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Quantitative description of the parameters affecting the adsorption behaviour of globular proteins

Roy J.B.M. Delahaije[a], Harry Gruppen[a], Marco L.F. Giuseppin[b], Peter A. Wierenga[a,*]

[a] Laboratory of Food Chemistry, Wageningen University, Bornse Weilanden 9, 6708 WG Wageningen, The Netherlands
[b] AVEBE, Prins Hendrikplein 20, 9641 GK Veendam, The Netherlands

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ABSTRACT

The adsorption behaviour of proteins depends significantly on their molecular properties and system conditions. To study this relation, the effect of relative exposed hydrophobicity, protein concentration and ionic strength on the adsorption rate and adsorbed amount is studied using β-lactoglobulin, ovalbumin and lysozyme. The curves of surface elastic modulus versus surface pressure of all three proteins, under different conditions (i.e. concentration and ionic strength) superimposed. This showed that the interactions between the adsorbed proteins are similar and that the adsorbed proteins retain their native state. In addition, the adsorption rate (k_{adsorb}) was shown to scale with the relative hydrophobicity and ionic strength. Moreover, the adsorbed amount was shown to be dependent on the protein charge and the ionic strength. Based on these results, a model is proposed to predict the maximum adsorbed amount (Γ_{max}). The model approximates the adsorbed amount as a close-packed monolayer using a hard-sphere approximation with an effective protein radius which depends on the electrostatic repulsion. The theoretical adsorbed amount was in agreement with experimental Γ_{max} (±10%).

1. Introduction

Protein adsorption is important for the stabilization of interfaces, and thereby the formation of foams and emulsions [1]. If the adsorbed amount is close to the maximum (Γ_{max}), emulsion droplets are considered to be stable against coalescence and flocculation. Of course, the timescale within which Γ_{max} is reached is equally important. This is described by the adsorption rate (k_{adsorb}). Despite the importance of protein adsorption, it is not well understood how the adsorption rate is affected by system conditions and the molecular properties of the protein. Therefore, this study focuses on elucidating the effect of protein relative exposed hydrophobicity for different proteins, protein concentration and ionic strength on the adsorption behaviour.

The adsorption rate has been described to be influenced by exposed hydrophobicity and the electrostatic repulsion (i.e. surface charge or pH and ionic strength) [1,2]. For a single protein, an increase of the exposed hydrophobicity has been described to decrease the barrier for adsorption to the air–water interface, resulting in a higher adsorption rate [2,3]. Similarly, a decrease of the surface charge or an increase of the ionic strength decreases the electrostatic barrier and thereby increases the adsorption rate [4–6]. This shows that the adsorption rate is a function of exposed hydrophobicity and the electrostatic repulsion.

The maximum amount of protein that can be adsorbed at the interface depends on the size of the protein. The maximum fraction of the surface area which is covered by spherical particles (such as globular proteins) at the jamming limit has been described to be 0.547 [7] (i.e. saturation coverage), assuming no diffusion of the proteins at the interface. This was derived from the random sequential adsorption model, where the adsorbing particles are hard particles that have no charge. Whereas, for proteins, the maximum adsorbed amount for proteins is not affected by the exposed hydrophobicity [3], it is affected by electrostatic repulsion [4,8], as was also observed for the adsorption rate. This shows that the maximum adsorbed amount of protein should be a function of the protein radius and the electrostatic repulsion. Despite the available experimental data, there is no model to predict the maximum adsorbed amount of any protein under given conditions (e.g. pH and ionic strength).

To obtain more information on the parameters determining the adsorption behaviour, this study systematically investigates the effect of ionic strength, protein concentration and relative exposed hydrophobicity on Γ_{max} and k_{adsorb}. To this end, three different globular proteins (β-lactoglobulin, ovalbumin and lysozyme) are

* Corresponding author. Tel.: +31 317 483786.
E-mail address: peter.wierenga@wur.nl (P.A. Wierenga).

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used. Based on these results, a model will be proposed to predict the maximum adsorbed amount.

2. Materials and methods

2.1. Materials

Lysozyme (Lys; L6876, Lot n° 051K7028; purity >95% based on size-exclusion chromatography), β-lactoglobulin (β-lg; L0130, Lot n° SLBC2933V; protein content of 99% (N × 6.38) [9], of which 94% β-lactoglobulin based on SDS-PAGE), ovalbumin (Ova; A5503 Lot n° 031M7008V; protein content of 98% (N × 6.22) [9], of which 92% ovalbumin based on agarose gel electrophoresis) and 8-anilino-1-napthalenesulfonic acid (ANSA; A5144) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and purchased from either Sigma–Aldrich or Merck (Darmstadt, Germany).

Lysozyme (5 g L^{-1}) was dissolved in 10 mM sodium phosphate buffer pH 5.7. The protein solution was divided into 60 mL samples, which were heated at 83 °C for 0, 15, 30, 60 and 90 min (further referred to as Lys_0, Lys_{15}, Lys_{30}, Lys_{60} and Lys_{90}). After the heat treatment, the protein solutions were cooled on ice-water for 5 min and stored at −20 °C.

2.2. Secondary and tertiary structure

The secondary and tertiary structure of the (heated) lysozyme variants was determined using far-UV and near-UV CD, respectively according to the method described previously [10]. The lysozyme solutions were diluted to a concentration of 0.1 g L^{-1} with 10 mM sodium phosphate buffer pH 7.0.

