Pure & Appl. Chem., Vol. 64, No. 11, pp. 1777–1781, 1992.
Printed in Great Britain.
© 1992 IUPAC

Adsorption of complex proteins at interfaces

J.D. Andrade, V. Hlady, A.P. Wei

Department of Bioengineering, 2480 MEB, University of Utah, Salt Lake City, Utah 84112, USA

Abstract - We have previously shown that the adsorption of model proteins at model interfaces can be quantitatively understood via a careful consideration of the proteins' three dimensional structure and its stability. Using computer molecular graphics, dynamic surface tension, fluorescence probes and labels, and solution denaturation data, we can relate the chemical and structural properties of proteins to their interface behavior. We have developed a novel means to present these data and correlations in a simple radial plot (the "Tatra" plot). We are now extending these approaches to complex multi-domain proteins. Albumin consists of three large domains with differences in electrostatic nature, charge-pH characteristics, and denaturability. The interfacial activity of albumin is due, at least in part, to the interfacial activity of its constituent domains. Consideration of the structure and interfacial activity of the various domains permits new and more precise hypotheses to be developed, with which new and better experiments can be designed. Such hypotheses allow one to evaluate and compare adsorption data, including kinetics and isotherms, adsorbed layer thickness, refractive index, multilayer formation, etc. We feel strongly that each different protein is a unique molecular personality, which must be understood and considered if we are to more fully understand and apply the interfacial behavior of complex proteins. Expanded treatments of these topics are available in References 1-4.

PRINCIPLES OF PROTEIN ADSORPTION

The principles of protein adsorption have been presented in a number of monographs, review papers, and conference proceedings [1-8].

The arrival of protein at the interface is assumed to be driven solely by diffusion processes, which are dependent on bulk concentration and diffusion coefficient. That results in a collision frequency [9]. The particular surface chemistry of the protein and of the surface dictates the residence time due to the initial interaction energy. The surface dynamics, or denaturability, of the protein itself, together with the residence time, probably controls the surface denaturability of the protein. The protein tends to denature with time at the interface. With increasing residence time, denaturation reaches a maximum. With increasing denaturation, the interaction energy in the adsorbed state is increased, and the probability for desorption, or the rate of desorption, is decreased.

The reality of the process of course is that proteins are not homogeneous particles. Not all collisions are equally effective in adsorption, and different protein "surfaces," or faces, result in different interaction energies with the protein, and therefore different tendencies for surface denaturation. We attempt to illustrate this in Figure 1, in which the protein is shown as having four "faces:" a hydrophobic face, a positively charged face, a negatively charged face, and a neutral hydrophilic face. Although all collisions are equally probable, it is only those collisions which result in interaction energies in the range of kT which will provide the residence times necessary for subsequent interfacial processes to occur. Protein adsorption on neutral hydrophilic surfaces, for example, tends to be relatively weak, whereas adsorption of proteins on hydrophobic surfaces tends to be very strong and often partially irreversible. Adsorption on charged surfaces tends to be a strong function of the charge character of the protein, the pH of the medium, and the ionic strength [8].

