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Adsorption behavior of milk proteins on polystyrene latex

A study based on sedimentation field-flow fractionation and dynamic light scattering

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ABSTRACT

Sedimentation field-flow fractionation (SdFFF) has been used to characterize the adsorption of the proteins β-casein (BCN) or β-lactoglobulin (BLG) on colloidal polystyrene latices; this system was used to model hydrophobic interactions between the proteins and the surfaces of fat droplets in protein-stabilized emulsions. It was found that the SdFFF technique could determine directly the surface concentrations of BCN and BLG irreversibly adsorbed to the latex surface, provided care was taken to maintain the ionic strength of the carrier at a level which suppressed particle–wall repulsion in the separation channel. The measured surface concentrations were similar for the two proteins (about 1 mg/m^2), and this was verified by quantitative amino acid analysis. These concentrations were smaller than those found in depletion studies (3 and 4 mg/m^2 respectively for BCN and BLG), in which loosely associated protein may have been included in the determinations. The thickness of the adsorbed layers was determined *in situ* by dynamic light scattering and was found to differ significantly for the two proteins (up to 15 nm for BCN *vs.* 2–3 nm for BLG). The implication of these findings in terms of different surface arrangements of the two proteins is discussed.

INTRODUCTION

Since the pioneering work of Kautzmann [1] it has been well known that proteins are marginally stable structures the conformation of which to a more or less pronounced degree is due to hydrophobic interaction between non-polar residues in their peptide chains. On the thermodynamic balance sheet, the folding of the peptide chain into a compact structure is a costly process in terms of entropy. Any opportunity for the molecule to interact with a hydrophobic surface is therefore likely to result in some relaxation of its folded structure, with a gain in entropy, as it exchanges intramolecular hydrophobic interactions for similar bonds with the surface. During this process, accomodation of the protein to the surface may lead to the exposure of previously buried hydrophobic residues which themselves can serve as adsorption sites for subsequent layers of protein molecules. Processes as different as emulsification, colloid stabilization and surface fouling may be affected by this type of protein adsorption, and the subject has therefore been given much attention [2].

If the hydrophobic groups are dispersed on an otherwise hydrophilic surface, such as a support used for hydrophobic interaction chromatography (HIC), the driving force behind the adsorption of

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proteins is ideally the weak interaction between matrix-bound non-polar ligands and hydrophobic patches on the protein surface which, for steric reasons, have failed to become buried in the interior of the molecule. With an appropriate spacing between ligands the adsorption process is reversible, and the protein structure remains essentially intact and biologically active during its contact with the matrix.

In the early 1970s Jerker Porath and coworkers [3–5] devoted much attention to the development of stationary phases for HIC. By increasing the degree of substitution of primarily agarose matrices from a level suitable for chromatography it was found that proteins could be irreversibly adsorbed with significant levels of retained activity, and could remain active for periods of months even under continuous percolation with the mobile phase [6–8]. Batch experiments showed the adsorption of several enzymes to be virtually instantaneous. After transfer of the adsorption complex into a minicolumn, a slow partial desorption was initially accomplished by the continuous introduction of protein-free buffer. Within hours, however, the desorption rate became negligible, and the remaining protein load was for all practical purposes immobilized. Although these and similar observations in chromatographic systems may give qualitative information regarding the behavior of proteins in contact with non-polar surfaces, the lack of a well defined surface area of the stationary phase precludes exact determinations of such parameters as the surface concentration and spatial extension of the adsorbed protein.

Processes underlying the formation and stability of protein-based emulsions are in part dependent on the conformational behavior of the proteins as they adsorb at the oil–water interface. Among the proteins which have been studied from this perspective are the different caseins and β-lactoglobulin from bovine milk [9,10]. Because of the difficulty in characterizing the adsorbed layer of protein in emulsions, which are composed of polydisperse mixtures of oil droplets, it has been assumed that the behavior of the oil droplet can be simulated by the behavior of similarly hydrophobic polystyrene (PS) latex particles [11] suspended in a comparable protein solution. Such latex particles are available in a variety of discrete and uniform sizes. The high level of cross-linking produced during the polymerization process causes these particles to behave as solid spheres with well defined surface areas. This makes them particularly useful as substrates in adsorption experiments intending to shed light on the surface concentration of the adsorbed component and the thickness of the adsorbed layer.

