Lipase−Surfactant Interactions Studied by Neutron Reflectivity and Ellipsometry

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Received: March 25, 1999

The interaction between a microbial lipase and an anionic and a cationic surfactant at the air−water interface has been studied by neutron reflectivity. Sodium dodecyl sulfate (SDS) and tetradecyltrimethylammonium bromide (TTAB) were used as anionic and cationic surfactant, respectively. The same enzyme−surfactant systems were also studied at the interface between a hydrophobic solid surface and water by ellipsometry, and the results from the two techniques were compared. Surface tension measurements were also performed in order to monitor complex formation in bulk. The data obtained from neutron reflectivity and from ellipsometry were in very good agreement with each other. Both techniques show that lipase adsorbs readily at the interfaces and that SDS at low concentration does not interact strongly with this protein layer. At higher SDS concentration, the protein is displaced from the surface. On the other hand, when TTAB is added at low concentration, a thick lipase−surfactant layer is formed at the surfaces. This compact layer is solubilized by further addition of the cationic surfactant.

Introduction

The interactions of lipases and amphiphiles have been studied by a variety of methods. As early as 1976 Borgström and Donnér used equilibrium dialysis to demonstrate that pancreatic lipase formed a complex in solution with the anionic surfactant sodium dodecyl sulfate (SDS). They also showed that this interaction caused a severe reduction in lipase activity with a complete inactivation taking place already at an SDS concentration of half the critical micelle concentration (cmc).[1] Interestingly, bile salts which can be regarded as natural anionic surfactants did not impair the activity of the same lipase. Later work showed that the activity of a microbial lipase, *Humicola lanuginosa* lipase, was not affected by SDS.[2] Evidently, the interaction of lipase and anionic surfactant is quite specific in nature and depends on the choice of both lipase and amphiphile.

Association between microbial lipases and surfactants with different headgroups has been investigated by surface tension measurements.[3,4] From the surface tension vs log (surfactant concentration) plots it was concluded that while cationic surfactants formed complexes with the lipases, anionic and nonionic surfactants did not. The interaction of lipase and cationic surfactant occurred over a broad pH range, including below the isoelectric point of the lipase where the enzyme carries a net positive charge.[3] Recent attempts to use titration microcalorimetry to obtain further proof of microbial lipases interacting with cationic but not with anionic surfactants in bulk solution were inconclusive.[5]

Lipase−surfactant interactions at the oil−water interface have been studied by measuring the electrophoretic mobility of droplets of emulsions prepared with different types of surfactants to which a lipase solution was added. It was found that droplets stabilized by an anionic or a nonionic surfactant were unaffected by the enzyme. On the other hand, for droplets stabilized by a cationic surfactant, the surface charge changed as a function of lipase concentration in solution.[6]

Ellipsometry has been used to investigate association between lipase and different types of surfactants at the interface between water and a hydrophobic solid surface.[3,4,7,8] The results obtained indicated behavior of the enzyme at the solid−water interface similar to that at the oil−water interface, i.e., strong interaction with surfactants carrying a positive charge and no or negligible interaction with surfactants carrying negative or no charge.

In the present paper we have used neutron reflectivity to investigate the interaction between a microbial lipase and an anionic and a cationic surfactant at the air−water interface. The same enzyme−surfactant systems were also studied at the interface between a hydrophobic solid surface and water by ellipsometry, and the results from the two techniques are compared. Surface tension measurements were also performed in order to monitor complex formation in bulk.

Experimental Section

Materials. *Humicola lanuginosa* lipase of high purity was kindly provided by Novo Nordisk. This enzyme is a single chain protein consisting of 269 amino acids with a molecular weight of 29 472 g/mol and an isoelectric point of 4.4.[9]

The normal surfactants, sodium dodecyl sulfate (SDS) and tetradecyltrimethylammonium bromide (TTAB), were both from Sigma. SDS was recrystallized once before use, while TTAB was used as received. The deuterated anionic surfactant SDS was purchased from ISOTEC Inc. and used as received, while the deuterated cationic surfactant TTAB was a generous gift from M. Dubois (SCM, Saclay, France) who carried out the synthesis. The water used was Millipore filtered.

