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Adsorption Behavior of Lysozyme and Tween 80 at Hydrophilic and Hydrophobic Silica–Water Interfaces

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Abstract Nonionic surfactants such as Tween 80 are used commercially to minimize protein loss through adsorption and aggregation and preserve native structure and activity. However, the specific mechanisms underlying Tween action in this context are not well understood. Here, we describe the interaction of the well-characterized, globular protein lysozyme with Tween 80 at solid–water interfaces. Hydrophilic and silanized, hydrophobic silica surfaces were used as substrates for protein and surfactant adsorption, which was monitored in situ, with ellipsometry. The method of lysozyme and Tween introduction to the surfaces was varied in order to identify the separate roles of protein, surfactant, and the protein–surfactant complex in the observed interfacial behavior. At the hydrophobic surface, the presence of Tween in the protein solution resulted in a reduction in amount of protein adsorbed, while lysozyme adsorption at the hydrophilic surface was entirely unaffected by the presence of Tween. In addition, while a Tween pre-coat prevented lysozyme adsorption on the hydrophobic surface, such a pre-coat was completely ineffective in reducing adsorption on the hydrophilic surface. These observations were attributed to surface-dependent differences in Tween binding strength and emphasize the importance of the direct interaction between surfactant and solid surface relative to surfactant–protein association in solution in the modulation of protein adsorption by Tween 80.

Keywords Adsorption · Desorption · Formulation · Protein binding · Proteins · Surfactant · In situ · Ellipsometry

Introduction

The loss of activity through adsorption and surface-induced structural alteration is a significant problem in the production, formulation, and administration of therapeutic

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proteins. The unfolding of proteins at interfaces results in an entropic gain and is recognized as one of the driving forces of protein adsorption [1, 2]. Multiple contacts with a surface may form due to the large size of protein molecules, leading to strong binding and irreversible loss. Another important factor relates to the action of "bound water," or the hydration layer in the vicinity of the surface, with which the protein molecules interact [3]. In this regard, protein adsorption is hypothesized to depend on the affinity of water for the surface: a hydrophilic surface would be expected to show less adsorption than a hydrophobic surface, as bound water is less readily removed from a hydrophilic surface.

Surfactants can modulate both protein adsorption and surface-induced structural alteration by their own action at interfaces as well as by their participation in the formation of protein–surfactant associations. The use of surfactants in upstream and downstream processing as well as formulation is thus very common. For example, weak, nonionic surfactants such as Tween 80 are used commercially to minimize adsorption loss and aggregation and preserve native structure and activity. However, the specific mechanisms underlying Tween action in this context are not well understood. In particular, even for formulations that are considered "optimized" for chemical and physical stability of the protein, the effectiveness of the surfactant will depend very strongly on the chemistries of the interfaces present (whether gas–liquid, liquid–liquid, and solid–liquid) in a given circumstance.

An important goal in process development and formulation engineering is to minimize protein loss that occurs through colloidal and interfacial mechanisms (e.g., aggregation, adsorption) [4, 5]. In order to achieve this, a fundamental understanding of the mechanisms underlying surfactant effectiveness is needed. In particular, the specific roles of surfactant, protein, and the surfactant–protein complex in modulating interfacial behavior must be better understood. In this way, we will generate a basis to provide direction for much needed process improvements in the manufacture and finishing of therapeutic proteins.

A number of experimental investigations of the interfacial behavior of surfactant and protein mixtures have been conducted, and these have identified three possible adsorption outcomes: complete hindrance, reduced amounts, or increased amounts of protein adsorption. Complete hindrance is attributed to the faster diffusion of the (smaller) surfactant molecules as compared to protein molecules, with the adsorbed surfactant layer sterically preventing protein adsorption. Reduced and increased amounts are usually attributed to the formation of surfactant–protein complexes with reduced or increased surface affinity, respectively, and in any case different from that for pure protein or pure surfactant in solution.

The sequential introduction of a surfactant after protein adsorption may result in removal of adsorbed protein, due to the formation of surfactant–protein complexes and subsequent solubilization of these complexes, and/or replacement of adsorbed protein by surfactant on account of a stronger surfactant–surface association. The extent of surfactant-mediated removal of adsorbed protein depends on protein, surfactant, and surface properties among other factors [6–11]. Most pertinent to the context of this report, the difference in adsorbed protein elution by anionic, cationic, and nonionic surfactants, in general, corresponds with the strength of surfactant binding to protein in solution [7, 9]. Nonionic surfactants which are known to bind rather weakly to proteins are least effective in removing adsorbed protein molecules from the interface. Nonionic surfactants, when introduced to an adsorbed protein layer, do not generally affect the amount adsorbed on hydrophilic surfaces but do have an effect on the amount adsorbed on hydrophobic surfaces, presumably because of the difference in surfactant binding strength at the interface [10]. In the case of elution of beta-lactoglobulin from the air–water interface by the nonionic surfactant Tween 20, for example, protein replacement by free Tween 20 molecules was determined to be more

important than formation of a protein–surfactant complex with reduced surface activity, even though very specific protein–surfactant binding was measured in those experiments [11].