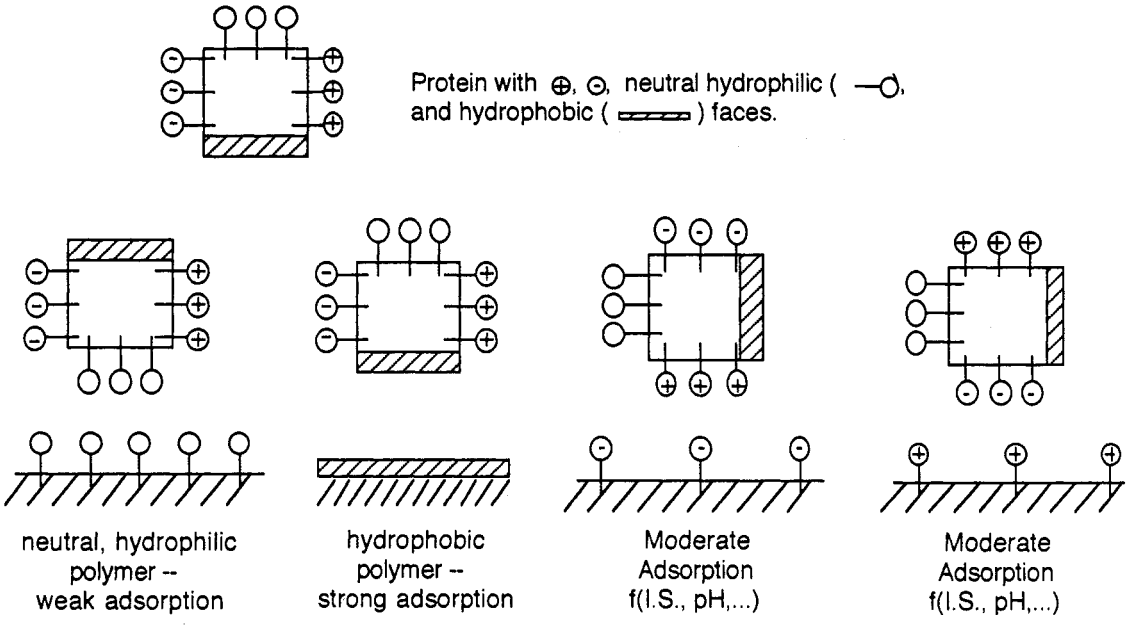

Figure 1: A schematic view of four-sided "protein," with one face being hydrophobic, one face being negatively charged, one face positively charged, and one face of neutral hydrophilic character, shown interacting with surfaces of comparable character. In the case of a neutral hydrophilic polymer surface, one would expect weak or little adsorption, whereas in the case of a hydrophobic polymer one would expect strong adsorption via the orientation shown. In the case of charged surfaces, one would expect moderate or variable adsorption, depending on the electrostatic nature of the interaction, a function of the ionic strength and pH of the solution, charge density, charge location, etc. (from Ref. 1).

In order to predict the initial contact event, or the orientation of adsorbed protein which would lead to the maximum interaction, we need to know something about the external surface chemistry of the proteins themselves. This is a simple problem for proteins whose three dimensional structures are well known, such as insulin, myoglobin, and lysozyme. In these cases the X-ray crystallographic coordinates of the protein are readily available, and can be displayed on a computer screen. One can very easily visualize the different faces or surfaces of the protein with respect to their hydrophobic, charge, and neutral hydrophilic character and readily formulate hypotheses as to their possible surface interaction [10,11].

We have studied a matrix of model proteins at air/water interfaces by dynamic surface tension techniques [13]. Our goal was to correlate the three-dimensional and surface structure of the protein in solution, its initial adsorption at air/water interfaces (determined by dynamic surface tension methods), its stability or denaturability in solution, and its tendency to denature upon long term contact at the air/water interface (again using dynamic surface tension). Denaturability was assessed by calorimetry and by urea and guanidinium chloride perturbation deduced by fluorescence changes. The surface chemical nature of the protein was assessed by examination of its external surface chemistry using molecular graphics and by the use of fluorescent probe titration. A relative, effective surface hydrophobicity (ESH) parameter was then deduced [11,12].

After consideration of a wide range of parameters, we selected twelve variables and began to qualitatively examine the correlations between them using radial axes with the axes arranged and scaled so as to emphasize and even exaggerate correlations among the various parameters. We call this multi-parameter radial plot a "Tatra Plot" [11].

Figure 2 presents the Tatra Plot for superoxide dismutase, a highly stable protein whose surface is extremely hydrophylic and thus exhibits little surface activity at the air/water interface. The upper left quadrant depicts protein surface hydrophobicity. The lower left quadrant depicts the stability of the protein. The upper right reflects surface activity. We are still experimenting with the variables, their placement, and their scaling, and the Tatra Plot is far from being completed or optimized at this time.