Surface Tension Measurements. Surface tension was measured with a KSV Sigma 70 instrument using a du Noüy ring. The Zuidema−Waters correction method for the ring was used. Surfactant addition was done with a Methrom dosimat titration

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10.1021/jp991032f CCC: $18.00
Published on Web 08/13/1999

unit. Measurements were performed in a glass beaker cleaned with bichromate sulfuric acid. The surface tension measurements of surfactant only were carried out starting with 1 mM phosphate buffer to which the surfactant dissolved in the same buffer was added. Measurements in the presence of enzyme were made with a solution of 0.05 wt % lipase in 1 mM phosphate buffer to which a concentrated solution of surfactant in the same buffer containing the same concentration of enzyme was added. All measurements were performed at pH 7.5.

Neutron Reflectivity Measurements. Specular neutron reflectvitiy experiments were carried out on the time-of-flight reflectometer DESIR in the ORPHEE reactor at Saclay. The solution sample was contained in a Teflon trough (15 cm × 5 cm × 0.3 cm) which was completely enclosed in an aluminum cell with quartz windows to allow the neutron beam to enter and exit the cell. The grazing incident angle was 1.3°, and the angular resolution was 3%.

Reflectivity is obtained as a function of the momentum transfer, q, defined as $q = 4\Pi \sin \theta / \lambda$, where θ is the incident angle and λ the neutron wavelength.

All measurements were made at the liquid−air interface of solutions of protonated lipase in D_2O at a concentration $C =$ 0.005 wt %., i.e., a concentration 10 times lower than that used in the surface tension and ellipsometry measurements. In this mixture, the lipase scattering length density was calculated to be 3.12×10^{-6} /Å^2, taking into account exchange of the labile hydrogen with the deuterated solvent. For solutions containing mixtures of lipase and surfactant, the surfactant was first dissolved in the lipase solution before the measurements. Deuterated surfactants were used in all studies. Since the scattering length densities of deuterated surfactants almost match that of D_2O, the signals measured in the q range employed in these studies are attributed to the lipase. Calibration of the experimental conditions to obtain the precise incident angle, the angular resolution, and the normalization factor was carried out with pure D_2O. The reference Fresnel curve R_F mentioned henceforth therefore incorporates both resolution and surface roughness effects, which in the present case is fixed at 3 Å. In the presence of deuterated surfactants, no deviation from the Fresnel curve was observed in the present q range used.

Ellipsometry. Ellipsometry is a technique for investigating thin films, which is based on the measurement of changes in the ellipticity of polarized light upon reflection at this interface.[10,11] The measurements were performed by means of so-called null ellipsometry.[10,11] The instrument used was a Rudolph thin-film ellipsometer, type 436, controlled by a computer. A xenon lamp, filtered to 4015 Å, was used as light source.

Ellipsometry measurements were performed at the solid−liquid interface of plates of hydrophobized silica.[12] The plates were cleaned by treatment for 5 min in boiling 25% NH_3:30% H_2O_2:H_2O = 1:1:5 after which the plates were rinsed with Millipore water. They were then treated for 5 min in boiling 25% HCl:30% H_2O_2:H_2O = 1:1:5 and rinsed with Millipore water and thereafter with ethanol. Subsequently, the plates were rinsed with trichloroethylene before they were methylated by exposure to a solution of 0.1 wt % dichlorodimethylsilane in trichloroethylene for 90 min. Finally, the plates were rinsed again with trichloroethylene and ethanol. They were stored in ethanol until use. The zero adsorption was recorded from the buffer solution before a solution of 0.05 wt % lipase in 1 mM phosphate buffer, pH 7.5, was introduced. After a plateau value of lipase adsorption had been reached, a concentrated solution of surfactant in the same buffer was added in increments. Calculation of the adsorbed amount was performed according to de Feijters, using a refractive index increment of 0.15 cm^3/g for the surfactants [13] and 0.19 cm^3/g for the lipase.[14]

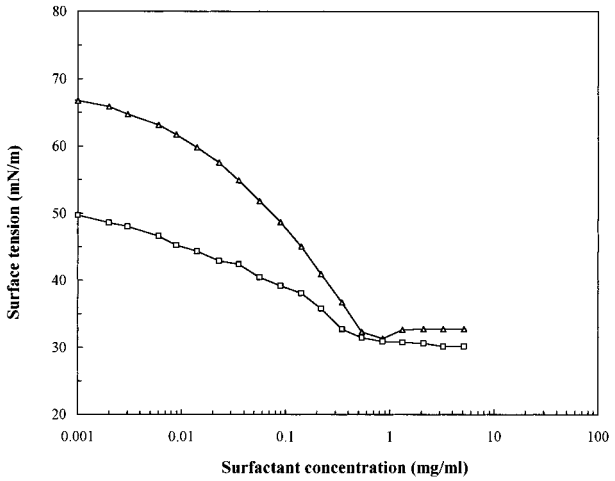

Figure 1. Surface tension vs log concentration of sodium dodecyl sulfate (SDS) with (squares) and without (triangles) lipase present.