Here, we describe the interaction of a globular protein, lysozyme, with the nonionic surfactant Tween 80 at solid–water interfaces. The concentration of surfactant, as well as the method of surfactant and protein introduction to the surfaces (in sequence or combined), was varied in order to identify the separate roles of protein, surfactant, and the protein–surfactant complex in determining adsorption outcomes. The interfacial behavior of lysozyme is perhaps better known than is the interfacial behavior of any other protein. This work was performed in order to form a much-needed foundation for interfacial study of complex therapeutic proteins in surfactant-containing formulations. Results of this direct, surface spectroscopic, in situ evaluation of lysozyme adsorption and elution kinetics at interfaces in the presence of Tween 80 are thus interpreted in a manner directly applicable to other combinations of surfactants and proteins.

Materials and Methods

Protein, Surfactant, and Buffers

Lysozyme from chicken egg white (Sigma, Lot number 051K7028) was dissolved in filtered (0.2 μm) sodium phosphate buffer (0.01 M, pH 7) at a concentration of 5 mg/ml and used as the protein stock solution for all adsorption experiments. Protein solutions were prepared fresh each day. Tween 80 was dissolved in distilled, deionized water (DDW) to obtain concentrated stock solutions of 10,000 and 50,000 ppm. Tween stock solutions were stored as 2 ml aliquots at −80 °C until use.

Preparation of Hydrophilic and Hydrophobic Silica

Silicon (Si) wafers (WaferNet, crystal grade, type N, boron doped, orientation 1-0-0, thickness 525±18 μm, resistivity 0.01–0.02 ohm-cm) were oxidized in air (1 atm, 1,000 °C) for 18 min to obtain an oxide film thickness of about 300 Å [12]. Wafers were cut into 1×3 cm plates using a tungsten pen, rinsed with acetone, then cleaned using a standard acid/base cleaning procedure. In brief, the plates were first immersed in a solution of $NH_4OH/H_2O_2/H_2O$ (1:1:5 volume ratio), held at 80 °C for 10 min, and rinsed in copious amounts of DDW. They were then transferred to a solution of $HCl:/H_2O_2/H_2O$ (1:1:5 volume ratio), held at 80 °C for 10 min, rinsed with DDW again and then dried under a flow of nitrogen. At this stage, the silica plates exhibit hydrophilic surfaces, as evidenced by a water contact angle between 0° and 10°. Hydrophilic silica samples were stored in ethanol until use.

Hydrophobic silica plates were made by immersion of hydrophilic silica plates in a solution containing 1% dichlorodimethylsilane in xylene for 1 h. The plates were then rinsed sequentially with xylene, acetone, and ethanol. The silanization procedure rendered the silica plates hydrophobic, as evidenced by a water contact angle between 90° and 100°. The hydrophobic plates were stored in ethanol until use.

Evaluation of Lysozyme Secondary and Tertiary Structure

Circular dichroism (CD) spectra of lysozyme (0.50 mg/ml) with Tween 80 concentrations of 0, 8, 20, and 80 ppm were performed to evaluate the effect of Tween 80 on protein

structure in solution. The CD spectra were obtained using a J-720 UV Spectrum spectropolarimeter (JASCO). All experiments were carried out at 25 °C. A cylindrical cuvette with a 100 μm pathlength was used. The CD spectra were recorded with every 1 nm increment in wavelength, starting at 290 nm and ending at 182 nm. In order to increase the signal-to-noise ratio, six scans were recorded for each sample and then averaged. The CD spectra of protein-free, Tween-containing buffer were subtracted from the lysozyme CD spectra in every case.

Fluorescence spectroscopy was used to evaluate the effect of Tween concentration on the tertiary structure of lysozyme. Emission spectra were obtained for lysozyme samples (0.5 mg/ml) containing 0, 8, 20, and 80 ppm Tween using a Photon Technology International (PTI) QuantaMaster fluorometer (Photon Technology International, New Jersey). The excitation wavelength was set at 295 nm. Emission spectra were recorded at 1 nm increments from 305 to 405 nm at a scan rate of 1 nm/s. The excitation and emission slit widths were set at 0.25 and 2.0 mm, respectively. Three scans were recorded and averaged in each case in order to increase the signal-to-noise ratio. The data were corrected for protein-free background. In order to estimate the wavelength at which maximum fluorescence emission (λ_{max}) occurred, the background-corrected data were differentiated using the FeliX32 software supplied by PTI. The wavelength at which the first-order derivative curve intersected the *x*-axis was recorded as λ_{max}.

