Proteins at Interfaces
Physicochemical and Biochemical Studies

EDITED BY
John L. Brash and
Thomas A. Horbett

ACS SYMPOSIUM SERIES 343

Proteins at Interfac

Physicochemical and Biochemical Studies

John L. Brash, EDITOR
McMaster University

Thomas A. Horbett, EDITOR
University of Washington

Developed from a symposium sponsored by
the Division of Colloid and Surface Chemistry
at the 192nd Meeting
of the American Chemical Society,
Anaheim, California,
September 7-12, 1986

American Chemical Society, Washington, DC 1987

Library of Congress Cataloging-in-Publication Data

Proteins at interfaces.
(ACS symposium series, ISSN 0097-6156; 343)

"Based on a symposium sponsored by the Division of Colloid and Surface Chemistry at the 192nd meeting of the American Chemical Society, Anaheim, California, September 7-12, 1986."

Includes bibliographies and indexes.

1. Proteins—Congresses. 2. Surface chemistry—Congresses. 3. Biological interfaces—Congresses.

I. Brash, John L., 1937- . II. Horbett, Thomas A., 1943- . III. American Chemical Society. Division of Colloid and Surface Chemistry. IV. American Chemical Society. Meeting (192nd: 1986: Anaheim, Calif.) V. Series.

QP.P89778 1987 574.19'245 87-14394
ISBN 0-8412-1403-4

ACS Symposium Series

M. Joan Comstock, *Series Editor*

1987 Advisory Board

Foreword

The ACS SYMPOSIUM SERIES was founded in 1974 to provide a medium for publishing symposia quickly in book form. The format of the Series parallels that of the continuing ADVANCES IN CHEMISTRY SERIES except that, in order to save time, the papers are not typeset but are reproduced as they are submitted by the authors in camera-ready form. Papers are reviewed under the supervision of the Editors with the assistance of the Series Advisory Board and are selected to maintain the integrity of the symposia; however, verbatim reproductions of previously published papers are not accepted. Both reviews and reports of research are acceptable, because symposia may embrace both types of presentation.

Preface

THE CURRENT STATE OF KNOWLEDGE of proteins at interfaces is reflected in this book. Developed from a symposium that was one of a continuing series entitled "Surface Chemistry in Biology, Dentistry, and Medicine," the book is organized around the subtopics of behavior, mechanisms, methods of study, blood-material interactions, and applications of proteins at solid-liquid, air-water, and oil-water interfaces.

We asked authors who had worked in this field for some time to provide minireviews or overviews of their previous work. We also accepted original contributions from them and from those new to the field. Some authors chose to forgo the minireview approach in favor of newer work, but in general many of the contributions provide the broader view we had hoped for.

The content of this book is quite diverse. Many factors contribute to this diversity, including the fact that the contributors' formal training varies widely. Investigators trained as immunologists, biochemists, polymer chemists, chemical engineers, and physical chemists provided chapters. Subjects range from the behavior of prothrombin at oil-water interfaces, to enhancement of albumin binding of certain biomaterials, to studies of protein foam stability. Finally, methods used also vary. Each technique is now recognized as inherently sensitive to certain aspects of proteins at interfaces but insensitive or inapplicable to the measurement of other aspects. For example, in situ ellipsometry is an exquisitely sensitive method but must be used with highly smooth, reflective surfaces and cannot be used easily to detect one protein among other proteins in a mixture.

The book's broad range can make easy understanding of the field difficult for nonspecialists. On the other hand, the book provides a rich source of information for those motivated enough to pursue the topic. We

hope that *Proteins at Interfaces* will be of interest and use to both experienced investigators and to newcomers who need to learn more about the field.

JOHN L. BRASH
Department of Chemical Engineering
McMaster University
Hamilton, Ontario L8S 4L7, Canada

THOMAS A. HORBETT
Department of Chemical Engineering, BF-10, and
Center for Bioengineering
University of Washington
Seattle, WA 98195

December 19, 1986

Contents

Chapter 1

Proteins at Interfaces: Current Issues and Future Prospects

Thomas A. Horbett[1] and John L. Brash[2]

[1]Department of Chemical Engineering, BF-10, and Center for Bioengineering, University of Washington, Seattle, WA 98195
[2]Department of Chemical Engineering, McMaster University, Hamilton, Ontario L8S 4L7, Canada

The ability of proteins to influence a wide variety of processes that occur at interfaces is well recognized. The biocompatibility of clinical implants, mammalian and bacterial cell adhesion to surfaces, initiation of blood coagulation, complement activation by surfaces, solid phase immunoassays, and protein binding to cell surface receptors all involve proteins at interfaces. Furthermore, practical problems such as contact lens fouling, foaming of protein solutions, and fouling of equipment in the food processing industry, are direct consequences of the relatively high surface activity of proteins. In general, any process involving an interface in which contact with a protein solution occurs is likely to be influenced by protein adsorption to the interface. Thus, several reviews of protein adsorption have been published (1-5).