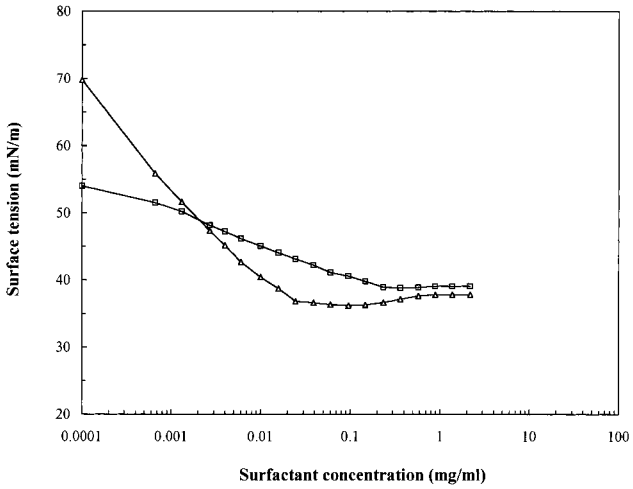

Figure 2. Surface tension vs log concentration of tetradecyltrimethylammonium bromide (TTAB) with (squares) and without (triangles) lipase present.

Results

Surface Tension Measurements. Surface tension vs surfactant concentration plots for SDS and TTAB with and without lipase present are shown in Figures1 and 2, respectively. Lipase only gives a surface tension of 54 mN/m. It has been shown previously that the surface tension for this enzyme varies insignificantly at a concentration above 0.01 g/dm^3.[3] The lipase concentration used here, 0.05 g/dm^3, is well above this value.

The curves for both SDS and TTAB alone show a dip at a concentration just around the cmc. This is an indication of the presence of an impurity which is more surface active than the surfactant and which is, thus, preferentially adsorbed at the air−water interface, reducing the surface tension to values lower than those obtained by only surfactant. When micelles start to form, the hydrophobic impurity becomes solubilized in the micelles, leading to an increase in surface tension. This behavior is well-known for SDS, in which case the impurity is usually dodecanol.[15] The contaminant in the TTAB sample is unknown. Note, however, that the magnitude of the minimum is rather small in both cases, indicating that the impurities are of marginal importance for the interfacial behavior of the surfactant preparations.

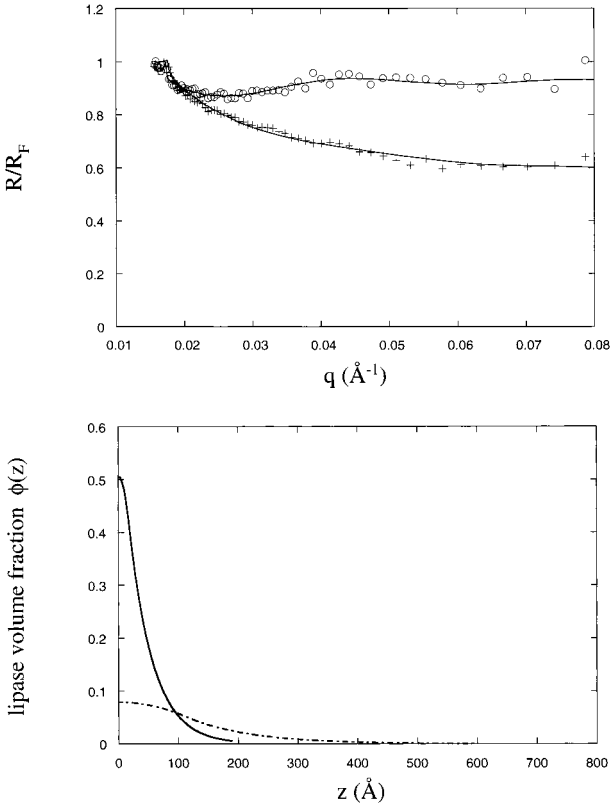

Figure 3. (a) Normalized reflectivity curves of lipase adsorbed at the air−solution interface. Lipase concentration, $C = 0.05$ mg/mL; SDS concentration, $C_s = 0$ (crosses) and $C_s = 3.0$ mg/mL (circles). The solid lines are the best fit curves using the concentration profiles shown in b. (b) Concentration profiles of adsorbed lipase at SDS concentration, $C_s = 0$ (solid line) and $C_s = 3.0$ mg/mL (dotted−dashed line).

