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Building a working understanding of protein adsorption with model systems and serendipity

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ABSTRACT

Here we will consider a working understanding of protein adsorption to be one that is adequate for practical use. Serendipity will be considered as a resource that can be used along with model systems in order to build such a working understanding. In particular, the term refers to a preparedness on the part of the researcher to make connections between a variety of everyday inputs from sources in and outside of the main concerns of the research, and a willingness to apply those connections toward the broader utility and impact of the work. In this paper we summarize the highlights and major conclusions of our work with proteins at interfaces – gained by use of comparatively very simple experimental systems while harnessing luck as effectively as we could along the way – and its relevance to meeting challenges in biopharma and biomedical technology.

1. The beginning

My interest in issues surrounding proteins at interfaces began while employed as a research technician in the Department of Food Science at North Carolina State University in 1984. My supervisor there had interest and activities underway in relation to fouling in heat exchangers during ultra-high temperature processing of milk. In order to help familiarize myself with the area, he handed me a three-ring binder which included the proceedings of an international workshop arranged by the University of Lund and the University of Wisconsin and held at Tylösand, Sweden in April 1981 entitled *Fundamentals and Applications of Surface Phenomena Associated with Fouling and Cleaning in Food Processing*. Listing "adsorption onto surfaces" first among the main research needs for the future – and including contributions transcending food applications from Willem Norde, Robert Baier, Kåre Larsson, and Ingemar Lundström – reading through the proceedings proved a transformational experience for me. My supervisor kindly allowed me to pursue a PhD program in chemical engineering while employed as a technician in food science. I chose to focus on surface properties affecting the fouling of milk components during ultra-high temperature processing, and in July 1985 participated in the Second International Conference on Fouling and Cleaning in Food Processing in Madison, Wisconsin – again arranged by the University of Lund and the University of Wisconsin. There I listened to presentations by Joe Andrade, Bob Baier, John Brash, and Brian Vincent, all from outside the realm of food science and technology. My universe was rendered two-dimensional at that meeting, and I resolved to explore the finer details of protein adsorption as soon as my dissertation research on milk fouling was complete.

I joined the Department of Food Science and Technology at Oregon State University in 1987. There we set up a system for *in situ* detection of protein adsorption by ellipsometry [1] and began quantifying surface and protein properties affecting the rate and extent of protein adsorption from single-protein solutions [2–7]. We worked mainly with the milk proteins α-lactalbumin, β-lactoglobulin, BSA, and β-casein. But the utility of our results was limited owing to the fact that these like so many other proteins vary in many ways. In addition, for any single protein, detection of the effect of a given property on its adsorption behavior requires altering its solution conditions. We were focused on quantifying molecular factors affecting protein adsorption and proposed reductive methylation of lysine residues in β-lactoglobulin using ^{14}C-formaldehyde, in this way generating sets of proteins varying in their "surface" hydrophobicity. After my first and only proposal based on that idea failed, I learned from a colleague about the variants of bacteriophage T4 lysozyme, at that time the largest,

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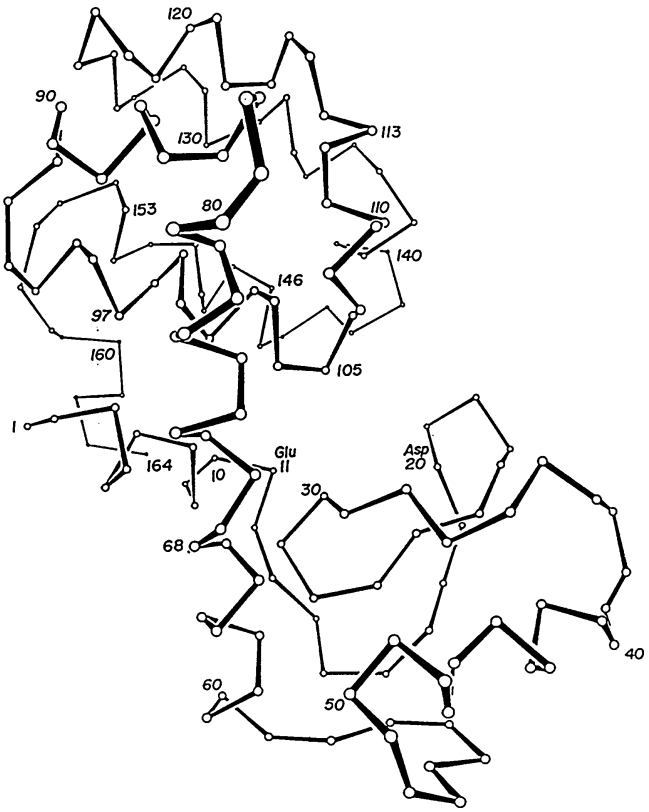

Fig. 1. The α-carbon backbone of the wild type lysozyme from bacteriophage T4. Adapted from an original provided by Brian W. Matthews.

most well-characterized set of synthetic mutants of a single protein available anywhere in the world.

