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Modulation of the adsorption properties at air–water interfaces of complexes of egg white ovalbumin with pectin by the dielectric constant

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Abstract

The possibility of modulating the mesoscopic properties of food colloidal systems by the dielectric constant is studied by determining the impact of small amounts of ethanol (10%) on the adsorption of egg white ovalbumin onto the air–water interface in the absence and presence of pectin. The adsorption kinetics was monitored using tensiometry. The addition of ethanol resulted in considerably slower adsorption of the protein onto the interface, and this effect was enhanced when the protein was in complex with the pectin. Time-resolved fluorescence measurements demonstrated that in the case of noncomplexed ovalbumin the addition of ethanol resulted in a more condensed protein surface layer where ovalbumin adopted a preferred orientation at the interface. In contrast, the effect of ethanol on the ovalbumin–pectin complex suggested a pronounced multipoint electrostatic interaction between protein and polyelectrolyte and the formation of a more rigid spatial arrangement within the complex, thereby leading to suppressed protein–protein interactions. From this work it is concluded that by the enhanced binding affinity between ovalbumin and pectin a strong effect on the adsorption properties of the protein can be accomplished. This work does therefore illustrate how solvent quality can be exploited effectively to enhance or suppress protein functional behavior in complex applications containing air–water interfaces.

Keywords: Air–water interface; Protein adsorption; Pectin; Solvent quality; Fluorescence; Surface tension

1. Introduction

Understanding and controlling the key factors that determine the formation and/or stability of foams and emulsions is one of the fundamental challenges in colloidal science. It is of great importance for industrial applications to be able to modulate the properties of the surface-active component, frequently protein, which would allow one to develop new products with better-defined quality and predicted behavior. In vivo the control over protein functional behavior is often mediated by the formation of supramolecular assemblies with large polysaccharides that frequently play a crucial role in the molecular organization of biological systems. Also, in many artificial systems encountered in industrial applications, a complex mixture of biopolymers is present. Control of the interaction between these biopolymers is thus crucial for desired performance.

Noncovalent complex formation of globular proteins with polyelectrolytes (PE), like polysaccharides, has been reported as an effective approach to modulating the adsorption kinetics and the molecular properties of the protein at the air–water interface [1–3]. It has been demonstrated that the interaction of egg white ovalbumin with pectin in bulk solution slows down the adsorption of the protein onto the air–water interface considerably, more than expected from the differences in diffusion. Complex formation in the bulk may lead to a different spatial arrangement of the structure at the interface, resulting in different interface stability properties [3]. Protein–polyelectrolyte complexes are formed spontaneously in aqueous solution and are generally stable in a broad interval of pH values due to cooperative multipoint electrostatic interaction between oppositely charged groups on the protein and PE (see Fig. 1) [4–6].

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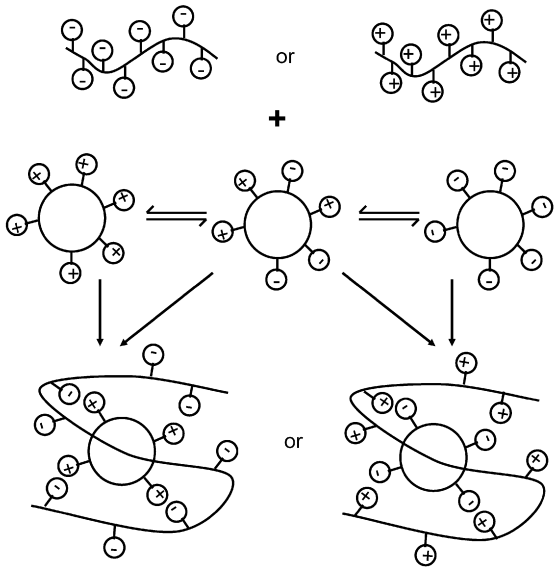

Fig. 1. The formation of protein–polyelectrolyte complexes.

The efficiency of protein–PE interaction is mainly determined by the number of possible electrostatic contacts of the protein and PE [7] and can be controlled by changing system parameters such as ionic strength or pH [6,8–10]. Complex formation can be effectively suppressed by increasing the ionic strength of the solvent due to screening of charges on both biopolymers, whereas the pH can be primarily used to control the charges on the protein. Alternatively, binding of protein to PE can be expected to be more efficient in an environment with a lower dielectric constant, where electrostatic interactions are more favorable than in pure water [7]. The addition of organic solvent to the medium could therefore amplify the effect of complex formation on the protein structure and its functional behavior.