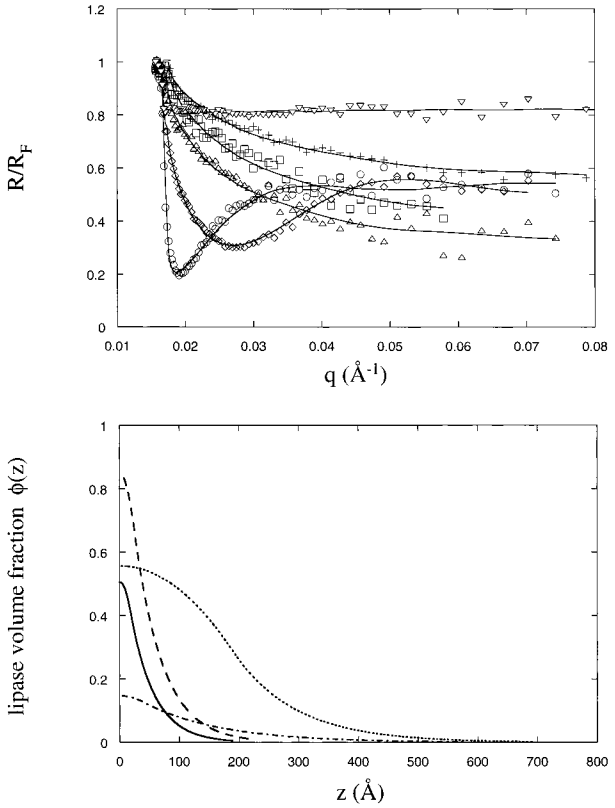

Figure 4. (a) Normalized reflectivity curves of lipase adsorbed at the air−solution interface. Lipase concentration, $C = 0.05$ mg/mL; TTAB concentration, $C_s = 0$ (crosses), $C_s = 0.05$ mg/mL (squares), $C_s = 0.10$ mg/mL (triangles), $C_s = 0.20$ mg/mL (diamonds), $C_s = 0.50$ mg/mL (circles), and $C_s = 2.0$ mg/mL (inverted triangles). The solid lines are the best fit curves using the concentration profiles shown in b. (b) Concentration profiles of adsorbed lipase at TTAB concentration, $C_s = 0$ (solid line), $C_s = 0.10$ mg/mL (dashed line), $C_s = 0.50$ mg/mL (dotted line), and $C_s = 2.0$ mg/mL (dotted−dashed line).

As can be seen from the plots, the two surfactants behave differently in the presence of the enzyme. For SDS the curve for surfactant only gradually approaches the curve for surfactant + lipase. This is the normal behavior when there is no interaction between the species in solution.[16] For TTAB the curves cross each other; i.e., over a wide range of surfactant concentration the surface tension for TTAB + lipase is higher than that for TTAB alone. For polymer−surfactant mixtures in general, this behavior indicates formation of a complex in solution at a surfactant concentration below the cmc.[16,17] Extensive surfactant packing at the air−water interface with a concomitant decrease in surface tension will not occur until the enzyme, both in solution and at the surface, is "saturated" with surfactant molecules. The more surface active surfactants will then remove the enzyme−surfactant complex from the surface, giving approximately the same surface tension as with surfactant only. A similar behavior toward the same lipase has previously been seen with other charged surfactants.[3]

Neutron Reflectivity. For the acquisition of the neutron reflectivity spectra, 2 h cycles were carried out for 8−10 h. Comparison of each 2 h spectrum shows no change with time, indicating that the adsorbed layer is formed within 2 h. The spectra were then summed for better statistics. Figure 3a shows the normalized reflectivity curves R/R_F versus q for lipase alone in D_2O at $C = 0.05$ mg/mL (crosses) and in the presence of SDS at $C_s = 3.0$ mg/mL (open circles). In this representation, the deviation from $R/R_F = 1$ is directly attributed to the lipase adsorbed at the interface. One can see that lipase adsorbs at the water−air interface in measurable amount. Addition of SDS from $C_s = 0.05-0.5$ mg/mL gives reflectivity curves which resemble that for $C_s = 0$. However, at $C_s = 2.0$ mg/mL (∼cmc), an abrupt decrease in the normalized reflectivity is measured, indicating a decrease in the amount of lipase at the surface. Subsequent increase in SDS to $C_s = 3.0$ mg/mL produces very little variation in the reflectivity. The solid lines passing through the curves are the best fit curves calculated using the concentration profiles shown in Figure 3b, where $\Phi(z)$ is the volume fraction of lipase in the adsorbed layer and z is the distance from the interface ($z = 0$) to the bulk solution ($z = \infty$). These profiles are parabolic with an exponential tail and are found to be adequate in fitting the present set of experimental data. Best fit curves are determined by minimization of the χ^2.