Since previous reviews provide excellent coverage of the generally well understood or frequently studied aspects of the interfacial behavior of proteins, this chapter will focus on several facets of protein adsorption that have so far not been examined in much detail. While this approach is atypical for an overview chapter, it is in keeping with the intent of this book to provide information to the reader that reflects more recent developments in this field. Furthermore, as will be seen, the topics to be discussed necessitate re-examination of previous studies and provide some unifying views of this rather diverse science.

The main topics to be presented include the origins of the surface activity of proteins, multiple states of adsorbed proteins, and the competitive adsorption behavior of proteins. These topics were chosen because it appears that a better understanding of each is necessary

0097-6156/87/0343-0001$09.25/0

to describe many of the interfacial phenomena involving proteins, yet the fundamental concepts underlying each have not been discussed as fully as we hope to do in this chapter. Finally, we describe some future areas of research that are likely to yield important advances in our understanding of protein behavior at interfaces.

On the Origins of Differences in the Surface Activity of Proteins

Molecular Properties Influencing Surface Activity of Proteins. The molecular properties of proteins that are thought to be responsible for their tendency to reside at surfaces are summarized in Table I. The size, charge, structure, and other chemical properties of proteins that presumably influence surface activity are all fundamentally related to their amino acid sequence, which is fixed for each type of protein but varies greatly among proteins. Thus, differences in surface activity among proteins arise from variations in their primary structure. At this point, further enquiries into the origin of surface activity differences among proteins become quite problematical because little detailed information is available that relates variation in the primary structure of proteins to changes in the surface activity of the molecules. However, a discussion of specific factors will serve to clarify our concepts in this regard.

Size is presumably an important determinant of surface activity because proteins and other macromolecules are thought to form multiple contact points when adsorbed to a surface. The irreversibility typically observed for proteins adsorbed to surfaces is thought to be due to the fact that simultaneous dissociation of all the contacts with the surface is an unlikely event. Multiple bonding is also indicated by the relatively large number of protein carbonyl groups that contact silica surfaces upon adsorption (6). The bound fraction of peptide bond carbonyl groups, as calculated from shifts in infrared frequencies after adsorption, has been found to be in the range of 0.05-0.20 (6). The bound fractions correspond to 77 contacts per adsorbed albumin molecule, and up to 703 contacts per adsorbed fibrinogen molecule (6). On the other hand, size is clearly not on overriding factor determining the surface activity differences among proteins. For example, hemoglobin appears to be far more surface active than fibrinogen (7), yet the molecular weight of hemoglobin (65,000) is approximately 1/5 that of fibrinogen (330,000). While albumin nearly is the same size as hemoglobin, it is much less surface active. Finally, slight variations in the amino acid sequence of hemoglobin make large differences in surface activity even though these variants have the same molecular weight (see below).

Table I. Molecular Properties of Proteins Possibly Influencing Their Surface Activity

1. Size: larger molecules may have more contact points.

2. Charge: molecules nearer their isoelectric pH may adsorb more easily.

3. Structure:

   a. Stability: less stable proteins may be more surface active.

   b. Unfolding rates: more rapid unfolding may favor surface activity.

   c. Cross-linking: -S-S- bonds may reduce surface activity.

   d. Subunits: more subunits may increase surface activity.

4. Other chemical properties:

   a. Amphipathicity: some proteins may have more of the types of side chains favored for bonding.

   b. "Oiliness": more "hydrophobic" proteins may be more surface active.

   c. Solubility: less soluble proteins may be more surface active.

The charge and charge distribution of proteins are likely to influence surface activity because it is known that most of the charged amino acids reside at the exterior of protein molecules. These charged residues must therefore come into close proximity with the surface in the process of adsorption. Experimentally, proteins have frequently been found to exhibit greater adsorption at or near the isoelectric pH, perhaps because charge-charge repulsion among the adsorbed molecules is minimized under these conditions. However, Norde has concluded that the reduction in adsorption at pH's away from the isoelectric is due to structural rearrangements in the adsorbing molecule, rather than charge repulsion (4). In this context, it is pertinent to note that the isoelectric pH (pI) of hemoglobin is near neutrality (7.2) and that this protein is much more surface active at pH 7.4 than either fibrinogen (pI = 5.5) or albumin (pI = 4.8). It would be of interest to compare the surface activity of these molecules at pH's other than 7.4 to determine whether the ranking of surface activities changed as the isoelectric pH of each protein was approached. The role of protein surface charge is especially important and probably predominant at interfaces with fixed ionic charges, as shown by the ability to adsorb proteins to ionized matrices. Adsorption to this type of surface is strongly affected by the degree of opposite charge on the protein and the degree of competition provided by like charged ions in the buffer. Adsorption to charged matrices is the basis for the widely applied separation of proteins by ion exchange chromatography.