Evaluation of Lysozyme Adsorption Kinetics

Lysozyme adsorption kinetics were recorded in situ by ellipsometry. Ellipsometry is used to determine the thickness and refractive index of very thin films by measuring the change in the state of polarization upon reflection of a laser beam from the surface. The effect of reflection is characterized by the angles Δ, defined as the change in phase, and Ψ, the arctangent of the factor by which the amplitude ratio changes, with reference to the two component plane waves into which the electric field oscillation is resolved. Ellipsometry allows the analysis of protein adsorption on solid, specular surfaces in real time and in situ in biologically relevant aqueous media [13]. An automatic ellipsometer (L-104SA, Gaertner Scientific) with a 1 mW He–Ne light source was used. The angle of reflection was set equal to the angle of incidence at 70°. Each silica plate (hydrophobic or hydrophilic) was suspended in a trapezoidal, fused quartz cuvette (Hellma) which was equipped with a magnetic stir-bar, and filtered phosphate buffer was added. After stable optical properties (Δ and Ψ) of the bare substrate were measured for 30 min (at 15 s intervals), a lysozyme, Tween, combined lysozyme + Tween, or protein-free, surfactant-free buffer solution was introduced to the cuvette as described in the following subsections.

The adsorbed layer changes the optical properties of the reflected laser beam which can then be related to the adsorbed mass [14]. A one-film-model ellipsometry program [15] was used for the calculation of adsorbed mass. The program uses the values of the ratio between molar mass and molar reflectivity (M/A) and partial specific volume (V) of the adsorbing species. The M/A and V used for lysozyme were 3.841 g/ml and 0.761 ml/g, respectively. Protein-specific values of V and M/A were used to determine the adsorbed mass in both the presence and absence of surfactant, as it is not possible to assign a correct value to these parameters for mixed, protein/surfactant films [6, 7]. This approach does not influence any of the trends observed in these experiments. Each experiment was performed at least twice on each type of surface, with an average deviation from the mean of about 0.005 μg/cm^2.

The experimental scheme involved the introduction of Tween together with lysozyme (co-adsorption), after introduction of lysozyme (sequential adsorption), and before the

introduction of lysozyme ("pre-coat"). For adsorption on hydrophobic silica, different Tween concentrations (0, 8, 20, and 80 ppm) were used in each case. For adsorption on hydrophilic silica, experiments were performed without Tween (0 ppm) or in the presence of Tween at a "high" concentration (80 ppm).

Tween + Lysozyme Co-adsorption

Lysozyme (0.5 ml, 5 mg/ml) was mixed with 20 μl of concentrated Tween solution to obtain 80, 200, or 800 ppm Tween-containing protein samples. These samples were vortexed briefly to ensure good mixing. The Tween + lysozyme mixture was then added to the cuvette (containing 4.5 ml filtered phosphate buffer) to obtain a final protein concentration of 0.5 mg/ml and a Tween concentration of 0, 8, 20, or 80 ppm. Adsorption was monitored for 30 min. The sample was then rinsed by flowing phosphate buffer through the cuvette at a flow rate of 30 ml/min for 5 min. Adsorbed mass was monitored for an additional 25 min.

Lysozyme–Tween Sequential Adsorption

A surfactant-free lysozyme solution was added to the cuvette to give a final concentration of 0.5 mg/ml, and adsorption kinetic data were recorded for 30 min. The sample was then rinsed by flowing phosphate buffer through the cuvette as above, with adsorbed mass monitored for an additional 25 min. A Tween solution (0.5 ml) was then added such that the final Tween concentration was 8, 20, or 80 ppm. Tween was allowed to contact the surface for 15 min, after which the sample was rinsed with buffer. Adsorbed mass was monitored for an additional 25 min.

Tween + Lysozyme Co-adsorption at a Surface Pre-coated with Tween

In this case, 4.4 ml filtered phosphate buffer was added to the trapezoidal cuvette. One-tenth milliliter (0.1 ml) Tween solution was then added such that the final Tween concentration in the cuvette after the addition of protein would be 8, 20, or 80 ppm. Tween adsorption was monitored for 45 min, after which 0.5 ml lysozyme solution was added to the cuvette to obtain a final protein concentration of 0.5 mg/ml. Adsorption from the Tween + lysozyme mixture (i.e., the lysozyme which was added and the Tween that was already present in the cuvette) was monitored for 30 min. The surface was then rinsed with buffer for 5 min and adsorbed mass monitored for an additional 25 min.

Lysozyme Adsorption at a Surface Pre-coated with Tween

This procedure was similar to that described in the preceding paragraph up to the time of protein addition. Here, after 45 min contact with Tween, the sample was rinsed with surfactant-free, protein-free phosphate buffer for 5 min and adsorbed mass monitored for an additional 25 min. Then 0.5 ml lysozyme solution was added to the cuvette to obtain a final protein concentration of 0.5 mg/ml. Adsorption was monitored (in this case, in the absence of Tween) for 30 min. The sample was then rinsed with phosphate buffer for 5 min and adsorbed mass monitored for an additional 25 min.

Results and Discussion

Lysozyme Structure as a Function of Tween Concentration

There are numerous reports [16–18] which describe the effect of surfactants on the structure of proteins in solution. In particular, ionic surfactants such as sodium dodecylsulfate and dodecyltrimethylammonium bromide are reported to have a denaturing effect on the structure of proteins. Tween 80 is a nonionic surfactant. Nonionic surfactants are generally weaker than ionic surfactants and not expected to alter protein secondary or tertiary structure in solution.