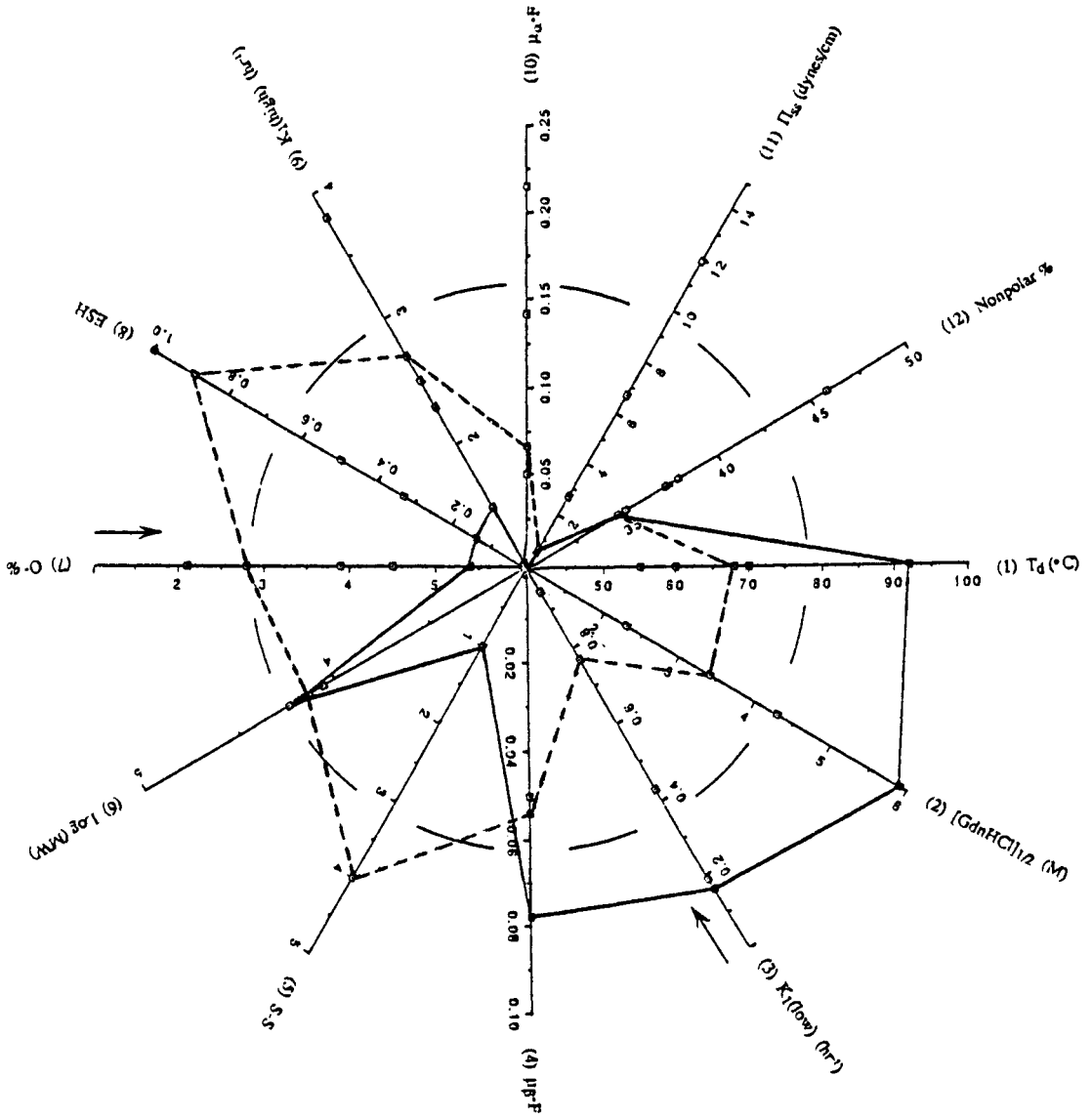

Figure 2: The Tatra Plot, Tatra parameters, and the data for superoxide dismutase and ribonuclease (dotted lines). Axes 1-5 are stability or stability related parameters. Axes 7-9 are parameters of effective surface hydrophobicity or related to it. Axes 10-12 are steady state surface activity. The directions for all axes, except for 3 and 7, are increasingly outward. It is clear that superoxide dismutase is a very stable, hydrophilic and relatively non-surface active protein (from Reference 11).

Figure 2 also gives comparable data for ribonuclease. It is less stable than superoxide dismutase, as indicated by the partial collapse of the points in the lower right quadrant; ribonuclease has slightly less surface hydrophobicity and less surface stability.

The proteins studied to date fall into three different categories in terms of their structure, function, and surface activity relationships [11].

