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Mechanical properties of protein adsorption layers at the air/water and oil/water interface: A comparison in light of the thermodynamical stability of proteins

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ABSTRACT

Over the last decades numerous studies on the interfacial rheological response of protein adsorption layers have been published. The comparison of these studies and the retrieval of a common parameter to compare protein interfacial activity are hampered by the fact that different boundary conditions (e.g. physico-chemical, instrumental, interfacial) were used. In the present work we review previous studies and attempt a unifying approach for the comparison between bulk protein properties and their adsorption films. Among many common food grade proteins we chose bovine serum albumin, β-lactoglobulin and lysozyme for their difference in thermodynamic stability and studied their adsorption at the air/water and limonene/water interface. In order to achieve this we have i) systematically analyzed protein adsorption kinetics in terms of surface pressure rise using a drop profile analysis tensiometer and ii) we addressed the interfacial layer properties under shear stress using an interfacial shear rheometer under the same experimental conditions. We could show that thermodynamically less stable proteins adsorb generally faster and yield films with higher shear rheological properties at air/water interface. The same proteins showed an analog behavior when adsorbing at the limonene/water interface but at slower rates.

Contents

1. Introduction

1.1. Proteins

Proteins are highly polymorphic biopolymers with a high functional and structural variability. Among other, proteins can function as catalysts, substrate binders, structure givers, transporters, and in addition, they constitute an indispensable part of our nutrition. The specific function and structure of each molecule depends on its amino acid sequence, which is called the primary structure. Amino acids are linked via covalent amide bonds and form the polypeptide chain. In an amide bond the carboxylic group of the preceding amino acid is linked to the amine group of the succeeding amino acid. A polypeptide chain, therefore, has a N-terminal (amine group of first amino acid) and a C-terminal (carboxyl group of last amino acid) region [1–3]. A polypeptide chain assembles into

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two dimensions and the structure is called secondary structure. Typical secondary structure motives can be α-helices and β-sheets. Folding of these motives into the three dimensional space results in the fully functional protein, called native, and the structure is called tertiary structure. The quaternary structure is the assembly of folded proteins into larger functional complexes. At their isoelectric point pI, which is the pH at which proteins have zero net charge, proteins adopt their most compact conformation.

Amino acids interact through non-covalent interactions such as hydrogen bonds, ionic and hydrophobic interactions. There are also covalent interactions for example disulfide bonds [1,2]. All of these interactions contribute to the stabilization of the folded protein [4]. Heat, pressure, the presence of interfaces, or solvents can lead to protein denaturation (unfolding) because the covalent and non-covalent interactions are disturbed [1]. Proteins can be classified as globular or random coil proteins, but it should be kept in mind that this classification is not absolute since there are well-structured non-globular proteins (e.g. ankyrin proteins [5]). For the sake of simplicity we will, nevertheless, use this classification in this review.

In this review the globular proteins bovine serum albumin (BSA), β-lactoglobulin, lysozyme, and the random coil, non-globular protein β-casein are discussed. These proteins are technologically important as foaming, stabilizing, and emulsifying agents in food systems and have been thoroughly studied in the past [3,4,6–14]. Some of their chemical characteristics, which are most relevant for the present study, are summarized in Table 1.

β-Casein, as an example of a non-globular (often also called random coil because of its almost inexistent tertiary structure) protein has an extremely flexible structure. It consists of a single polypeptide chain with 224 amino acids. There are two types of β-casein (A1 and A2), which differ in their amino acid composition at position 67: type A1 has a histidine while type A2 a proline amino acid. Most interfacial studies examine type A1 β-casein, because it is the main component of bovine milk and, thus, is especially important for the food industry. A2 β-casein is present in human, sheep, goats or other species' milk. The C-terminal part is long and has mainly hydrophobic residues compared to the N-terminal part which has mainly hydrophilic residues. The hydrophilic N-terminus of the protein is electrically charged [12] and tends to spread into the aqueous phase of an interface. The N-terminal combined with the flexibility of β-casein governs the protein adsorption and subsequent layer formation at any interface.