Bacteriophage T4 lysozyme is extremely well characterized: the protein's 3-D structure and surface morphology are known, and importantly, numerous variants of this protein had been synthesized and characterized with respect to their deviations in crystal structure and other properties (e.g., thermodynamic stability, charge) from the wild type. In addition, the source of the T4 lysozyme variants was controlled by Professor Brian Matthews at the University of Oregon, less than an hour's drive from my laboratory at OSU. By the early 1990s we had secured a number of T4 lysozyme expression vectors, and were routinely growing suitably transformed *E. coli* cells, and expressing and purifying selected mutants of T4 lysozyme at will.

2. Quantifying the molecular origins of protein adsorption with bacteriophage T4 lysozyme

A schematic of the α-carbon backbone of bacteriophage T4 lysozyme is shown in Fig. 1. T4 lysozyme is comprised of two distinct domains: the C-terminal and N-terminal lobes, joined by an α-helix (residues 60–80) that traverses the length of the molecule [8]. T4 lysozyme has 164 amino acid residues with a molecular mass of about 18,700 Da [9]. Crystallographic data suggest dimensions for the protein in solution are consistent with that of an ellipsoid about 5.4 nm × 2.8 nm [9–11]. T4 lysozyme is a basic molecule with an isoelectric point above 9.0, and an excess of nine positive charges at neutral pH.

Important contributions to our understanding of molecular influences on protein adsorption had evolved from several earlier, comparative studies of protein interfacial behavior, in which similar or otherwise very well-characterized proteins [12–15], genetic variants [16,17] or site-directed mutants [18] of a single protein had been selected for study. While a number of factors are known to affect protein adsorption, those studies stressed the importance of protein charge, hydrophobicity, and structural stability in interfacial behavior.

We focused most of our attention on selected stability mutants of T4 lysozyme, produced by amino acid substitution of the isoleucine residue at position three (I3). I3 is largely buried within the interior of the molecule where it contributes to a major hydrophobic core, but is sufficiently close to the surface that different substitutions can be accommodated with little change in protein structure, presumably owing to I3 being so close to the amino terminus [19]. The result was a set of proteins differing in structural stability, but that were otherwise virtually identical. The structural stability of each mutant was quantified by $\Delta\Delta G$: the difference between the free energy of unfolding of the mutant protein and that of the wild type at the melting temperature of the wild type [8]. Among the most notable T4 lysozyme variants we used to study protein adsorption and function were a mutant with cysteine substituted for I3 (I3C) within which a disulfide link is formed with C97 yielding a more stable protein than the wild type ($\Delta\Delta G$ = +1.2 kcal/mol at pH 6.5), and a mutant with tryptophan substituted for I3 (I3W), which at that time was one of the least stable, fully functional lysozymes ever characterized ($\Delta\Delta G$ = −2.8 kcal/mol at pH 6.5).

2.1. Measurement of protein adsorption and binding strength

We used a number of techniques to study the interfacial behavior of charge-changed as well as structural stability mutants of T4 lysozyme, including *in situ* ellipsometry [20–24], air–water interfacial tensiometry [25,26], radioisotope labeling with ^{125}I [24] and ^{14}C [26], circular dichroism (CD) [27,28], the interferometric surface force technique [29], and various assays of enzymatic activity of T4 lysozyme mutants bound to surfaces [30,31]. The elution of adsorbed protein by surfactant had been used by Horbett and coworkers [32–36] to provide an index of protein binding strength, and the earliest work with T4 lysozyme at interfaces was focused on ellipsometric detection of the adsorption and dodecyltrimethylammonium bromide-mediated elution of T4 lysozyme stability variants at hydrophilic and hydrophobic surfaces [20]. Thomas Arnebrant and Marie Wahlgren had recently completed a mechanistic study of protein-surfactant interactions at solid surfaces [37–39] and the first work with T4 lysozyme was carried out with them during a fabulously productive sabbatical year at the University of Lund.

The essential steps of this type of an experiment are illustrated in Fig. 2. Adsorption is allowed to occur, followed by an elution step with protein-free buffer. A surfactant solution is then introduced, after which adsorbed protein is displaced and/or solubilized. This is followed by another buffer elution step, after which the amounts of protein present before surfactant addition and after the final rinse are compared. We found the surface behavior of each stability mutant differed substantially from that of the wild type. This alone was perceived as somewhat remarkable, since the structural and functional properties of each mutant in solution were virtually identical. At both hydrophobic and hydrophilic surfaces, I3W showed the greatest resistance to elution, while I3C showed the least resistance, i.e., the surfactant-mediated elution of each variant at each surface increased with protein stability.

2.2. Comparison of adsorption results to simple models

With a view toward gaining a better quantitative understanding of how (mainly) structural stability affects protein adsorption and interfacial function, and influenced strongly by mechanisms and associated models for single-protein and competitive adsorption