Several studies have followed the build-up of adsorbed layers of protein on such hydrophobic colloids by means of photon correlation spectroscopy (PCS) [12,13]. These have shown that it is possible to make *in situ* measurements of increases in particle hydrodynamic radius resulting from increased concentrations of protein in the suspension medium. If the thicknesses of the adsorbed layers are in excess of 2–3 nm, and the core particles are less than about 300 nm in diameter (so that uncertainties because of experimental error can be avoided), PCS will reproducibly define the increase in particle size. However, this increase can result either from irreversible adsorption or from a loose association of protein with the particle, or both, and PCS alone cannot define which of the two occurs. The surface concentration of adsorbed material can in principle be calculated from the layer thickness, but this requires making certain assumptions about the protein arrangement on the surface, which in view of the different measured thicknesses of adsorbed layers of different proteins [12,13] are not likely to be valid.

Recently, we have demonstrated that sedimentation field-flow fractionation (SdFFF) can be used to determine directly the surface concentration of materials adsorbed to colloidal particles [14,15]. The substrates in these studies were monodisperse PS latex spheres, and the adsorbed layer was a synthetic block co-polymer with known colloid stabilizing properties. If the adsorbed layer represents at least 15% of the mass of the core particles, and if the core particles can be well retained by the system, which for substrates such as PS the density of which is so close to that of the aqueous suspension medium (1.053 *vs.* 0.997 g/ml) implies particles larger than about 125 nm, the SdFFF retention is a direct measure of the mass adsorbed to each particle. In general, the process involves a separation of the adsorption complex from its supernatant using a mobile phase which is free from the adsorbing substance. As a result, the determined surface concentration of this substance is that which represents an irreversibly adsorbed layer.

In this study, we will examine the interaction between PS latex particles and the two milk proteins β-casein (BCN) and β-lactoglobulin (BLG) using both the PCS and the SdFFF technique. Although the proteins are of similar molecular weight (23 000 *vs.* 18 000 dalton), their structures are very different. BCN is believed to possess very little structural order [16], and is known to be highly surface active in spread films [17] and emulsions [18], while BLG with its tightly folded β-barrel core [19] is less surface active, but once adsorbed gives a much stronger interfacial layer [20]. As expected, the two experimental techniques discussed here give complementary information on the surface arrangement of these proteins.

EXPERIMENTAL

Sizing methods

SdFFF. At the end of the protein adsorption process, 5-μl samples of the 1% (w/v) suspended particles were injected directly into the thin separation chamber the highly polished Hastelloy walls of which were clamped together around a Mylar spacer which defined the geometry of the separation channel. The 94 × 2 × 0.0254 cm channel was curved to fit inside a rotor basket, allowing it to spin at some preset centrifugal acceleration G, which can be considered constant across the thin channel. The system was configured to allow the flow of mobile phase through the channel while it was spinning. Samples were injected with a syringe directly into the stationary channel under a slow flow of mobile phase (0.2 ml/min). After 30 s the flow was turned off and the rotor was accelerated to the selected spin rate.

Under the influence of the field, the injected components migrate to one of the channel walls and concentrate into exponentially distributed particle clouds the average thickness of which (l) depends on the interplay between the field-induced force on the particles and the sample's diffusivity [21]. This concentration distribution $c(x)$ in the direction of the field varies with distance x from the accumulation wall in the following manner

$$c(x) = c(0) \exp(-x/l) = c(0) \exp(-x/\lambda w) \quad (1)$$

where $c(0)$ is the concentration at the wall, and λ is the dimensionless layer thickness, defined as the ratio of l and the channel thickness w.

Parameter λ can be given a general definition, valid for all types of field-flow fractionation

$$\lambda = kT/Fw \quad (2)$$

where F is the force acting on a particle in the field, and k and T have the usual meaning of Boltzmann constant and temperature. In the case of a sedimentation field of acceleration G, the reduced layer thickness is described by

$$\lambda = kT/m'Gw = kT/m(\Delta\rho/\rho_s)Gw = 6kT/d^3\Delta\rho\pi Gw \quad (3)$$

The leftmost of these three expressions casts λ as a function of the buoyant mass m' of the sample particle, while in the middle m' is replaced by the product of the actual mass m and the bouyancy factor, consisting of the density difference $\Delta\rho$ between the particle (density ρ_s) and the mobile phase. The right hand expression, obtained by replacing m with the product of volume and density, is particularly useful for the sizing of spherical particles, as λ is seen to depend inversely on particle diameter d raised to the third power.