Nearly 50% of the whey proteins present in bovine milk is β-lactoglobulin. In total, six genetic variants can be found but two of them labeled A and B (differing at amino acid positions 64 and 118) are the most frequent ones [19]. β-Lactoglobulin has two disulfide bonds and one free cysteine residue. In the folded molecule, 15% of the polypeptide chain arranges into α-helices and 50% into β-sheets (www.ncbi.nlm.nih.gov/protein; www.expasy.org/cgi-bin/protparam). The protein has a net charge of $-8e^-$ at pH 7.5 [19]. At room temperature ($T \approx 25$ °C) and pH 7.0, β-lactoglobulin appears as a dimer and only at higher temperatures ($T \approx 30$ °C) as a monomer. The quaternary structure is influenced by the electrostatic forces between the folded molecules [23].

The three most important types of lysozyme are c-type (chicken), g-type (goose), and p-type (phage). C-type lysozyme is used as a preserving agent for cheese and tinned vegetables [24,25]. It is a small, globular protein consisting of 129 amino acids (9 are acidic, 18 are basic). The protein has four disulfide bonds and in the folded molecule, 45.7% of the polypeptide chain arranges into α-helices, 19.4% into β-sheets and 22.5% into β-turns. The molecular weight of c-type lysozyme is approximately 14.6 kDa and it has an isoelectrical point pI of 11.35 [17]. The protein has an excess of 8 positive charges at pH 7.0 [26]. The interior of c-type lysozyme is nearly non-polar while there are also hydrophobic patches on its surface [27].

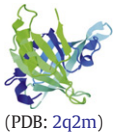
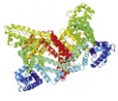
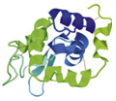
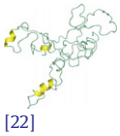
Bovine serum albumin (BSA) and human serum albumin (HSA) are the most important types of albumin. Both types occur at a concentration of 42 g/l in bovine or human blood. Function and composition of both albumins are similar and 76% of their amino acid sequences are identical. The folded BSA molecule, which consists of 580 amino acid residues, contains 17 disulphide bonds. 67% of the secondary structure consists of α-helices and 16% of β-sheets [15]. The molecule is hydrophilic and has a high solubility in water.

1.2. Adsorption of proteins at interfaces

A protein's surface activity depends on its thermodynamic stability, flexibility, amphipathicity, molecular size, and charge [3,14,28–30]. The protein adsorption process from the bulk at an interface is a multistep process [14,27,31–33]. In general it can be divided into the following steps: (i) proteins diffuse from the bulk solution towards the interface, (ii) adsorb to the interface, (iii) change their molecular structure, and (iv) spread at the interface. During the adsorption, molecules may hinder each other sterically and the adsorption rate, thus, decreases [19]. The conformational changes undergone by a protein depend on the interface nature (e.g., air/liquid, liquid/liquid or solid/liquid surfaces), on the bulk phase conditions (e.g. protein concentration, ionic strength of solution, temperature pH) [4,9], and on the protein's intrinsic properties. When a protein adsorbs to an interface, the surface or interfacial tension σ decreases. As a consequence, the surface pressure Π increases ($\Pi = \sigma_0 - \sigma$ in mN/m, where σ_0 is the surface or interfacial tension of the pure solvent) until it reaches a plateau level after several minutes to several hours [34]. Moreover, many proteins show strong lateral interactions at the interface and unfold irreversibly upon adsorption [27].

As mentioned before, proteins are often classified in food science as random coil proteins (also termed flexible or soft) or globular proteins (also termed rigid or hard) [14]. In this context, a protein with high conformational stability is called hard or rigid in contrast to those easily undergoing conformational changes called soft or flexible proteins such as β-casein or other less thermodynamically stable proteins. The terms

Table 1
Chemical characteristics of β-lactoglobulin, bovine serum albumin (BSA), lysozyme, and β-casein [15–21]. In the 3D representations of the proteins arrows indicate β-sheets, loops indicate α-helices, and red lines indicates disulphide bonds.

Characteristics	β-Lactoglobulin	BSA	Lysozyme	β-Casein
Occurrence	Bovine milk	Bovine blood	Hen egg white	Bovine milk
Molecular weight [kDa]	18.36	66.4	14.6	23.8
Number of amino acids	162	580	129	209
Cysteine	5	34	8	1
Isoelectrical point	5.6	4.7	11.35	5.3
Net charge (pH 7.0)	−8	−18	8	−13 (pH 6.7)
				
	(PDB: 2q2m)	(PDB: 1ao6)	(PDB: 3ijv)	[22]