These results show that lipase forms a moderately dense layer at the water−air interface with a maximum volume fraction of about 0.5 in the surface region and a thickness of about 70 Å. This value is of the order of the size of a lipase molecule.[18] In the presence of SDS below the cmc, no detectable interaction of the surfactant with the lipase at the surface is registered. Above the cmc, adsorption of lipase is decreased and the residual lipase at the surface appears to form a very dilute and extended layer.

Figure 4a shows R/R_F curves for lipase in the presence of TTAB. The solid lines are calculated curves using the concentration profiles shown in Figure 4b. In contrast to SDS, TTAB shows a significant effect on the reflectivity curves, with the normalized reflectivity signal increasing with TTAB concentration. We see from the profiles in Figure 4b that addition of TTAB (0.10 mg/mL; long dashes) increases the lipase fraction in the surface region but does not increase significantly the

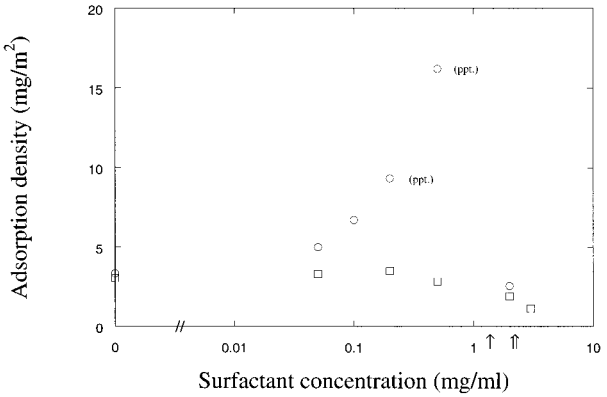

Figure 5. Adsorption density of lipase in the presence of surfactant: SDS (squares) and TTAB (circles). (ppt.) indicates a turbid solution. The single arrow indicates the cmc of TTAB and the double arrow the cmc of SDS.

thickness of the adsorbed layer. This increase in volume fraction suggests a more compact packing of the lipase molecules due to neutralization of their charges by the cationic surfactant. At TTAB concentrations in the range of about 0.20 to about 0.50 mg/mL TTAB, bulk precipitation is obtained. Under these conditions, thick adsorbed layers are obtained resulting in strong oscillations in the reflectivity curves (diamonds and circles). The concentration profile shown in Figure 4b for $C_s = 0.50$ mg/mL (dotted curve) shows that the adsorbed lipase layer is indeed rather thick, more than 5 times the thickness obtained for lipase alone. Note that the lipase fraction close to the interface decreases compared to that for 0.10 mg/mL TTAB, indicating a less compact packing. At $C_s = 2.0$ mg/mL, the precipitate is redissolved and adsorption decreases (inverted triangle). Interestingly, the adsorbed layer resembles that obtained in the presence of high concentration of SDS: very dilute and extended.

These studies show clearly the strong interactions of lipase and TTAB due to electrostatic forces. These data also show the important changes in the features of the adsorbed lipase layer in the presence of TTAB. The addition of a cationic surfactant neutralizes the lipase charges, allowing for the formation of a more compact layer; this takes place before the bulk-phase separation. Second, the adsorbed lipase−surfactant complex which is in equilibrium with the bulk precipitate forms very thick adsorbed layers of moderate compactness. When the precipitate is resolubilized (above the cmc), the residual adsorbed lipase appears extended, as in the case of SDS.