After a "relaxation time" of 20 min, during which the sample equilibrates under the influence of the field, the mobile phase flow is initiated at a rate of 2.6 ml/min while the system remains spinning. The thin channel ensures laminar flow of liquid, which implies that the various particle clouds are transported downstream at rates governed by their level of compression near the wall. The more compact its distribution, the slower will a zone move through the channel and the larger will be its retention volume V_r. For the "infinite parallel plate" type channels used here [21], the retention ratio R bears the following relationship to λ:

$$R = V_0/V_r = 6\lambda[\coth(1/2\lambda) - 2\lambda] \approx 6\lambda \quad (4)$$

Here, the approximate relationship between R and λ is accurate to within 5% for R values less than 0.1. Experimentally R, the ratio between the channel void volume V_0 and the observed retention volume, can be directly converted into a value for parameter λ which, in turn, gives information on particle mass or size through use of eqn. 3.

While the basic sample characteristic given by this approach is the buoyant mass m', this quantity is frequently less useful than the size d or actual mass m which are obtainable only for particles of known density. If the density is unknown, as is the case for colloidal substrates with coatings or adsorbed layers of unknown thickness, the retained particle fraction can readily be sized by independent techniques, *e.g.* electron microscopy or PCS. Although these techniques are applicable to direct sizing of the sample without prior fractionation [22], the introduction of the separation step ensures removal both of loosely associated protein and of the particle aggregates which may have formed during the adsorption process and which would disturb the sizing. In a recent study [15] we have demonstrated that the mass m_2 of material adsorbed to a colloidal particle is amenable to determination from SdFFF retention data, provided the densities of the (unsolvated) coating (ρ_2) and the moile phase (ρ_3) are both known

$$m_2 = [kT/Gw(1-\rho_3/\rho_2)](1/\lambda_2 - 1/\lambda_1) \quad (5)$$

Here, λ_1 and λ_2 are the retention parameters recorded at a fixed field strength G for the bare and coated particles, respectively. If the field strength is chosen so that both particle types are retained more than 10 column volumes, an approximate value for m_2 is given by a combination of eqns. 4 and 5.

$$m_2 \approx [6kT/V_0Gw(1-\rho_3/\rho_2)]\Delta V_r \quad (6)$$

where ΔV_r is the difference in retention volume between coated and bare particles. Since the retention volume for the bare particles gives their size d, provided their density is known, one can easily evaluate the surface area A per particle

$$A = \pi d^2 \approx \pi(6kT/\lambda_1 \pi \Delta\rho Gw)^{2/3} \quad (7)$$

Therefore, the SdFFF observations lead directly to determinations of the surface concentration Γ ($=m_2/A$) of adsorbed material. In the limit of well retained zones for which the approximate form of eqn. 4 applies, the value for Γ can be simply expressed in terms of ΔV_r

$$m_2/A = \Gamma \approx 0.55(kT/\pi V_0 Gw)^{1/3}(\Delta\rho/V_1)^{2/3}\,(1-\rho_3/\rho_2)^{-1}\,\Delta V_r \quad (8)$$

In the above approximate expression, $\Delta\rho$ symbolizes the density difference between bare particles and mobile phase, and V_1 is the retention volume of the bare particles.

Photon correlation spectroscopy. PCS measurements were made at a scattering angle of 90° on a spectrometer attached to a 7032 Multi-8 autocorrelation system (Malvern Instruments). Diffusion coefficients were calculated from the correlation functions using the method of cumulants [23], and apparent diameters of the particles were calculated from the diffusion coefficients using the Stokes equation. All measurements were made at a temperature of 25°C.

For the depletion experiments, a sample of latex (20 μl of a 10% suspension of diameter 190 nm) was suspended in 10 ml of buffer (20 m*M* imidazole, pH 7.0), and 20 μl of of a 10 mg/ml solution of BCN were added. The diameter of the latex was measured before and after addition of the protein. The mixture was then centrifuged using an Eppendorf 5414 centrifuge, operating at 16 000 *g*, and the supernatant liquid was removed. The latex was then resuspended in buffer containing no protein. This washing procedure was repeated six times, and the diameter of the residual latex–protein complex was measured and compared with the original values.

