrated in Figure II.1.2.5, polystyrene and other hydrophobic surfaces become more wettable after exposure to protein-containing media. However, for certain materials, such as cleaned Germanium oxide prisms that initially have low contact angles with water (11°) and are thus fairly wettable to begin with, exposure to protein-containing solutions has been reported to cause the contact angle to increase (to around 60°). How can this be explained?

6. Proteins are tightly bound to many biomaterials, and are considered irreversible in that the proteins are not washed off in the buffer used for adsorption, even after long soaking periods. Give a molecular explanation of why proteins are so tightly bound.

SOLUTIONS TO PROBLEMS

1. The three main factors that are believed to control the outcome of competitive adsorption processes are: (1) the intrinsic affinity of each protein for surfaces, meaning that some proteins are more surface active and more adsorptive than others; (2) the chemical nature of the adsorbing surface, due to the fact that the variations in surface affinity among proteins one sees for a given surface are only fixed for that surface, and will differ if another surface is used; (3) the relative bulk concentration of each protein, meaning that even a protein with high affinity for a surface will not be present in high amounts on the surface if it is present in low bulk concentration compared to the other proteins, since the excess concentration of the competing proteins will overcome the affinity differences.

2. Close packing means that the molecules touch each other at their boundaries, so the problem can be solved by calculating the cross-sectional area of one molecule of the protein and dividing that into square centimeter of area, which then gives the number of molecules in that area. Using the molecular weight of the protein allows conversion of number of molecules into mass of molecule, and thus you end up getting mass per unit area of a close packed monolayer.

Protein Monolayer Calculation

The specific monolayer (volume per gram) of a protein can be used to calculate the volume of an individual protein molecule by using the molecular weight and Avogadro’s number:

\[ V_{\text{molecule}} = V_{\text{specific}} \times M / N_{\text{Avogadro}} \]

\[ = (0.73 \text{ ml/g}) \times (10^5 \text{ g/mole}) / 6.02 \times 10^{23} \]

\[ = 1.22 \times 10^{-19} \text{ ml/molecule} \]

The volume of an individual protein molecule can be used to calculate the radius of the molecule \( r \), assuming it is spherical:

\[ r = \left( \frac{3}{4\pi V_{\text{molecule}}} \right)^{1/3} \]

\[ = 3.08 \times 10^{-7} \text{ cm} \]

(This radius estimate can be compared to dimensions of proteins measured by X-ray crystallography. For proteins in the range of 100,000 molecular weight, average long dimensions are around 70 Å, giving a radius of around 35 Å, fairly close to what we just calculated using the specific volume of proteins.)

The radius of the protein molecule can then be used to calculate the projected area of an individual protein molecule \( A_{\text{molecule}} \), which is the “footprint” or area occupied on the surface by the protein molecules:

\[ A_{\text{molecule}} = \pi r^2 \]

\[ = 2.97 \times 10^{-13} \text{ cm}^2 \]

With the footprint area, you then can calculate the number of protein molecules that can fit into a
square centimeter $n_{\text{protein}}$, assuming close packing of circles representing the footprint area occupied by each spherical protein molecule. (This is a small overestimate of the occupyable area, because the tightest packing of circles on a planar surface leaves about 9% area unoccupied, according to a theory by Gauss.)

\[
    n_{\text{protein}} = \frac{1 \text{ cm}^2}{A_{\text{molecule}}} = 3.36 \times 10^{12} \text{ molecules}
\]

Then convert the number of molecules per unit area $n_{\text{protein}}$ into mass of protein per unit area $m_{\text{protein}}$ using Avogadro’s number and the molecular weight of the protein:

\[
    m_{\text{protein}} = n_{\text{protein}} \times \frac{M}{N_{\text{Avogadro}}} = 5.6 \times 10^{-7} \text{ g} = 0.56 \text{ microgram}
\]

Thus, according to this calculation, there should be around 0.56 micrograms per square centimeter in a close packed monolayer of a spherical protein of molecular weight 100,000.

3. Due to differences in relative competitive affinity of proteins for various surfaces, the amount of adsorption of each protein varies with surface chemistry. For example, some surfaces have more adsorbed fibrinogen and others more adsorbed albumin, and since platelets bind only to fibrinogen, surfaces enriched in fibrinogen are more platelet reactive. Thus, the effect of surface chemistry is to vary the composition of the adsorbed protein, and this is why the reactivity of cells varies with surface chemistry.

4. Three methods to show the role of adsorbed proteins are as follows.
   a. If surfaces are preadsorbed with various purified proteins, it is found that most inhibit cell adhesion to the surface, but a few such as fibronectin, fibrinogen, and vitronectin cause the cell adhesion to be much higher than for other proteins. Proteins that block adhesion are called passivating or blocking proteins, while those that mediate it are called adhesion proteins.
   b. Preadsorption with complex protein mixtures, such as blood plasma selectively deficient in only one protein, is a more physiologically relevant way to show if a protein is contributing an important role to platelet adhesion in the presence of many other potential adhesion proteins in plasma. If cell adhesion is greatly reduced when the surface is adsorbed with the protein mixture that is missing one protein such as fibronectin, it means this protein is playing an important role in that it is not replaced by the action of other proteins still in the mixture and on the surface.
   c. Addition of antibodies specific to a given adhesion protein or to its receptor to a cell suspension incubating with a biomaterial will result in a decrease in adhesion that is proportional to the concentration of added antibody. Because such antibody blocking studies can be done with surfaces adsorbed with mixtures of proteins such as plasma, yet they selectively interfere in adhesion mediated by only one adhesion protein, they provide information relevant to the functional role of the adhesion protein under conditions closer to the physiologic situation, where biomaterials are exposed to complex mixtures of proteins.

5. An increase in contact angle after exposure to protein-containing media can be explained by adsorption of protein if the adsorbed protein layer is less wettable than the starting surface. For certain solid materials, such as cleaned Germanium oxide prisms, the interaction is very strong with water, and so the contact angle is low. After protein adsorption occurs, the higher contact angle must mean that adsorbed proteins interact with water less strongly than the starting Germanium oxide surface. Thus, while proteins are much more wettable than polystyrene, they evidently are not as wettable as some solid surfaces.

6. The irreversible, tight binding of proteins to surfaces is due to the large size of protein molecules, so that many noncovalent bonds are formed between the surface and each molecule, i.e., “multipoint” attachment occurs. Although each bond is relatively weak and reversible, the chance that all the bonds would be broken simultaneously is low, so multivalent bonding results in strong bonding.

Note: Latour (2008) adds another way to look at this issue. He notes that because of the large number of contacts between functional groups of a protein and a surface, even weak interactions will hold the protein. For example, if the interactions with the surface are equal in energy to their interactions with water in the bulk phase, and we assign the probability of a surface functional group being bonded to a protein versus a water molecule to be 0.5, the probability for all of the functional groups of an adsorbed protein to dissociate from the protein at the same time would be $P = (0.5)^n$, with $n$ being the number of functional group contacts between the protein and surface. So even if $n$ is as small as 20, the probability that they would all dissociate at the same time is very small. Thus, even a hydrophilic surface with OH groups (e.g., OH-SAM) will tend to irreversibly adsorb a large protein, while for hydrophobic surfaces that have favorable thermodynamics of adsorption for a nonpolar amino acid residue versus water, the likelihood of desorption is even lower.