All CD spectra recorded for lysozyme at different concentrations of Tween were essentially identical (Fig. 1), indicating that the presence of Tween did not induce any secondary structure change in lysozyme. Hillgren et al. [19] reported similar behavior in the case of Tween 80 interaction with lactate dehydrogenase (LDH). Pyrene fluorescence measurements were carried out to get an indication of the local polarity in the Tween + LDH system. A hydrophobic interaction between LDH and Tween was observed at and below the Tween critical micelle concentration, but this interaction was not strong enough to denature the protein.

The fluorescence spectra of lysozyme samples containing different Tween concentrations were comparable to the sample containing no added Tween (Fig. 2a), and estimates of λ_{max} in each case were similar as well (Fig. 2b). This indicates that the addition of Tween did not alter the tertiary structure of lysozyme. In summary, with regard to the present study, while it is possible that Tween associates with lysozyme to form a surfactant–protein complex, our results rule out the possibility that a "new" lysozyme species with different structural characteristics is generated as a result of Tween addition. This fact allows for a more direct interpretation of the adsorption kinetics described in the following sections.

Tween + Lysozyme Co-adsorption on Hydrophobic Silica

The adsorption kinetics of lysozyme in the presence of 0, 8, 20, and 80 ppm Tween on hydrophobic silica are shown in Fig. 3. A reduction in adsorption with an increase in Tween concentration was observed, with reduced amounts remaining at the interface after rinse. These observations would be consistent with Tween being able to locate at the interface

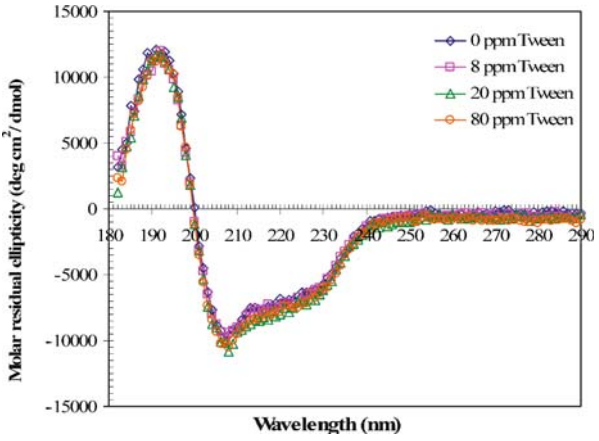

Fig. 1 Circular dichroism spectra of lysozyme in the presence of Tween 80

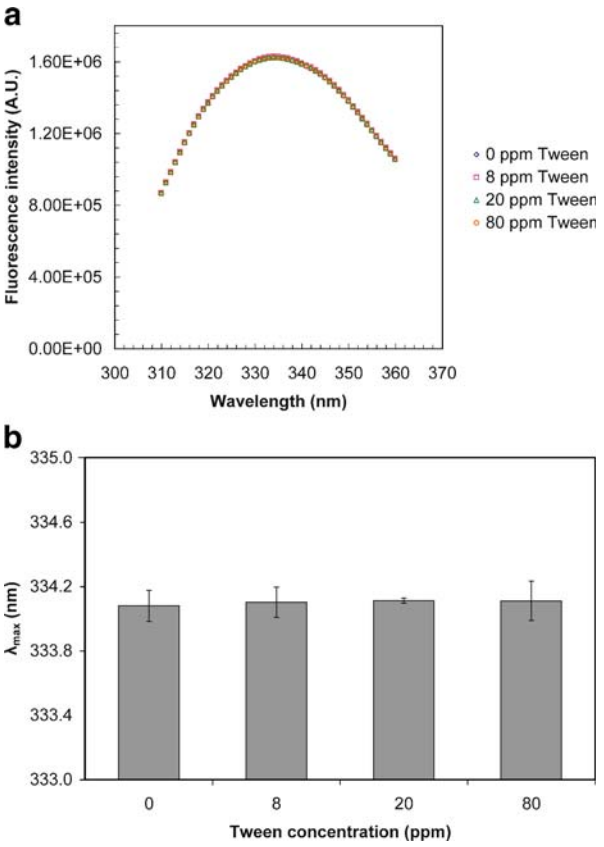

Fig. 2 The effect of Tween 80 concentration on **a** fluorescence spectra of lysozyme and **b** estimates of λ_{max}

more rapidly than lysozyme (owing to its smaller size and higher diffusivity), in this way inhibiting lysozyme adsorption and/or Tween associating with lysozyme in solution-forming complexes of reduced surface affinity. The adsorption kinetics for the lysozyme–Tween solutions at 80 ppm Tween were comparable to that for pure Tween adsorption,

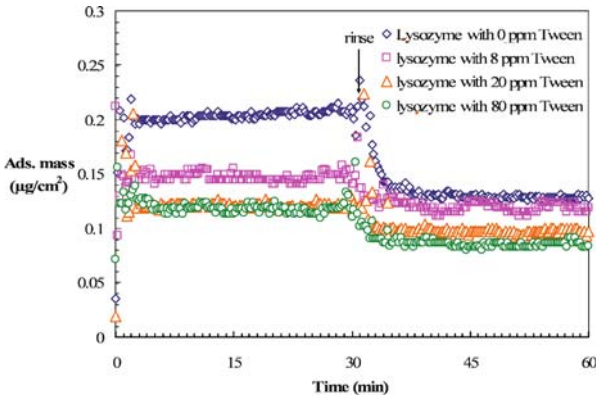

Fig. 3 Adsorption kinetics of lysozyme on hydrophobic silica in the presence of 0, 8, 20, and 80 ppm Tween

suggesting that Tween may be dominating the adsorption at high concentrations (Tween adsorption in the absence of protein appears in Fig. 6).

