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Simulation of Protein Adsorption. The Denaturation Correlation*

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We suggest means to model and simulate the adsorption of simple proteins at model interfaces. We suggest that molecular computer graphics is a very powerful method with which to study initial contact and interactions of proteins with model surfaces. We present and review kinetic models for protein adsorption and briefly discuss the role of surface-induced conformational change on such models. We suggest that data on the solution denaturation of proteins may be important in estimating protein lability and stability and, together with information on fhe surface tension and interfacial tension behavior of proteins, will help develop hypotheses and correlations with the actual solid/liquid interface behavior.

INTRODUCTION

The modelling and simulation of protein adsorption[1–4], including molecular computer graphics studies of the adsorption of hen and human lisozyme[5,6], have developed rapidly since the Conference presentation.

The simulation of protein adsorption by molecular graphics relies on the X-ray crystallographic coordinates of the protein, readily available in computer-readable format from the Protein Data Bank[7]. These coordinates can be displayed and imaged via a suitable computer and graphics system. Algorithms and software are readily available with which to display the molecule in either stick figure or space filling modes. In both modes, the various amino acids or amino acid sidechains can be color-coded to denote characteristics of interest. We have recently employed a color scheme based on Eisenberg's atomic solvation parameters[8] which was very helpful in visualizing how hen and human lysozyme might interact with a series of model surfaces[6]. This is a very powerful approach and is being extended to the study of other model proteins.

The major limitation of this approach, however, is that, for the time being, the protein has to be treated as a relatively rigid object, interacting with a rigid model surface. The question of conformational adaptation or

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denaturation at the interface has not yet been addressed by such computer modelling methods.

The main advantage and application of the computer structural simulation approach is to deduce the nature of the initial contact and interaction between the protein and the surface. In the case of a relatively rigid protein, such as hen lysozyme, conformational changes upon adsorption may be minimal, and this is probably why the computer predictions and the adsorption data obtained at model interfaces are in reasonable agreement[6].

Most of what we know about protein adsorption is summarized in Figure 1., 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 interaction required for initial stabilization of the protein/surface complex[9,10]. The protein can desorb at this stage; an appropriate desorption rate constant is indicated in Figure 1.

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[11], and an energy of 5—15 kcal/mol is sufficient to denature them in normal buffer solutions[10–12]. Therefore, interactions with the surface, particularly interactions of a hydrophobic nature, can significantly affect the

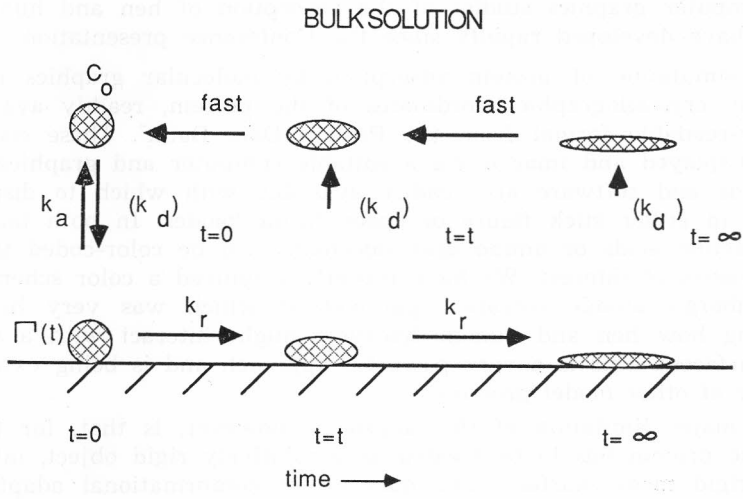

Figure 1. A kinetic protein adsorption model based in part on the ideas of Lundstrom, Walton, and Jennissen[1,14,19,21]. C_o is the bulk protein concentration; Γ is the surface concentration, which is a function of time, t. k_a is the on-rate constant and $k_d(t)$ are the off-rate constants, which are a function of contact time (residence time) (from Ref. 4).

solution equilibrium of the protein[10]. As the interfacial interactions may be very different from the interaction in solution, the solution lability may not be important in many adsorption situations.