2.3. Apparent molecular mass distribution

The (heated) lysozyme solutions were analysed by high-performance size-exclusion chromatography using an Äkta Micro equipped with a Superdex 75 PC 3.2/30 column (GE Healthcare, Uppsala, Sweden). Prior to analysis, the solutions (5 g L^{-1}) were centrifuged. Then, the solutions (20 μL) were injected and eluted with 10 mM sodium phosphate buffer pH 7.0 containing 90 mM NaCl at a flow rate of 0.06 mL min^{-1}. The elution was monitored using UV absorbance at 280 nm. The column was calibrated with globular proteins with a mass range of 13.7–67 kDa (GE Healthcare).

2.4. Quantification of exposed hydrophobicity

The increase in fluorescence intensity upon binding of 8-anilino-1-napthalenesulfonic acid (ANSA) to the accessible hydrophobic regions of the protein is used as a measure of the protein surface hydrophobicity [11]. The (heated) lysozyme solutions were diluted with 10 mM sodium phosphate buffer pH 7.0 to a concentration of 0.1 g L^{-1}. The measurements were performed on a Varian Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) as described elsewhere [3]. The emission spectrum was measured from 400 to 650 nm and the measurements were performed at 25 °C. The fluorescence spectrum with the highest area was corrected with the area of the buffer. Subsequently, the relative exposed hydrophobicity (Q_H) was expressed as the area of the sample relative to the area of the sample with the maximum area (i.e. β-lactoglobulin).

2.5. Zeta potential

Zeta potentials of the proteins in solution were determined with a Zetasizer Nano ZSP (Malvern Instruments, Worcestershire, UK) using the laser Doppler velocimetry technique. The proteins (10 g L^{-1}) were dissolved in 10 mM sodium phosphate buffer pH 7.0. The measurements were performed at 25 °C and 40 V. The results of five sequential runs were averaged. Zeta potentials were calculated with Henry's equation [12] (Eq. (1)).

$$\zeta = \frac{3\eta\mu_e}{2\varepsilon F(\kappa\alpha)} \quad (1)$$

in which ζ is the zeta potential [V], η is the viscosity [0.8872×10^{-3} Pa s], μ_e is the electrophoretic mobility [$m^2 V^{-1} s^{-1}$], ε is the dielectric constant of the medium [7.08×10^{-10} $C^2 J^{-1} m^{-1}$] and $F(\kappa\alpha)$ is Henry's function [–], which equals 1.5 using the Smoluchowski approximation [12].

2.6. Dynamic light scattering

The hydrodynamic radius of the (heated) lysozyme variants was determined with a Zetasizer Nano ZS (Malvern Instruments). The pH of the (heated) lysozyme solutions in 10 mM sodium phosphate buffer pH 5.7 (5 g L^{-1}) was adjusted to pH 7.0 with 0.1 M NaOH. The results of at least five sequential runs were averaged. The measurements were performed at 25 °C.

2.7. Adsorbed amount

2.7.1. Experimental adsorbed amount

The amount of protein adsorbed at the air–water interface was experimentally determined using a Multiskop ellipsometer (Optrel, Sinzing, Germany). Adsorption of protein to the air–water interface results in an increase of the ellipsometric angles (Δ and ψ) of the reflected monochromatic laser light (λ = 632.8 nm, angle of incidence = 50°). From the ellipsometric angles, the refractive index ($n_{adsorbed}$) and thickness ($d_{adsorbed}$) of the adsorbed layers are fitted using a model based on two bulk phases (i.e. air and water) and one adsorbed layer. The fitting parameters for the model were: $n_{air} = 1.000$, $n_{buffer} \approx n_{water} = 1.333$, $dn/dc = 0.185$ mL g^{-1} (typical for globular proteins [13,14]) and the angle of incidence was 50°.

The adsorbed amount (Γ) is calculated from the fitted refractive index ($n_{adsorbed}$) and thickness ($d_{adsorbed}$) of the adsorbed layer using Eq. (2) [13].

$$\Gamma(t) = \frac{(n_{adsorbed}(t) - n_{buffer})}{dn/dc} d_{adsorbed}(t) \quad (2)$$

where t is the time [s], Γ is the adsorbed amount [mg m^{-2}], $n_{adsorbed}$ and n_{buffer} are the refractive index of the adsorbed layer and the buffer [–], respectively, dn/dc is the refractive index increment [L g^{-1}] and $d_{adsorbed}$ is the thickness of the adsorbed layer [m].

For all measurements, the buffer was measured for 600 s. Next, the concentrated protein solutions were added. After 24 h, the ellipsometric angles were determined for 3600 s. The maximum adsorbed amount (Γ_{max}) was averaged over 3600 s (i.e. ~200 measurements). Four different sets of experiments were performed:

2.7.1.1. Effect of protein concentration. β-Lactoglobulin was dissolved in 10 mM sodium phosphate buffer pH 7.0 at concentrations of 3, 7.5, 15, 30 and 150 g L^{-1}. After measuring the buffer, protein solution was added to reach final concentrations of 0.1, 0.25, 0.5, 1 and 5 g L^{-1} in a constant volume.

2.7.1.2. Effect of ionic strength. β-Lactoglobulin (3 g L^{-1}) was dissolved in 10 mM sodium phosphate buffer pH 7.0 containing 0, 20, 40, 90 or 190 mM NaCl. After measuring the buffer, protein solution was added to reach a final concentration of 0.1 g L^{-1}.

2.7.1.3. Effect of exposed hydrophobicity. β-Lactoglobulin, ovalbumin and lysozyme (3 g L^{-1}) were dissolved in 10 mM sodium