Quantification of colloid surface area

The freshly acquired suspension of PS latex particles had a manufacturer-assigned solids content of 100 mg/ml. From this stock a series of samples was prepared the concentrations of which were based on the assigned value for the stock, and the optical densities of which were determined at a wavelength of 232 nm, using a Perkin-Elmer Model Lambda 6/PECSS spectrophotometer. From these measurements a calibration curve was established which allowed the determination of particle concentration, and thus surface area, in a sample of unknown particle concentration.

Quantification of adsorbed protein

Two techniques were employed to quantify the amount of protein adsorbed to a given amount of particles. The first method, which assessed the total amount of protein taken up by the particles, was based on a depletion study. Here, the concentration of a given protein solution was determined by ami-

no acid analysis (AAA). To 1-ml samples of each of these two solutions, containing around 1.6 mg/ml protein, were added 100-μl portions of a 10% (w/v) suspension of latex spheres. The samples were gently mixed for 10 min on a rotating shaker, and at the end of this incubation period the particles, with their protein load, were pelletted by spinning at 16 000 *g* in the Eppendorf centrifuge. The protein content in the supernatant was again determined by AAA, and the amount adsorbed calculated from the difference in concentration before and after exposure to the particles.

The second method involved determination of the amount of protein that was irreversibly bound to the particles. Here, the coated particles were carefully washed, either by multiple suspension/centrifugation steps in which the supernatant was removed between spins and replaced by fresh buffer, or by the fractionation process through which the 5 μl injected sample was carried downstream by the mobile phase to elute at a retention volume of around 40 ml. In either case, the turbidity of the washed suspension was determined spectrophotometrically as described above, whereupon the sample was freeze-dried and submitted to amino acid analysis. The AAA procedure has been described elsewhere [24,25], and is based on a 20-h hydrolysis of the freeze-dried sample in 6 *M* HCl at 105°C, followed by derivatization with phenyl isothiocyanate and reversed phase liquid chromatography using a Hewlett-Packard Model HP 1050 liquid chromatograph. The total protein content was determined as the total area under peaks corresponding to the standard amino acids (*i.e.* all common amino acids with the exception of Trp).

Microcalorimetry

The thermal stability of the two proteins, in solution and in their adsorption complexes with PS latex particles, was determined using a differential scanning microcalorimeter (Model 4207) from Hart Scientific. The protein was dissolved in a 20 m*M* imidazole buffer of pH 7.0, and portions (final concentrations 12 mg/ml) were filled into the three measuring cells (volumes 1.0 ml each) prior to ramping up the temperature by 1°C/min. The calorimetric enthalpy, ΔH_{cal}, was found by integration of the heat capacity curve after baseline identification and correction for the change in heat capacity ΔC_p between the fully native and fully denatured forms of the protein [26].

Materials

PS latex samples were obtained from Duke Scientific and from Seradyn; they were used without further preparation. BLG was obtained from Sigma, while BCN was prepared by isolating the whole casein fraction from skimmed bovine milk by acid precipitation, followed by chromatography on a column of S Sepharose-FF (Pharmacia LKB Biotechnology) in a buffer containing 20 m*M* acetate and 6 *M* urea at pH 5.0 [27]. This separated the four different caseins. The BCN fraction was collected and dialyzed exhaustively against four changes of distilled water, and was lyophilized. Analysis of the protein using fast protein liquid chromatography [28] did not show any other protein components to be present. The densities of both proteins were taken as 1.365 g/cm^3, while densities for the latex and buffer were 1.053 g/cm^3 and 0.997 g/cm^3, respectively.

RESULTS AND DISCUSSION

The two whey proteins BCN and BLG were adsorbed onto PS latex particles with a nominal diam-

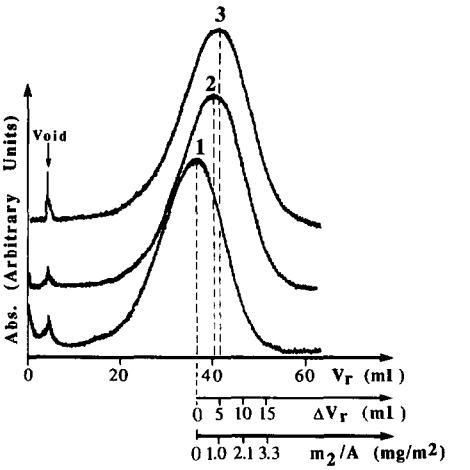

Fig. 1. Fractograms of PS bare and protein-coated PS particles with nominal diameter of 272 nm. Traces 1–3 represent bare, BCN-coated and BLG-coated particles, respectively. SdFFF parameters used were: field strength, 173 g; flow-rate, 2.9 ml/min. The ΔV_r expresses the excess retention volume caused by the uptake of protein, which in turn proportional to the mass (m_2) adsorbed per unit area.