Lysozyme–Tween Sequential Adsorption on Hydrophobic Silica

The kinetics of lysozyme adsorption followed by buffer elution and the introduction of Tween at concentrations of 8, 20, and 80 ppm are shown in Fig. 4. The adsorption and elution kinetics recorded during the first 60 min in each case were similar, since they represent identical experimental conditions [adsorption of lysozyme (0.5 mg/ml) followed by buffer rinse]. It is important to note that since the lysozyme solution was replaced with buffer during the rinsing procedure, there was no lysozyme in solution at the time of Tween addition. When Tween solutions at 8 and 20 ppm were added, adsorbed mass was observed to increase, consistent with Tween adsorption at the empty sites on the surface and/or on the adsorbed protein layer. After rinsing, adsorption values decreased to those recorded prior to Tween addition suggesting that at these concentrations the Tween was loosely held and had no apparent effect on the amount of protein adsorbed.

Upon introduction of Tween at 80 ppm, after a brief initial increase in adsorbed amount, a decreasing trend in the kinetic plot was observed consistent with the removal of adsorbed protein. After the buffer rinse, the adsorbed amount was less than that recorded prior to Tween addition and consistent with that expected for (protein-free) Tween adsorption. This suggests that the adsorbed lysozyme molecules may have been replaced by Tween in this case.

Figure 5 shows results of a special case in which 8 ppm Tween was introduced as the rinse buffer. Lysozyme adsorption and elution kinetics for this case are compared with that obtained with no Tween in the rinse buffer but with 8 ppm Tween introduced after the rinse step (i.e., after 60 min). Comparison between the two plots shows that elution kinetics for the first 5 min were indistinguishable. However, after that time, the rate of elution appeared to decrease for the case where Tween was included in the rinse buffer. This may be attributed to lysozyme elution and concomitant Tween adsorption at the empty sites and is supported by the observation that the adsorbed amount after 60 min for the case where Tween is included in the rinse buffer is similar to that recorded when Tween is added after completion of the buffer rinse.

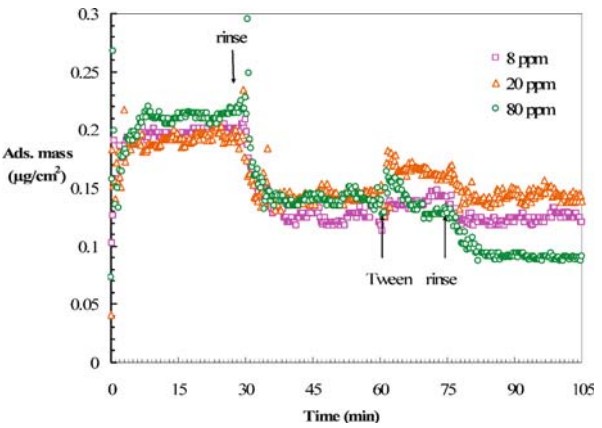

Fig. 4 Adsorption kinetics of lysozyme on hydrophobic silica followed by buffer elution and the introduction of Tween at concentrations of 8, 20, and 80 ppm

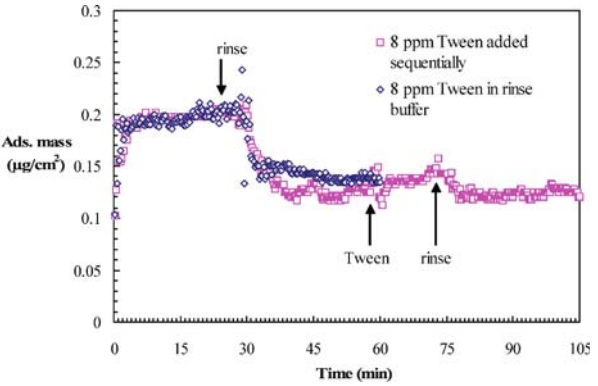

Fig. 5 Lysozyme adsorption followed by elution with 8 ppm Tween in the rinse buffer, compared with lysozyme adsorption followed by elution with no added Tween in the rinse buffer (but with 8 ppm Tween introduced after the rinse step)

Tween + Lysozyme Co-adsorption on Hydrophobic Silica Pre-coated with Tween

Tween adsorption at the hydrophobic surface followed by the addition of lysozyme and elution with buffer are shown in Fig. 6. As there was no competition for the surface during the initial adsorption phase and Tween remained in solution when lysozyme was added, it is not surprising that only little lysozyme adsorption at the Tween pre-coated surface was recorded. These results suggest that lysozyme is not able to replace Tween adsorbed at a hydrophobic interface to any appreciable extent when Tween is also present in solution. Lysozyme that did adsorb was apparently rinsed away as the adsorbed amounts reached final values similar to that expected for pure Tween (after rinsing). The origins of the anomalous pattern recorded for 20 ppm Tween are not clear.