Given the apparent success of the multi-variate Tatra Plot approach to correlating the behavior of model proteins at air/water interfaces, we are now extending the approach to the solid/liquid interface [1]. We have taken a limited set of parameters and used them to develop a preliminary Tatra Plot representation for model protein adsorption on polystyrene lattices, using the data of Norde and co-workers [8,13].

In the air/water interface case, hydrophobic interactions are believed to dominate the adsorption behavior and this is clearly reflected in the strong correlation with the hydrophobic surface character of the protein. In the case of charged surfaces electrostatics plays a major role, and this is strongly reflected in the shape of these preliminary Tatra Plots. Hydrophobic interactions also play a major role

We are still in the process of optimizing the Tatra Plot representation for solid/liquid interfaces [14].

COMPLEX PROTEINS

What do these concepts and results have to do with the more practical problem of the behavior of complex plasma and tear proteins at biomaterials surfaces? We feel strongly that the way to understand the behavior of a complex protein is to look at its various structural domain building blocks. In the last 10 years it has become evident in structural biochemistry that, although each protein is a unique and distinct molecular machine and molecular personality, proteins can be considered as constructed of a multiplicity of smaller domain subunits. For example, in the case of coagulation proteins, functional and structural domains include heparin binding domains, growth factor domains, kringle sequences, carboxy-glutamic acid-rich calcium binding domains, and others. Fibrinogen is an excellent example. High sensitivity calorimetry studies of fibrinogen and of its protease derived fragments suggest 12 domains in the fibrinogen molecule with denaturation temperatures of 45°, 55°, 90°, and 100° C [15]. We are only now beginning t o analyze fibrinogen in terms of its domain structure, with the hope of beginning to understand its behavior at solid/liquid interfaces. Fibronectin is another example. It has at least 20 calorimetrically identified domains [16], and it is likely that its complex adsorption behavior will be partially understood through a domain approach.

The optimistic view is perhaps best described by Chothia [17]: "The apparently complex structure of proteins is in fact governed by a set of relatively simple principles. Individual proteins arise from particular combinations of and variations on these principles. An analogous situation is found in linguistics, where a set of simple grammatical rules govern the generation of different , and sometimes complex, sentences." Others have suggested a protein structural linguistics [18].

We have attempted to apply some of these concepts to the analysis of the interfacial behavior of albumin [19]. Albumin is perhaps the simplest of the multi-domain proteins with which to initiate this analysis. It is a major component of blood plasma; it has no bound carbohydrate; it consists of three, roughly 20 kilodalton domains; it is high in alphahelix content, high in disulfide cross-link content. It has a high degree of alpha-helicity and is somewhat myoglobin-like; it binds a variety of ligands, including fatty acids and calcium. The crystal structure, refined to the four angstrom level, for human albumin is now available [20].

We have taken the three domain model of albumin and done a very preliminary analysis from an electrostatic point of view. A computerized simulated titration of the three domains as a function of pH, and a simple analysis of the possible electrostatic behavior of those domains at various interfaces, allowed us to begin to formulate a number of hypotheses regarding the possible interfacial activity of albumin. These hypotheses now allow us to probe into the voluminous albumin adsorption literature and try to begin to make sense of that enormous data base. The analysis is continuing.

It is clear that a domain approach to protein adsorption and immobilization helps to greatly simplify the apparent complexity of the process. In fact, we have been quite successful in applying these concepts to a variety of problems involving the covalent immobilization of antibodies for biosensor and related applications [21].

SUMMARY

We feel that the adsorption of simple proteins at simple interfaces is qualitatively understood. This understanding is being extended to the behavior of complex proteins, even at complex solid interfaces, by the careful consideration of domains, domain properties, patchiness of the surface, and domain-patch interactions. In fact, this can be used to develop the concept of statistical specificity of surface interactions [2]. The various domains of complex proteins and the various domains and patches on complex surfaces each have their own surface activity and "denaturability" which must be characterized and incorporated in the analysis.

We must also note that surfaces which greatly decrease adsorption and may even be resistant to protein interactions are becoming available. Models and simulations, as well as experimental results of protein interactions with PEO surfaces, suggest that such surfaces do indeed work, and can be further optimized and enhanced for biomaterials and related applications [22-24].