TABLE I

ADSORPTION OF MILK PROTEINS

Surface concentrations Γ of the two proteins on PS latex are determined by four methods: Γ_{FFF} by FFF, via eqns. 5 and 7; $\Gamma_{FFF/AAA}$ by AAA on coated particles after fractionation; $\Gamma_{AAA}(W)$ by AAA on particles extensively washed after adsorption; $\Gamma_{AAA}(T)$ by AAA of the supernatant, before and after protein adsorption to the latex. Coating thickness δ (PCS, nm) is determined from the size difference between bare and coated particles.

Proteins	Mol.wt. (dalton)	Γ_{FFF} (mg/m^2)	$\Gamma_{FFF/AAA}$ (mg/m^2)	$\Gamma_{AAA}(W)$ (mg/m^2)	$\Gamma_{AAA}(T)$ (mg/m^2)	δ (PCS, nm)
BCN	23 000	1.00	1.28	1.26	2.99	10
BLG	18 000	0.88	1.06	1.00	4.12	3

eter of 272 nm from solutions containing 8 mg/ml of protein and 1% (w/v) (surface area 0.21 m^2/ml) of the latex. The adsorption was rapid for both proteins, and no difference was seen in the fractionation behavior whether sampling was done after 5 min or several hours after mixing. Fig. 1 illustrates the typical fractionation patterns gathered for the bare PS latex particles, as well as for the particles coated with BCN and BLG, respectively. The approximate relationship (eqn. 6) between adsorbed mass, on the one hand, and the difference in retention volume between bare and coated particles on the other, serves as a qualitative indication of the similarities in amounts adsorbed encountered for the two proteins. A more exact assessment of the particle uptake of each protein is obtained via eqn. 5, in conjunction with the retention volumes measured for the bare particles and for each of the protein–particle complexes, respectively. The surface concentrations for the two proteins, obtained from eqns. 5 and 7, are listed in Table I. These concentrations are seen to be very similar. The similarity is particularly striking if they are expressed as area per molecule. However, the values of around 1 mg/m^2 are significantly lower than the concentrations (around 3 mg/m^2) reported by others performing adsorption of these milk proteins on PS substrates [29,30]. The explanation to this discrepancy may be that these other studies were based on the depletion of protein from a solution exposed to a known amount of particles, and therefore include both irreversibly and loosely bound protein, although the binding isotherms do not show biphasic adsorption.

There are, however, some potential sources of error that can affect the SdFFF measurement, and which therefore must be examined. First, it is known that mobile phases of low ionic strength can give rise to significant Coulombic repulsions between sample particles and the channel wall [31,32]. Such repulsions add a term to the flux equation which forms the basis for establishing the concentration distribution given by eqn. 1; if present, they lead to premature elution of a sample the size or mass of which therefore appears smaller than its actual value. By systematically varying the ionic strength (I) of the carrier and recording the effect of I on retention, one can easily detect whether such unwanted effects are present. This process is illustrated in Fig. 2 for the adsorption complex between BCN and the 272-nm PS latex; a similar curve was recorded for BLG. Although premature elution is clearly observed in mobile phases of low ionic strength, the gradual increase in I leads to a plateau value for the retention indicative of a complete suppression of any Coulombic interaction with the

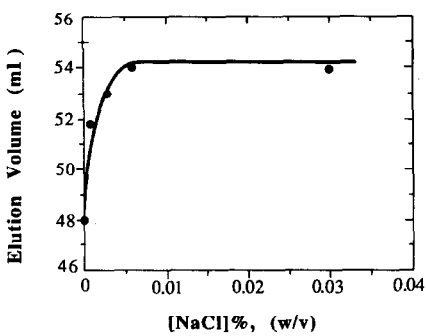

Fig. 2. Effect of carrier ionic strength on the elution volume of casein-coated PPS particles. The plateau is reached for salt concentrations above 0.015% (w/v).