Lysozyme Adsorption on Hydrophobic Silica Pre-coated with Tween

In order to more clearly isolate the contribution of interfacial Tween to the observed reduction in lysozyme adsorption, a rinsing step was introduced after the formation of a Tween pre-coat and prior to the addition of lysozyme. This step removed Tween present in solution as well as any Tween loosely held at the interface. Figure 7 shows that a large fraction of Tween molecules remained adsorbed even after the hydrophobic surface was

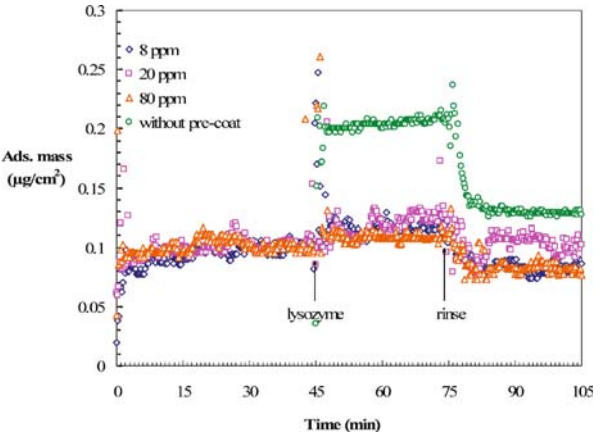

Fig. 6 Tween adsorption on hydrophobic silica followed by the introduction of lysozyme and elution with buffer

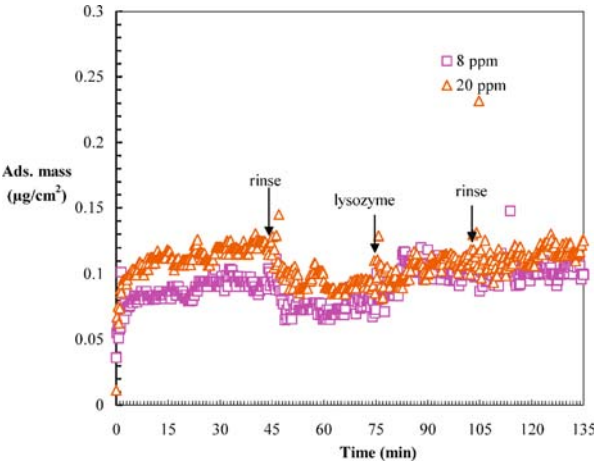

Fig. 7 Tween adsorption on hydrophobic silica followed by elution with buffer then introduction of lysozyme and second elution with buffer

rinsed with buffer. This partial irreversibility to buffer elution is contrary to the behavior of common ionic surfactants, for which adsorption to hydrophobic materials is typically reversible.

An increase in adsorption was recorded upon introduction of lysozyme, to an amount comparable to Tween and lysozyme co-adsorption on a Tween pre-coated surface. This indicates that the reduction in protein adsorption at a Tween pre-coated surface is a consequence of the presence of Tween at the interface as opposed to formation of a protein–surfactant complex of low adsorption affinity. However, when the surface was rinsed following lysozyme adsorption (at 105 min), no desorption was observed. In this case, lysozyme is likely to have adsorbed to empty sites on the surface, becoming tightly bound relative to lysozyme adsorbed to a Tween layer (Fig. 6).

Identity of the Adsorbed Layers

A limitation in interpreting ellipsometry results with multi-component systems is that the technique detects the total adsorbed mass and does not differentiate between individual adsorbing species. Every experiment conducted with ellipsometry was concluded with an elution step, expected to remove loosely held protein and surfactant molecules from the surface. Here, we will consider the adsorbed amount at the end of the final elution step as a semi-quantitative indicator of adsorbed layer identity. These adsorption values were determined as the average of the last 3 min of the experiment (i.e., the last 12 data points) and are presented in Fig. 8 along with the final adsorbed amounts recorded for single-component Tween adsorption and for single-component lysozyme adsorption. Using single analysis of variance ($p<0.05$ considered significant), the identity of the adsorbed layer was considered to be lysozyme if the adsorbed amount was significantly dissimilar to pure Tween but not significantly dissimilar to lysozyme. Accordingly, the data of Fig. 8 suggest that the final adsorbed layer consisted of mostly lysozyme for co-adsorption with 8 ppm Tween, sequential adsorption with 8 and 20 ppm Tween, and for surfaces pre-coated with Tween then rinsed prior to lysozyme adsorption. The same kind of analysis suggests the final adsorbed layer consisted of mostly Tween for co-adsorption with 80 ppm Tween, sequential adsorption with 80 ppm Tween, and co-adsorption on surfaces pre-coated with 8 and 80 ppm Tween. Note that the final layers for surfaces pre-coated with Tween and rinsed prior to lysozyme adsorption appeared to consist of lysozyme or of both lysozyme