Structural factors important in the surface activity of proteins are not well understood. We may speculate that proteins likely to unfold to a greater degree or that unfold more rapidly would be more surface active because more contacts per molecule could be formed and because the configurational entropy gain favors the adsorption. Thus, disulfide cross linked proteins would be less likely to unfold as rapidly or completely and therefore be less surface active. This prediction is amenable to experimental test since reduction of disulfide bonds can be done specifically and completely with very mild reagents. The only known test of this idea was the observation that disulfide bond reduction by thioglycollic acid increased the number of bonds formed by albumin adsorbed to silica by about 50% (6). On the other hand, additional cross-linking of albumin with diethyl malonimidate did not reduce the number of bonds formed (6), perhaps because native albumin is already heavily cross-linked by 16 disulfide linkages (8). Finally, the existence of non-covalently bonded subunits in a protein may favor surface activity because rearrangements of the inter-subunit contacts to allow more contact of each subunit with the surface can

probably occur more readily than rearrangements within each subunit. Measurement of the relative surface activity of the subunits of hemoglobin in comparison to the tetrameric whole molecule might provide an interesting test of this idea.

Chemical differences among proteins arising from the particular balance of amino acid residues in each protein probably are also important factors influencing the surface activity of proteins. The amphipathic nature of proteins, due to the presence of hydrophobic, hydrophilic and charged amino acid side chains, provides an opportunity for bonding to sites that vary considerably in chemical nature. Thus, for a particular surface, some proteins may have more of the type of residue that favors bonding to the kind of adsorption sites prevalent on this surface, and therefore would be more surface active than other proteins. More generally, the idea that proteins have a hydrophobic or oily core suggests that proteins that are more hydrophobic may be preferred on many surfaces, especially in view of the apparent importance of hydrophobic interactions in protein interactions with some surfaces (9). Lastly, since the solubility of a protein in the bulk phase is a complex function of its overall chemical composition, and because adsorption to an interface can be thought of as insolubilization or phase separation, it could be that differences in solubility are important indicators of differences in surface activity. However, the rather high solubility of hemoglobin (ca 300 mg/ml inside red cells) argues against this idea because this protein is quite surface active (7).

Surface Activity of Hemoglobin Genetic Variants. The best experimental evidence on the molecular origins of differences in the surface activity of proteins has come from study of the behavior of hemoglobin genetic variants at the air/water interface (10-14). The differences in surface activity of these variants were originally indicated by the fortuitous observation by Asakura *et al.* that hemoglobin S solutions tend to form precipitates when shaken, unlike solutions of the normal hemoglobin A variant (10). Hemoglobin S is predominant in the red cells of humans with the sickle cell disease. The rate of precipitation induced by mechanical shaking is referred to as "mechanical stability" in this literature.

Since shaking of protein solutions induces bubble formation, and because agitation without bubble formation (by slow stirring) causes a much slower rate of precipitation, the enhanced precipitation rate of hemoglobin S solutions was attributed to an enhanced rate of surface denaturation at the air/water-liquid interface. This idea was confirmed by direct measurements of the properties of hemoglobin films at the air/water interface with a surface balance. The surface

balance experiments showed that surface pressure kinetics (π - t) and isotherms (π - A) for hemoglobin S and other variants were markedly different from hemoglobin A (14). The decrease in surface pressure following injection of hemoglobin solutions into the subphase occurred more quickly and was greater at steady state for hemoglobin S than for hemoglobin A. Furthermore, the π - A curves for the two variants became much more alike when done at lower temperatures (14), in agreement with the observation that differences in mechanical stability among the genetic variants tend to disappear at lower temperatures (10). The pressure-area isotherms for the variants, obtained by compression of the protein films, also showed distinct differences. The sharp increase in the resistance to further compression (attributed to monolayer formation) occurred at an area of 8000 $Å^2$/molecule for hemoglobin S compared to 5000 $Å^2$/molecule for hemoglobin A. The greater area per molecule suggests a greater degree of unfolding of the hemoglobin S molecule compared to hemoglobin A.

Study of the mechanical stability of other hemoglobin variants has resulted in the following ranking: HbA ≈ HbC (β6Glu→ Lys) ≈ HbF (γ chain replaces β chain) ≈ HbA_2 (δ chain replaces β chain) ≈ Hb Deer Lodge (β2 His→Asp)< Hb Korle Bu (β73 Asn → Asp) < HbS (β6Glu → Val) < Hb C_{Harlem} (β6Glu → Val; β73 Asp → Asn) (13). The notations in parenthesis indicate the amino acid substitutions e.g., β6Glu → Val means the glutamic acid at position 6 in the β subunit has been replaced with a valine residue. The majority of these differences in mechanical stability are attributable to differences in surface activity i.e., the π-A or π-t isotherms at the air/water interface have been shown to vary considerably for these variants. However, some apparent exceptions to this correlation exist, e.g. no difference in the surface activity of Hb Korle Bu and HbA was observed despite their difference in mechanical stability.

The large difference in the surface activities of HbA and HbS apparently arises from a single Glu → Val amino acid substitution at position 6 in the β chain. Similarly, the variant Hb C_{Harlem}, which has an additional Asn → Asp substitution at β73, is even more unstable. In contrast, Hb Korle Bu, having only the Asn → Asp substitution at β73, is much more stable than HbS. These results clearly indicate that seemingly minor changes in primary structure can induce large changes in the surface activity of proteins. On the other hand, the data also show that the multiple differences resulting