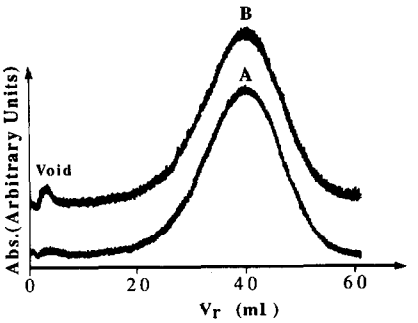

Fig. 3. Fractograms of BCN-cated PS particles in carriers of different compositions. Traces: A = 20 m*M* imidazole + 0.015% NaCl; B = as A, buth with the addition of 2 mg/ml BCN. SdFFF conditions were: field strength, 173 g; flow-rate, 2.9 ml/min.

channel wall. It is this plateau retention which is used to compute the surface concentrations given in Table I.

A second source of error in these measurements would be encountered if the adsorbed protein were to repartition from the particle surface to the channel wall during the fractionation. In order to ensure that this did not occur, fractograms were collected for the PS–BCN complex using mobile phases consisting of imidazole buffer alone or with the addition of 2 mg/ml of soluble BCN. The slight difference in retention, and therefore in adsorbed mass, which is seen in Fig. 3 may account for some loosely associated protein withstanding the shear of the carrier and thus co-migrating with the particles in the BCN-containing buffer. However, the difference in too small to in any way indicate that a significant loss of protein occurs from the particles during the SdFFF procedure in protein-free buffers.

To further scrutinize the validity of the FFF derived surface concentrations of BCN and BLG, PS latex samples of known concentration were incubated with solutions of each of the two proteins (8.03 and 7.79 mg/ml, respectively), as described in the Experimental section. The protein solutions were sampled before and after exposure to the particles, and these samples were subjected to amino acid analysis for quantification of the protein loss due to adsorption. In addition, the coated particles were removed from the supernatant by centrifugation and washed thoroughly with pure imidazole buffer to remove any loosely adsorbed protein. Given portions of coated particles, quantified by their turbidity, were also submitted to amino acid analysis. Similarly, samples which had been subjected to the SdFFF procedure were collected at the peak elution positions in the fractograms. From the turbidities of these fractions, the amounts of particles were determined prior to amino acid analysis of their protein loads. Thus it was found that the depletion experiments do indeed indicate higher surface concentrations of both proteins (2.94 ± 0.03 mg/m^2 for BCN and 4.12 ± 0.05 mg/m^2 for BLG) than that found after extensive wash (1.26 mg/m^2 for BCN *vs.* 0.96 mg/m^2 for BLG). The latter pair of data is very similar to that found for the fractions from SdFFF, either by AAA or from the level of retention (See Table I). The slight discrepancy between the retention-based values and those based on a turbidity-related surface area, may well be due to an error in the assumed particle concentration for the latex sample, as it is of the same sign and similar magnitude for both proteins.

Errors in substrate concentration, and therefore in surface area available for adsorption, are easy to make with samples the limited availability of which make dry weight determinations and subsequent concentration assignments impractical. In relying on SdFFF retention to determine surface concentration of adsorbed materials this source of error is eliminated, since the difference in elution volume between bare and coated particles is a measure of the amount of protein adsorbed per particle, *i.e.* per a well defined surface area.

In addition to determinations of protein surface concentration, the two latex–protein adsorption complexes were examined by PCS to determine the thickness of the adsorbed protein layer on the 272-nm latex particles. Here, the previously noted differences between the two proteins [33] were confirmed, as seen in Table I. Thus, while the surface concentrations of BCN and BLG were very similar, the spatial extension of these molecules from the PS surface was quite different. Indeed, the BLG layer was within the measurement error of PCS, and can therefore not exceed 2–3 nm in thickness, whereas the BCN layer appeared to be around 15 nm thick. The latter value agrees with previous PCS measurements reported on by one of us [12]. Experiments where the latex–protein complexes were exhaustively washed gave a layer thickness of 13.5 nm before