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 be provided, in part, by interactions with a partially hydrophobic surface. Dill has modelled and considered the configurational entropy aspects of globular protein structure[11], as well as of hydrophobic surfaces containing alkyl chains, such as commonly used in chromatography[13].

With increasing contact times, the probability for desorption decreases, as indicated in Figure 1. Assuming that the adsorption process does not result in any covalent bond changes in the protein molecule[14], then the model assumes that if a denatured or partially denatured protein does desorb, it rapidly renatures to the equilibrium globular state.

Competitive adsorption in two component systems has been modelled by Cuypers[2]. His results suggest that the »Vroman effect«[16,17] 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[18].

The effect of a surface-induced conformational change has been treated in a preliminary way by the models developed by Lundstom et al.[1,19], 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 model the adsorption behavior of labile globular proteins in single component systems.

Figure 2. begins to ask the question of what happens during a competitive adsorption process and is based on the ideas of Bagnall[20] and Jennissen[21], 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[18]. One would expect that the spreading characteristics would be related to the solution denaturability[12] of the protein, and particularly to its behavior at water/air and water/oil interfaces[9,20,22,23]. 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[16] can be explained. Clearly, the next step is to develop a model which incorporates the ideas of Cuypers[2], Lundstrom[1,19], and Bagnall[20], but generalized for complex multi-component systems.

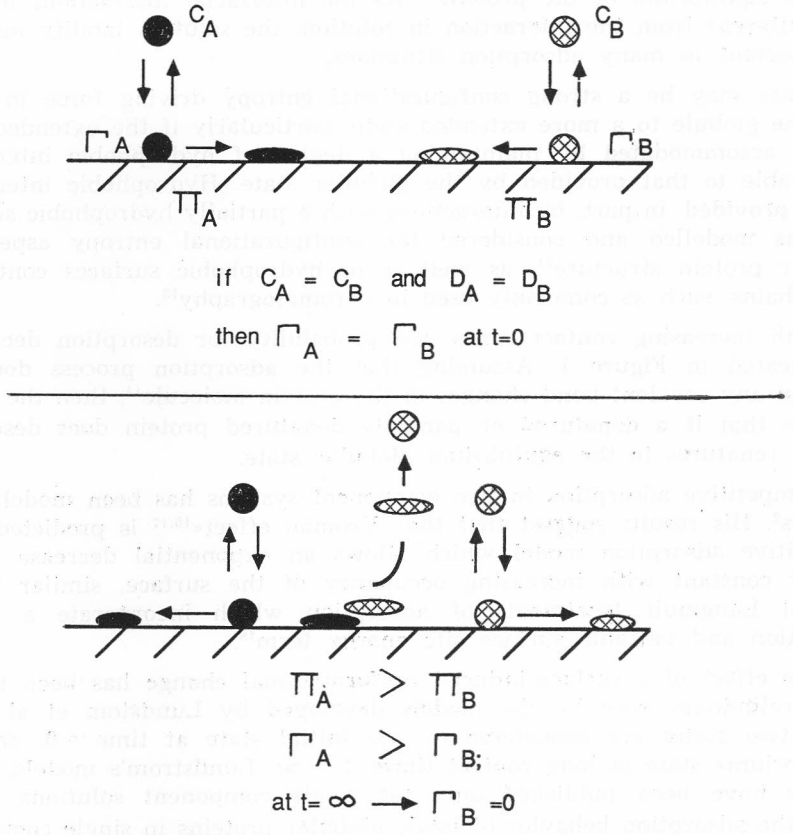

Figure 2. The competitive surface spreading hypothesis of Bagnall[20]. Top: Two proteins, A and B, have the same bulk concentration and diffusion coefficients and, therefore, have the same surface concentration (Γ) at $t = 0$ (initial contact). If the spreading pressure (π) of one is greater than the other, then eventually the protein with the large π will dominate the interface. Here we show, since $\pi_A > \pi_B$ that A displaces B (from Ref. 4).