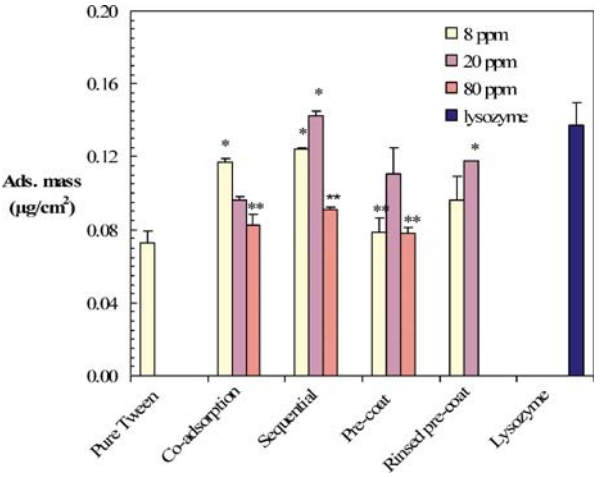

Fig. 8 Adsorbed amounts on hydrophobic silica recorded at the end of the final elution step, arranged according to the manner of lysozyme/Tween introduction to the surface. Adsorbed amounts that are not significantly dissimilar to lysozyme but significantly dissimilar to Tween are identified with an *asterisk* (*); adsorbed amounts that are significantly dissimilar to lysozyme but not significantly dissimilar to Tween are identified with *two asterisks* (**)

and Tween. This underlines the importance of having Tween present in solution if lysozyme repulsion is desired.

Tween + Lysozyme Co-adsorption on Hydrophilic Silica

Figure 9 shows Tween + lysozyme co-adsorption and single-component lysozyme adsorption kinetics on hydrophilic silica, plotted with similar experiments performed with hydrophobic silica (replotted from Fig. 3). While the adsorption and elution kinetics of lysozyme and (80 ppm) Tween closely correspond to that of pure Tween, no such reduction in adsorbed amount was observed at hydrophilic silica. Indeed, Fig. 9 indicates no difference between the adsorption and elution kinetics at 0 and 80 ppm Tween on hydrophilic silica. The dynamics of the protein–surfactant interaction in solution, as well as diffusion of each component, should remain independent of surface hydrophobic–hydrophilic balance. So the differences in adsorption behavior at hydrophobic and hydrophilic surfaces is attributed to the direct interaction between the surfactant and

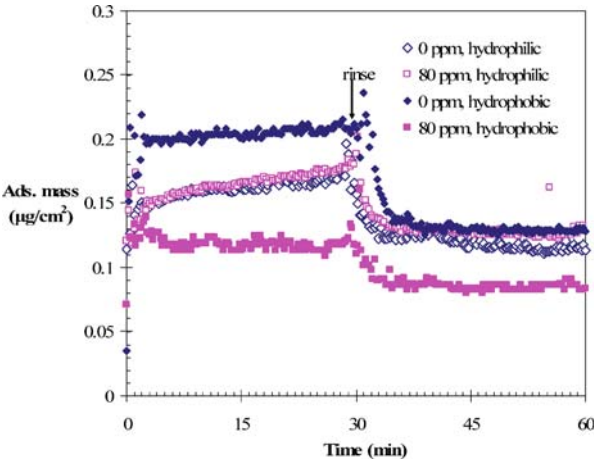

Fig. 9 Comparison of Tween + lysozyme co-adsorption and single-component lysozyme adsorption kinetics on hydrophobic and hydrophilic silica

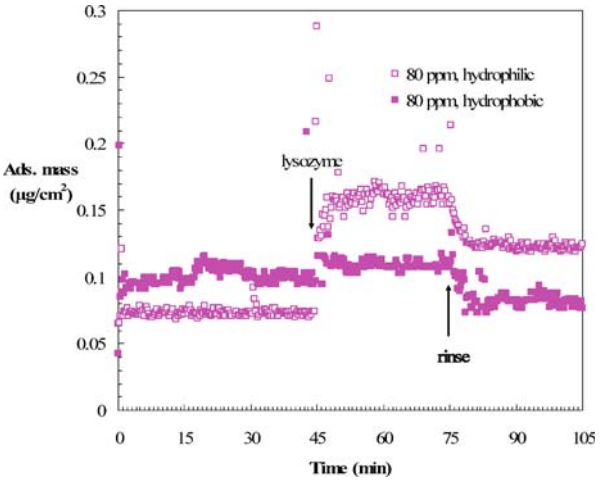

Fig. 10 Tween adsorption followed by Tween + lysozyme co-adsorption on hydrophobic and hydrophilic surfaces

surface. In summary, the Tween–hydrophilic surface association is weak in comparison to the Tween–hydrophobic surface association. Even if Tween is adsorbed at the hydrophilic surface, lysozyme is able to eventually replace it owing to the weak Tween–surface interaction in that case.