In the present work the influence of lowering the dielectric constant of the solvent by addition of small amounts of ethanol to water on adsorption onto air–water interfaces of egg white ovalbumin (OVA) in complexes with pectin is studied. This would give insight into new opportunities to control protein behavior in bulk solution and at interfaces. The effect of complex formation on OVA adsorption onto the air–water interface was monitored using automated drop tensiometry (ADT). To unravel the molecular origin behind the observed surface rheological effects of complex formation, time resolved fluorescence anisotropy (TRFA) was applied to observe complexes both in the bulk and while residing at air–water interfaces. For this latter application TRFA in the reflection mode is used to monitor the properties of supramolecular complexes directly at the interface [3,11,12]. With this technique both the total fluorescence and fluorescence anisotropy decays of a fluorophore covalently linked to a protein are monitored. The first yields fluorescence lifetimes that provide insight into the local environment of the fluorophore. Analysis of fluorescence anisotropy decays yields information on internal and overall rotational mobility of, or within, the protein that, as in our case, could be complexed to a polysaccharide. Due to its high sensitivity and selectivity, TRFA provides information on the dynamics, spatial arrangement, and molecular interactions of the fluorescent particles, making this method particularly informative when studying supramolecular assemblies. The results obtained in the present work demonstrate that small changes in the dielectric constant have a major impact on the interaction between protein and polyelectrolytes and allow effective control over their spatial organization both in the bulk phase and at air–water interfaces that cannot easily be reached by other means. The impact of these observations on industrial applications is discussed.

2. Materials and methods

2.1. Ovalbumin purification

A batch of ovalbumin (OVA) was purified from fresh hen eggs using the following semi-large-scale procedure, based on published purification protocols [13]. From nine day-old hen eggs the egg white was separated from yolk by hand. To the total egg white fraction (about 300 mL), 600 mL of a 50 mM Tris-HCl buffer (pH 7.5) containing 10 mM β-mercaptoethanol was added. This solution was stirred for 24 h at 4 °C. Subsequently, the solution was centrifuged for 30 min at 14,000g and 4 °C. The pellet was discarded, and 1800 mL of 50 mM Tris-HCl (pH 7.5) was added to the supernatant. After a 30-min period of gentle stirring, the solution was filtered over a paper filter (Schleicher & Schuell) to remove undissolved materials. To the filtrate, 500 g of DEAE Sepharose CL-6B (Pharmacia) was added, followed by overnight incubation at 4 °C under gentle stirring. Next, the solution was filtered over a glass filter (G2), followed by extensive washing with 10 L demineralized water and 5 L 0.1 M NaCl, successively. The protein was eluted stepwise with subsequent steps of 1 L of 0.1, 0.15, 0.2, 0.25, and 0.35 M NaCl. The last two eluents did contain some ovalbumin but appeared slightly yellowish and were discarded. The 0.15 and 0.2 M NaCl batches were pooled and concentrated using a Millipore ultrafiltration unit with a 30-kDa molecular mass cutoff membrane. The concentrated solution was dialyzed extensively against demineralized water, freeze-dried, and stored at −40 °C until further usage. The yield of this procedure is generally about 1.0 g ovalbumin per egg and the efficiency of isolation is over 90%. Purity of the protein was over 98%, as estimated from densitometric analysis of SDS-PAGE gels.

2.2. Pectin

Low-methoxyl pectin was supplied by CP Kelco (Lille Skensved, Denmark). The degree of methylation is 30.4% (the nonmethylated galacturonic acid monomers possess a free carboxyl group) and the uronic acid content is 78.5% [14]. The average molar mass (M_n) is 1.5×10^5 g/mol, the polydispersity (M_w/M_n) 2.4, and the pK_a ~4.5 [15]. Pectin solutions were prepared by dispersion in water, followed by heating at 70 °C for 30 min and subsequent cooling to 4 °C. After overnight storage, the samples were centrifuged at 6000g for 10 min and stored at 4 °C until further use.