We must also note that the new technique of scanning force microscopy shows potential not only in the direct imaging of proteins and complex biomaterial surfaces, but, perhaps even more importantly, in the manipulation, processing, and fabrication of protein interfaces [25,26].

Acknowledgement

We thank J.N. Herron and Kap Lim for our interactions and collaboration in the area of molecular graphics and model protein studies, Willem Norde for access to the data of model protein interactions with polystyrene lattices [13], and the Center for Biopolymers at Interfaces for partial support of the work. JDA thanks J. and P. Kopecek for a wonderful trip to Czechoslovakia's High Tatra Mountains, where the Tatra Plot approach was initially formulated.

REFERENCES

1. J.D. Andrade, V. Hlady, A-P Wei, C-H Ho, A.S. Lea, S.I. Jeon, Y.S. Lin, E. Stroup, in E. Piskin, ed., *Biologically Modified Biomaterial Surfaces*, Elsevier (1991) in press..
2. J.D. Andrade, in E. Piskin, ed., *Biologically Modified Biomaterial Surfaces*, Elsevier (1991) in press..
3. V. Hlady, J.D. Andrade, C-H Ho, L. Feng, and K. Tingey, *J. Clin. Mat.* (1991) in press.
4. J.D. Andrade and V. Hlady, *J. Biomat. Science-Polymer Ed. 2,* 161 (1991).
5. J.D. Andrade and V. Hlady, *Adv. Polymer Sci. 79,* 1(1986).
6. J.D. Andrade, ed., *Protein Adsorption*, Plenum Press, (1985).
7. T.S. Horbett and J. Brash, eds., *Proteins at Interfaces*, Amer. Chem. Soc. Symp. Series 343, (1987).
8. W. Norde in E. Piskin, ed., *Biologically Modified Biomaterial Surfaces*, Elsevier (1991) in press.
9. J.D. Andrade and V. Hlady, *Ann. N.Y. Acad. Sci. 516*, 158 (1987).
10. D. Horsley, J. Herron, V. Hlady, and J.D. Andrade, in *Proteins at Interfaces*, T.S. Horbett and J. Brash (Eds), *Am.Chem. Soc. Symp. Series 343*, 290 (1987).
11. A-P Wei, M.Sc. Thesis, University of Utah (1990).
12. A.P. Wei, J.N. Herron and J.D. Andrade in D.J.A. Crommelin and H. Schellekens, eds., *From Clone to Clinic* , 305-313 (1990).
13. T.Arai and W. Norde, *Colloids and Surface 51,* 1-15 (1990).
14. V. Hlady, C.H. Ho, J.D. Andrade, and W. Norde, in preparation (1991).
15. P.L. Privalov and L.V. Medved., *J. Mol. Biol. 159* , 665 (1982).
16. L.V. Tatunashvili, V.V. Filimonov, P.L. Privalov, *J. Mol. Biol.211,* 161-169 (1990).
17. C. Chothia, *Ann. Rev. Biochem 53,* 537 (1984).
18. M.H. Zehfus, *Proteins 3,* 90 (1987).
19. J.D. Andrade, V. Hlady, A-P Wei, C-G Golander, *Croatica Chem. Acta. 63*, 527 (1990).
20. D.C. Carter, X-M He, S.H. Munson, P.D. Twigg, K.M. Gernert, M.B. Brown, T.Y. Miller, *Science 244,* 1195 (1989).
21. J-N Lin, I-N Chang, J.D. Andrade, J.N. Herron, and D.A. Christensen, *J. Chromatography 542,* 41-54 (1990).
22. J.H. Lee, J. Kopecek, and J.D. Andrade, *J. Biomed. Mater. Res. 23,* 351 (1989).
23. S.I. Jeon, J.H. Lee, J.D. Andrade, and P.G. deGennes, *J.Colloid Interface Science 142* , 159 (1991).
24. J.M. Harris, ed., *Biomedical Applications of Polyethylene Glycol Chemistry* (1991) in press.
25. J.N. Lin, B. Drake, A.S. Lea, P.K. Hansma, and J.D. Andrade, *Langmuir 6*, 509 (1990).
26. A.S. Lea, A. Pungor, V. Hlady, J.D. Andrade, J.N. Herron, and E.W. Voss, Jr., *Langmuir* (1991) in press.