Figure 10 shows that when Tween + lysozyme co-adsorption takes place on hydrophilic silica pre-coated by the adsorption of Tween (as opposed to bare hydrophilic silica), lysozyme apparently replaced Tween at the hydrophilic surface, adsorbing to the same extent as recorded on bare hydrophilic silica (Fig. 9). This indicates that lysozyme rapidly replaces Tween and underlines the importance of direct interactions between adsorbing species and the surface relative to surfactant–protein association in solution. Also note Tween adsorbed to a lesser extent on hydrophilic relative to hydrophobic silica, indicating a greater affinity for the hydrophobic surface [While we are confident that the affinity of Tween for the hydrophilic surface is low in comparison to its affinity for the hydrophobic surface, data recorded for Tween adsorption to hydrophilic silica were not reproducible due to very low values of adsorbed mass. In particular, when the change in optical properties of a film (relative to the optical properties of the bare substrate) is below a certain threshold, the mathematical routine used to determine film thickness and refractive index does not yield reliable results, instead often reporting negative values accompanied by noise. The data shown in Fig. 10 for Tween adsorption to hydrophilic silica should therefore be taken only as an indication that the affinity of Tween for the hydrophilic surface is low in comparison to its affinity for the hydrophobic surface].

Lysozyme–Tween Sequential Adsorption on Hydrophilic Silica

Figure 11 presents results for the case where 80 ppm Tween was added sequentially following lysozyme adsorption and elution from hydrophilic silica, along with the plot from the similar experiment performed with hydrophobic silica (replotted from Fig. 4). While Tween was apparently able to remove the adsorbed protein layer at hydrophobic silica, no such effect was seen at hydrophilic silica. Instead, a small increase in adsorbed amount was recorded upon introduction of Tween, followed upon rinsing by a decrease to that value recorded before Tween addition. These results, like those recorded for Tween + lysozyme

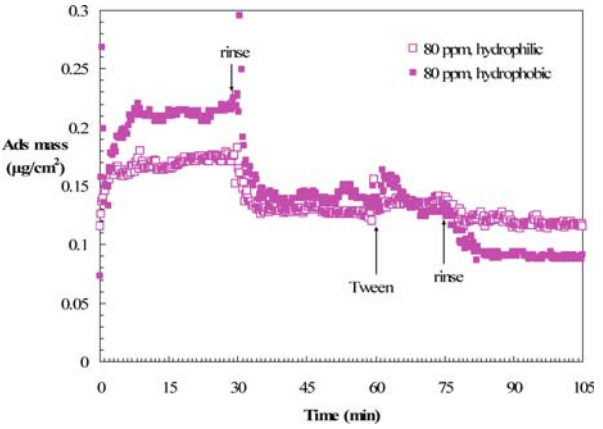

Fig. 11 Lysozyme adsorption and elution followed by introduction of 80 ppm Tween and subsequent buffer elution at hydrophilic and hydrophobic silica

co-adsorption at hydrophilic silica, indicate that a strong Tween–surface interaction is required to effect protein repulsion or removal from a surface. These results also suggest that a "replacement mechanism" in which surfactant is able to remove adsorbed protein molecules on account of high surfactant–surface affinity may be more relevant here than a "solubilization mechanism," in which surfactant binding to adsorbed protein results with a more soluble, readily elutable species. Cho et al. [20] recorded comparable behavior using surface pressure and surface concentration measurements of bovine serum albumin (BSA) adsorbed onto lecithin monolayers. At the air–water interface, an adsorption synergism was observed at short time intervals, leading to a BSA surface concentration greater than the steady state value in the absence of lecithin. At longer times, BSA desorbed slowly. Those authors postulated that the BSA desorption was likely caused by surface pressure and steric exclusion effects produced by the spread lecithin monolayer. Similar observations regarding the enhancement in protein adsorption at short times after spreading monoglyceride were also made in the case of beta-lactoglobulin [21]. In this system as well, the spreading of a close packed lipid monolayer caused the desorption of protein at longer time intervals.

Conclusions

The addition of Tween decreased lysozyme adsorption on hydrophobic silica, and this reduction in adsorbed protein increased with Tween concentration in solution. Sequential adsorption experiments showed that, at sufficiently high concentration, Tween was able to remove adsorbed lysozyme from a hydrophobic surface. In addition, if Tween is introduced to the hydrophobic surface prior to lysozyme addition, lysozyme adsorption can be reduced or prevented. On the other hand, adsorption of lysozyme on hydrophilic silica showed no dependence on the presence of Tween in solution. In addition, sequential adsorption experiments showed the presence of Tween, whether introduced to the interface before or after lysozyme, had no effect on lysozyme adsorption. These data indicate that strong Tween–surface association is necessary to inhibit protein adsorption, while the Tween–protein association in solution is of relatively small consequence. Accordingly, the rapid diffusion of Tween (relative to protein) to the interface is likely to contribute to a reduction in protein adsorption only if Tween–surface affinity is sufficiently high.

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