Integration of the concentration profiles gives the adsorption density of the lipase. These results are shown in Figure 5 where the adsorbed amount (mg/m^2) is plotted as a function of surfactant concentration for SDS (squares) and TTAB (circles). In the presence of SDS below the cmc, the adsorption density of lipase is unaffected. In a micellar solution however, adsorption decreases. In the presence of TTAB, the adsorption goes through a maximum, with a divergence at bulk-phase separation followed by a sharp decrease upon resolubilization of the precipitate around the cmc. The corresponding adsorbed layer thicknesses are plotted in Figure 6. Note the corresponding divergence of the thickness of the precipitated layer. Interestingly, the adsorbed layer remains rather extended even after cmc in both cases. In Figure 7 is plotted the surface concentration, $\Phi(z)$ for $z \rightarrow 0$ of the adsorbed lipase layer. These data show that the adsorbed layer is of moderate density and increases in compactness at intermediate TTAB concentration. At cmc or above, the adsorbed layer is extremely dilute and very extended as seen in Figure 6.

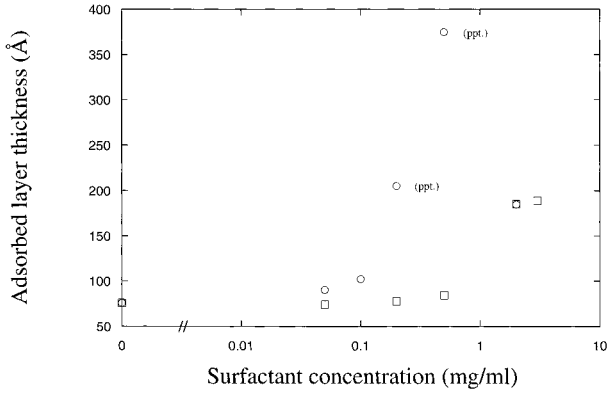

Figure 6. Thickness of the adsorbed layer of lipase in the presence of surfactant: SDS (squares) and TTAB (circles). (ppt.) indicates a turbid solution.

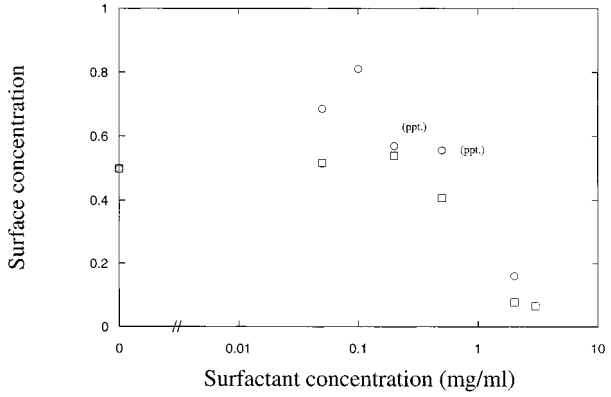

Figure 7. Surface concentration, $\Phi(z)$ at $z = 0$ of the adsorbed layer of lipase in the presence of surfactant: SDS (squares) and TTAB (circles). (ppt.) indicates a turbid solution.

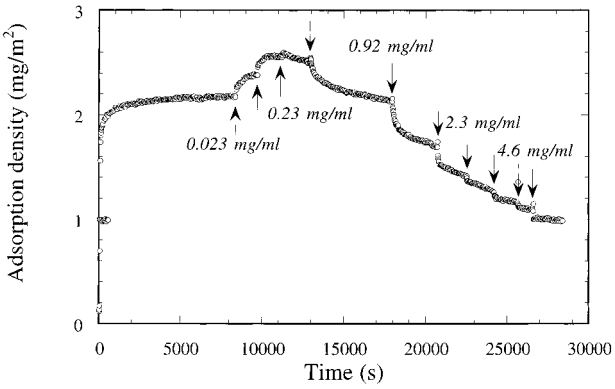

Figure 8. Adsorption of *Humicola lanuginosa* lipase followed by sequential additions of sodium dodecyl sulfate (SDS) with times for additions and surfactant concentrations indicated on the graph.