SURFACE DENATURABILITY

In addition to the on- and off-rate constants described in Figure 1., 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 obtain 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[24]. Total internal reflection fluorescence (TIRF) also provides evidence for changes with time at the interface[25,26], 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 References 2, 19).

Another approach is the use of monoclonal antibodies to probe the conformation of adsorbed proteins. Such studies do indeed suggest that epitopes, which are normally masked in solution, can be made accessable upon adsorption at certain interfaces[28,29]. A recent review of conformational changes upon adsorption is available[30]. A number of recent papers in the chromatography literature have appeared which clearly document surface induced conformational changes or denaturation upon adsorption to chromatographic supports[31-34].

As suggested earlier, the intrinsic solution stability of a protein is probably very important in understanding its adsorption behavior. There is a wealth of information in the solution biochemistry literature on the denaturation of proteins. The intensive activity on protein folding and on protein unfolding and refolding has provided an enormous data base which may be helpful to the protein adsorption community[35-39]. Proteins can be denatured as a result of major changes in a number of solution parameters, including temperature, pH, urea concentration, quanidinium chloride concentration, and low molecular weight surfactants. In the case of thermal denaturation, detailed thermodynamic analysis and modelling of the data is available[37].

Through modern specialized instrumentation, it is now possible to follow the denaturation process in real time by monitoring a number of conformationally sensitive parameters simultaneously[39]. It is clear from a brief perusal of this literature that some proteins are very robust and require major changes in solution conditions before they denature. Other proteins are quite labile and denature readily. Although the process of solution denaturation and denaturation at a solid/liquid interface is very different, one would expect some correlation between the surface denaturability of a protein and its intrinsic stability in aqueous solutions, as suggested in Figure 3.

We feel that another useful approach is to study the behavior of proteins at water/air, water/oil, water/fluorocarbon liquid, and water/siloxane liquid interfaces, using standard, proven, and inexpensive surface and interfacial tension techniques[18,20,22,23]. 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 doesn't relate directly to the interface between 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 limited, and there is no compilation of information on the interfacial activity of plasma proteins. If such data were available, the modelling and simulation of protein interfacial processes might actually be straightforward.

Our previous argument that interfacial denaturation should be related to solution denaturation is suggested from results available in the protein surface tension and protein monolayer literature. Lysozyme has been particu-

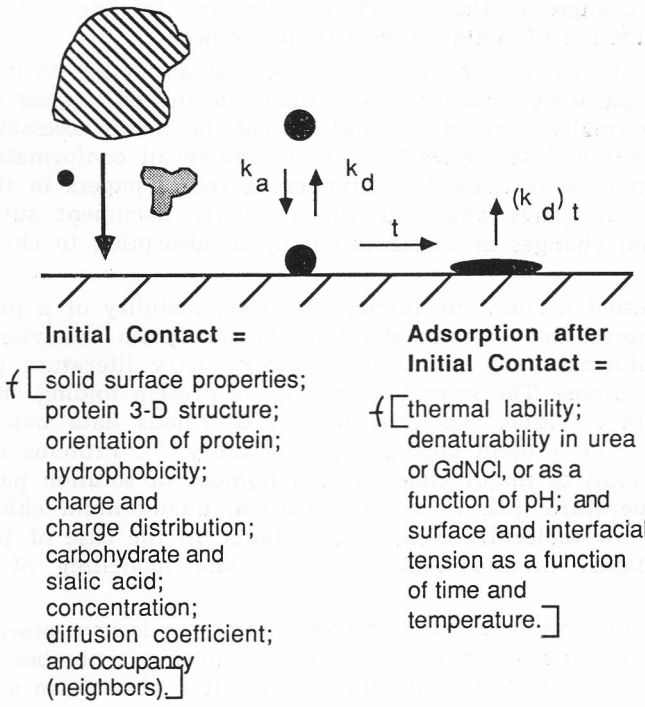

Figure 3. Suggested parameters involved in the initial contact phase of adsorption (left side) and in the time-dependent surface and protein »denaturation« processes (right side) (from Ref. 4).