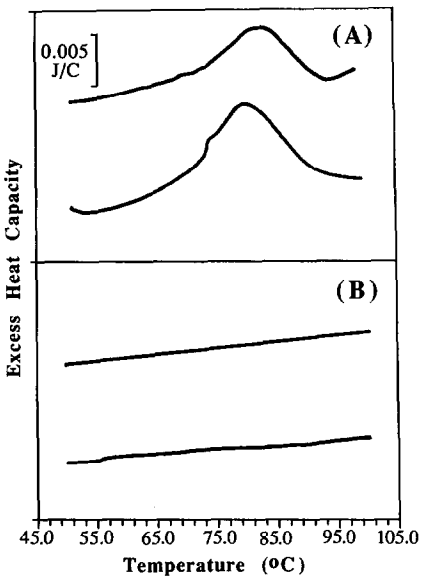

Fig. 4. Thermograms of proteins in free and adsorbed forms: (A) the lower trace represents free BLG in 20 m*M* imidazole buffer and the upper trace shows the protein adsorbed to PS latex with diameter of 90 nm. The unfolding enthalpies were 3.5 J/g and 1.8 J/g for the free and adsorbed forms, respectively; (B) as (A) but for BCN. The unfolding enthalpies are zero for both forms of the protein. The scan rate of the instrument is 1°C/min. The excess heat capacity (J/C) is in Joules per centigrade.

washing and 9.5 nm afterwards. While this confirmed that there was a change in the surface as a result of washing, it was clear that the thickness of the layer was not diminished proportionately to the amount of protein which was lost, according to the surface coverage figures quoted above.

Although of comparable molecular weight, the two proteins of interest here are structurally very different. BCN appears to be a flexible molecule, with a highly hydrophobic "tail", which may be the site of adsorption to fat droplets and similarly nonpolar surfaces, while the remaining hydrophilic portion of the molecule reaches out into an aqueous environment in which the fat is solubilized [34]. In contrast, the BLG molecule is known from X-ray crystallography [19] to be folded into a stable β-barrel. It is thought that after adsorption on to a hydrophobic surface, the BLG undergoes a slow structural rearrangement [35]. However, calorimetric examination of BLG adsorbed to the 90-nm PS latex shows that a significant amount of structure

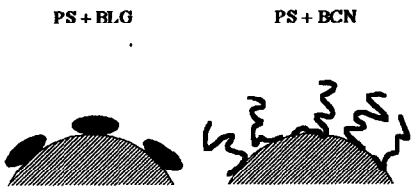

Fig. 5. Proposed surface arrangements for BCN and BLG on the PS latex particles. The BLG is heavily structured and compact even in the adsorbed state, while the BCN, a good surfactant protein, remains flexible and structureless on the particle surface.

remains in the molecule even after adsorption. In fact, the melting temperature for the protein in its adsorbed state was found to be somewhat higher (by 2.6°C) than for the protein in solution, as seen in Fig. 4. This observation supports the notion of a molecule which remains relatively compact even when it is adsorbed on a solid substrate. This notion is illustrated by the cartoon in Fig. 5.

In these experiments we have considered the adsorption of two proteins of similar chain length. Both adsorb with about the same surface density, but one retains a significant amount of structure, although in the adsorbed state it may be forced to flatten out somewhat for better contact with the surface. The other, in turn, is virtually structureless, allowing its long and highly hydrophilic tails to penetrate deeply into the aqueous solvent which surrounds the complex.

CONCLUSIONS

The ability to accurately assess the surface concentration and layer thickness of adsorbed macromolecules is crucial for the understanding of their function in the adsorbed state. This is particularly true for molecules of surfactant character, the ability of which to create a stable emulsion is directly linked to their ability to interact both with the oil and the water phase. Here, the milk surfactant protein β-casein is compared in its adsorption behavior to the structurally more rigid β-lactoglobulin. With the help of SdFFF we have been able to quantify the surface concentration of irreversibly adsorbed protein of both types and found it to differ significantly from adsorption data gathered earlier from depletion experiments. The SdFFF data are verified by the significantly more labor intensive amino acid

analysis procedure. It is noted that in determining surface concentrations by means of SdFFF one needs no prior knowledge of the exact amount of colloidal surface area exposed to protein. Rather, the measurement indicates mass increase per particle, where the surface area is easily determined from observations on the bare particles. The size increase associated with the uptake of protein can not be determined by SdFFF, since the composite density of the adsorption complex is unknown. Instead, the use of PCS has made it possible to demonstrate that the thickness of the β-casein adsorbed layer is at least five times that of the more structured companion protein.

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