Ellipsometry. Adsorption of lipase followed by surfactant is shown in Figures 8 and 9 for SDS and TTAB, respectively. The concentration of lipase used was the same as that employed in the surface tension experiments, i.e., 0.05 wt %. As can be seen, a plateau adsorption of around 2.2 mg/m^2 is built up in 2−4000 s in both experiments. This is a reasonable value for a monolayer of a relatively small protein such as the *Humicola lanuginosa* lipase. The same value has been obtained by other workers, and it fits well with the calculated value for side-on addition of the lipase.[19] The surfactant, SDS or TTAB, is then added in increments at the times and amounts indicated on the figures. Figure 8 shows that the first additions of the anionic surfactant, SDS, result in adsorption of a small amount of

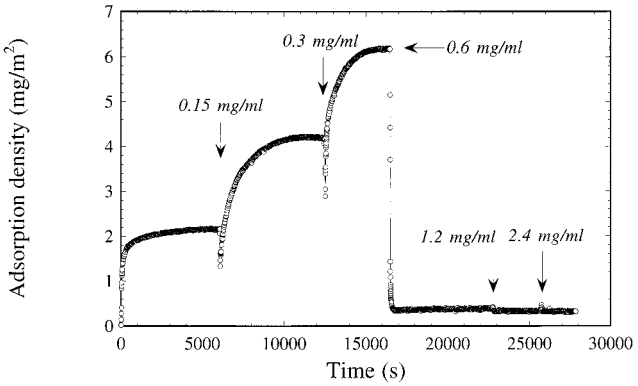

Figure 9. Adsorption of *Humicola lanuginosa* lipase followed by sequential additions of tetradecyltrimethylammonium bromide (TTAB) with times for additions and surfactant concentrations indicated on the graph.

surfactant either on top of the lipase monolayer or at free, uncovered spots of the hydrophobized silica surface. Further addition of surfactant leads to a reduction of the total amount of material (lipase + SDS) on the surface. The plateau value obtained at high SDS concentrations, around 1.0 mg/m^2, is similar to that obtained by adsorbing SDS to methylated silica from a solution around the cmc of the surfactant. Since the neutron reflectivity measurements indicate the presence of residual protein at the air−water interface also at high SDS concentration, the plateau value seen in the ellipsometry measurements can be assumed to be composed of both protein and surfactant.

The curve for lipase followed by the cationic surfactant, TTAB (Figure 9), is very different from the lipase + SDS curve. Sequential addition of the surfactant results in an increase of material on the surface from 2.2 to 6.1 mg/m^2. This behavior suggests formation of a surfactant−lipase complex at the surface. (Note that there is free lipase in solution in equilibrium with adsorbed lipase on the surface. The subsequent addition of surfactant may therefore result in adsorption of more lipase, along with surfactant, at the lipase−covered surface.) At a surfactant concentration of about 0.6 mg/mL, the amount on the surface drops dramatically and reaches a low value where it stays constant. The plateau value of 0.4 mg/m^2 probably corresponds to a combination of loosely packed protein and surfactant, as discussed above for SDS. It has been observed before that a cationic surfactant at high solution concentration gives a loose packing of surfactant molecules at a hydrophobic surface.[20]

Discussion and Conclusions

In the present paper three different techniques, i.e., surface tension measurements, neutron reflectivity, and ellipsometry, are used to study the interaction between *Humicola lanuginosa* lipase and ionic surfactants in bulk, at the air−water interface and at the hydrophobic solid surface−water interface. The results obtained are coherent and demonstrate that while the cationic surfactant TTAB forms aggregates with the lipase over a wide range of surfactant concentration both in bulk solution and at the two interfaces, there is no indication of complex formation between the lipase and the anionic amphiphile SDS at any concentration below the surfactant cmc. These observations are in line with previous results, obtained with a microbial lipase and other types of ionic surfactants.[3] The results are different from those obtained with pancreatic lipase and SDS, however, in which case complex formation was clearly demonstrated.[1]

The agreement in information between the neutron reflectivity and the ellipsometry data is particularly striking. The former technique shows that lipase adsorbs readily at the air−water interface and that SDS at low concentration does not interact strongly with this protein layer. At higher SDS concentration, the protein is displaced from the surface. On the other hand, when TTAB is added at low concentration a thick lipase−surfactant layer is formed at the surface. This compact layer can be solubilized by further addition of the cationic surfactant. Ellipsometry gives, in qualitative terms, analogous information about lipase adsorption and interaction between lipase and the two surfactants at the hydrophobic solid−water interface. Quantitatively, there are differences, of course. The adsorbed amount deduced from neutron reflectivity is higher than that obtained by ellipsometry. This can be due to the difference in the type of interfaces used in the experiments. Taken together, the results give a clear indication that (i) the events at the air−water and the hydrophobic solid−water interfaces are similar in character and (ii) data from neutron reflectivity and ellipsometry experiments correlate well with each other.