larly well-studied. Early work has indicated that lysozyme is difficult to spread and unfolds very slowly at interfaces, perhaps due to its rigid structure[40,41]. The air/water interface techniques allow one to measure total adsorption and surface tension independently. The amount adsorbed can reach a steady state value while the surface tension or the spreading pressure is continuing to change significantly, demonstrating the slow rate determining nature of the conformational changes occurring at the interface[41]. One can measure surface and interfacial tension as a function of temperature and in different solution environments, such as various pH, urea, and guanidinium chloride conditions. In this way one can begin to correlate the lability or denaturability of a molecule in solution with its air/water interface or water/oil interface behavior[20,23,42]. The correlation is not necessarily obvious or straightforward, however, as Arnebrant et al.[43] in a preliminary study found a correlation between the adsorption of casein on a hydrophobic chrome surface and its surface tension reduction at the air/water interface, but the maxima in each case were not directly correlated.

Deyme et al.[44,45] have used the method to study collagen adsorption in competition with albumin and fibrinogen, measuring both adsorption and surface and interfacial tension changes separately and dynamically. The methodology, therefore, has great potential for studying competitive adsorption processes.

We cannot expect surface tension studies to mimic solid/liquid interface studies. Generally the solids of interest are rigid and immobile, whereas the air/water and water/oil interfaces are highly mobile and dynamic. Nevertheless, surface and interfacial tension studies are straightforward, easy to perform, relatively easy to interpret, and should be helpful in helping us understand and predict the general interfacial behavior of proteins.

MODEL SYSTEMS

We are now expanding our efforts with hen and human lysozyme as model proteins[5,6] to a larger group of proteins whose crystallographic structures are known and which therefore can be studied by molecular computer graphics techniques. In addition, this set of proteins will be studied for their solution denaturation characteristics, as well as for their surface tension behavior at various air/solution interfaces. Eventually the same set will be studied for their solid/liquid adsorption properties, using a series of model solid supports based primarily on commercially available hydrophobic, ion exchange, and change-transfer chromatographic matrices. Given such data and the models and concepts presented in Figures 1—3., together with the theoretical models and treatments previously cited, we are confident that the general predictive understanding of the adsorption of small, simple, globular, single-domain proteins[47] will be within reach. Indeed, Keshavarz and Nakai have already shown a good correlation between interfacial tension at the oil/protein solution interface and hydrophobicity as measured by retention on hydrophobic chromatography supports[46].

CONCLUSIONS

Figure 3. 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. We suggest that the time dependent conformational adaptation to the surface is related to the bulk solution denaturation tendencies of the protein, including thermal denaturation, denaturation in urea and 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.

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 control direct covalent

chemistries, such as possibly the interaction of Complement-C3 with nucleophilic surfaces[48]. The surface activation models, which are being developed by Sefton[49], Mann[50], and coworkers, coupled with the modelling and simulation suggested here, will be an important next step in attempting to treat truly practical protein-material interfaces.

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SAŽETAK

Simulacija adsorpcije proteina. Korelacija s denaturacijom

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Predložen je način modeliranja i simuliranja procesa adsorpcije jednostavnih proteina na modelnim površinama. Vrlo je korisno pritom istraživati početni kontakt i međusobno djelovanje proteina i modelne površine s pomoću molekulske računalske grafike. Dan je pregled kinetičkih modela adsorpcije proteina i ukratko je razmotrena uloga konformacijskih promjena proteina uzrokovanih prisutnošću površine. Ističe se da podaci o denaturaciji proteina u otopinama mogu biti izuzetno važni pri ocjenjivanju stabilnosti i labilnosti proteina na površinama. Ti parametri, kombinirani s površinskom napetosti i utjecajem proteina na međupovršinsku napetost, pomažu pri razvijanju hipoteza o adsorpciji proteina i koreliranju tih hipoteza sa stvarnim ponašanjem proteina na međupovršinama krute i tekuće faze.