The neutron reflectivity data indicate that at high concentration of either SDS or TTAB the residual lipase at the surface forms a dilute and extended layer. This may be interpreted as unfolding of the enzyme. Unfolded lipase is likely to be more surface active than lipase in the native conformation. However, the presence of charged surfactants on the lipase molecule would render it less surface active. A small amount of macromolecule at the surface may be difficult to detect in the surface tension vs surfactant concentration plots. It is also difficult to tell from the ellipsometry results whether a minor amount of lipase remains at the hydrophobic solid surface at high surfactant concentrations.

The results presented above indicate a compact structure of the lipase−TTAB complex at the surface at low and intermediate surfactant concentration with a possible protein unfolding around and above the cmc. The question of the structure of the lipase−surfactant complex in solution has not been addressed in this study. In the literature several suggestions for the models of such aggregates are given.[21−24] Some of these, such as the "necklace model" [21] and the "flexible helix model",[22] involve considerable protein unfolding and would probably affect the biological activity of the lipase. Indeed, a necklace model would require long and flexible polymer chains, such as those proposed in the case of poly(ethylene oxide)[25] and poly(*n*-isopropylacrylamide),[26] and may be unlikely with a globular protein such as a microbial lipase in its native state. In the unfolded state, such a necklace model is possible. No investigation of the effect of the surfactants on lipase activity has been made in this work, but we have previously seen that cationic but not anionic surfactants cause a major loss of activity of a microbial lipase.[4] Thus, the formation of a complex between the lipase and the cationic surfactant TTAB at sub-cmc concentrations may cause a considerable conformational change of the lipase, but this has not been demonstrated experimentally. Another possible cause of reduced lipase activity after complex formation with a cationic surfactant is that one or more surfactant molecules have bound at or close to the active site of the lipase. It is known from other enzyme−surfactant systems that such a binding may lead to reduced biological activity of the protein.[27] One may also speculate that the aggregates that form particularly with TTAB at higher surfactant concentration may reduce the rate of transport of substrate and product to and from the lipase active site.

Studies of the behavior of lipases at hydrophobic surfaces (air, oil, or hydrophobic solids) are of particular interest since it is known that the enzyme needs such an interface in order to open the lid which covers the active site.[18,28] The interfacial

events which trigger the opening of the lid are likely to be a delicate process,[29,30] and formation of an enzyme−surfactant complex, in bulk solution or at the interface, is likely to interfere with this process. This could be another reason for the deactivation of microbial lipases by cationic surfactants.

Apart from the structural aspects discussed extensively above, an important physicochemical aspect of the systems investigated is the origin of the interaction between the surfactant and the protein. In particular, the relative importance of hydrophobic and electrostatic interactions, as well as of surfactant−protein and surfactant−surfactant interactions, is of interest. Previous investigations have shown that at least for some systems a high affinity binding occurs well below cmc, indicating the importance in this regime of protein−surfactant interactions, which may be of either hydrophobic or electrostatic nature. This is followed at higher surfactant concentrations by a cooperative binding, which is largely related to surfactant−surfactant interactions, essentially resulting in a "mixed micelle formation" or a "protein-template micellization process".[31]

Since the measurements in the present investigation are performed well above the isoelectric point of the protein, its net charge is negative, although local variations in charge density are present. Therefore the surfactant binding is favored by electrostatic interactions in the case of TTAB, but disfavored for SDS. On the other hand, hydrophobic protein−surfactant interactions favor the surfactant binding for both TTAB and SDS. Since both surfactants cause a protein conformational change resulting in an increased exposure of hydrophobic moieties, thus facilitating further surfactant binding, the latter effect is rather complex, not the least given that cationic and anionic surfactants frequently interact differently with the protein, which may result in differences in the surfactant-induced protein conformational change.[31−33]

Even more important than the effects of the protein−surfactant interactions is the cooperative surfactant self-assembly. Since both the surfactants investigated in the present study are ionic and since such surfactants display a cmc which is markedly affected by the electrostatic screening,[34] one would expect this cooperative binding to be affected also by the presence of the protein. These effects can be modeled to some extent by considering the asymmetric counterion distribution around the protein, and analytical solutions for the effective Debye length exist.[35] However, such an analysis is based on the notion of the protein not undergoing any major conformational changes, an assumption which is probably not true in the presently investigated system. We therefore feel that although this approach is certainly interesting, particularly for more rigid and stable proteins, our system is too complex for such an analysis to be relevant.

Acknowledgment. Mr. Dries Muller is thanked for help with some of the ellipsometry measurements.

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