Interaction between Lactate Dehydrogenase and Tween 80 in Aqueous Solution

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Purpose. The weak aqueous interaction between the protein lactate dehydrogenase (LDH) and the nonionic surfactant Tween 80 has been investigated, because weak protein-amphiphile interactions are of significant importance in pharmaceutical formulations, but are experimentally hard to determine. The system LDH/sodium dodecyl sulphate (SDS) was used as reference because SDS, by its strong protein binding, denatures LDH completely.
Methods. Fluorescence spectroscopy with pyrene and 1,3-bis(1-phenyl)propane (P3P) as probes, intrinsic protein fluorescence and NMR spectroscopy have been used.
Results. The fluorescence probe pyrene monitors a weak Tween-LDH interaction, detectable below the critical micelle concentration of ordinary Tween micelles. The microviscosity probe P3P shows a surfactant-induced denaturation in the case of LDH/SDS but not in the case of LDH/Tween 80. Intrinsic LDH fluorescence verifies this behavior. Pulsed-gradient spin-echo NMR was also used to verify the weak LDH-Tween 80 interaction.
Conclusions. A weak interaction between LDH and Tween 80 occurs at hydrophobic zones of the protein, but it is not strong enough to denature LDH. The experimental outline used here provides a useful approach for mapping the very weak protein-amphiphile interactions often present in pharmaceutical formulations.

KEY WORDS: protein-surfactant interaction; protein denaturation; fluorescent probes; pulsed-field-gradient spin-echo NMR.

INTRODUCTION

An increasing number of potential drugs are proteins or peptides which, up until now at least, are best administered by injection. The high demands on stability and efficiency of parenteral dosage forms require them to be freeze-dried for storage, and redissolved prior to administration. The process of freeze-drying puts severe stress on protein molecules. Protective additives are often necessary to recover sufficient activity of the polypeptide. Several studies deal with the ability of surfactants and amphiphilic polymers to protect proteins at freeze-drying and freeze-thawing (1–3).

Protein-surfactant interaction in solution has been of considerable interest in the literature the last two decades due to its number of technical applications, and the possibility of a better understanding of proteins in general, including their aggregation properties. Recent reviews (4–6) cover the area of globulins interacting with surfactants. Charged surfactants, especially anionic ones tend to denature proteins totally and e.g., sodium dodecyl sulphate (SDS), is used in gel electrophoresis, where complete denaturation of the proteins is necessary for a correct mobility and separation. Nonionic surfactants are generally "milder" in their interaction with proteins meaning they most often do not denature the protein, e.g., octylglycosides are used to recover otherwise insoluble membrane integral proteins for crystallization.

In a current freeze-thawing study a system is used in which lactate dehydrogenase (LDH) is a model protein in a solution where polyoxyethylene 20 sorbitan monooleate, Tween 80 is added. LDH is an enzyme built up by four subunits each of about 35,000 g/mol (7). Tween 80 is a nonionic surfactant with a monomer molecular weight of 1310 g/mol known for its cryoprotective properties (3,8). To elaborate the starting conditions of this system in the freeze-thawing process from room temperature to the freezing point, the interaction between LDH and Tween should be investigated. LDH/SDS is used as a reference system because SDS totally denatures LDH, and the denaturation of several proteins by SDS is well characterized (5,9,10).

A combination of fluorescence probe methods is used together with the intrinsic fluorescence of LDH to map the Tween-LDH interaction. The data are supported by NMR self-diffusion measurements in which the limitations of fluorescence probe methods are avoided, e.g., knowledge about the probe solubilization site. DSC is used to investigate the eventual phase transitions that are occurring in the studied temperature interval.

EXPERIMENTAL

Materials

LDH from rabbit muscle was obtained (ICN Biomedicals, Inc., Costa Mesa, California), as a crystalline suspension in 65% ammonium sulphate, pH 7.2. The LDH suspension was dialyzed against 10 mM sodium citrate buffer pH 6.5 and concentrated during centrifugation using Microsep Centrifugal Concentrators (Pall Filtron Company, Northborough, Massacheusetts). The concentration of the protein was determined spectrophotometrically with a Spectronic Genesys Spectrophotometer (Milton Roy Company, Rochester, New York). The UV absorbance at 280 nm was linearly related to LDH concentration in the range 0.1–1.1 mg/ml. The LDH concentration was 1 mg/ml throughout the study.

Tween 80 was obtained from KEBO AB, Sweden. SDS 99.9% was bought from MERCK, Germany. Pyrene (+98%) (Acros Chimica, Belgium) was recrystallized twice from absolute ethanol and filtered pyrene-saturated water containing $< 10^{-6}$ M pyrene. P3P (Molecular Probes, Eugene, Oregon) was added in aliquots from a P3P-acetone solution and stirred for 3 days to reduce microcrystals, a known problem with this probe. D_2O 99,9% (Isotec Inc., Miamisburg, Ohio) was used in the NMR experiments.

Methods

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) measurements were done on a Seiko (Japan) DSC 220C calorimeter tem-

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perature- and heat-calibrated with indium, tin, gallium, and mercury as standards. The gravimetrically determined samples, typically some 20 mg, were kept in aluminum pans in a nitrogen atmosphere. The temperature history included freezing of the sample from 30–40°C to −60°C followed by heating to 30–40°C. The cooling and heating rate was 5°C/min. The samples were run in triplicate, and thermograms were recorded at both cooling and heating.

Fluorescence Measurements

Steady state fluorescence measurements were performed on a SPEX Fluorolog-2 system equipped with the dm3000f software (Edison, New York). Pyrene was excited at 334 nm and emission spectra were recorded at 360–500 nm at room temperature. It has been found (11) that the first to third peak intensity ratio, I_1/I_3, of the fine vibrational emission spectrum of pyrene is sensitive to the polarity of the surrounding condensed medium. Pyrene dissolved in water gives $I_1/I_3 \approx 1.9$ (as well as in the citrate buffer used here), and pyrene dissolved in toluene gives $I_1/I_3 \approx 1.0$. In a 1 mg/ml LDH-citrate buffer solution, $I_1/I_3 \approx 1.6$. If pyrene is added to an aqueous micellar solution, I_1/I_3 will suddenly drop when the cmc is passed an addition of surfactant. I_1/I_3 has been used as an indicator of the local polarity in aqueous surfactant, polymer-surfactant, and protein-surfactant systems (5).

P3P was excited at 348 nm. Emission spectra were recorded for pyrene at room temperature. P3P consists of two pyrene molecules connected by a propane chain. With one of the pyrene entities electronically excited and the other in the ground state, the molecule is called an excimer if the two pyrenes are close in space in a sandwich-like manner. The excimer distributes its excited state over a larger π-electron system as compared to the monomer conformation, and its emission thus occurs at longer wavelengths. The intramolecular excimer formation of P3P is sensitive to the local molecular rigidity. The monomer ($\lambda = 377$ nm) to excimer ($\lambda = 490$ nm) peak intensity ratio, I_M/I_E, of the emission spectrum of P3P is a qualitative index of the microviscosity at the probe solubilization site (12). In citrate buffer, $I_M/I_E \approx 1.6$, and in 1 mg/ml LDH-citrate buffer solution, $I_M/I_E \approx 0.6$.

LDH was excited at 285 nm and emission spectra were recorded at 4, 10, and 21°C, respectively, in the range 300–700 nm.

Time-resolved fluorescence measurements were recorded on a Photon Technology International C72 spectrometer equipped with the TMaster software and a pulsed nitrogen light source coupled to a dye laser at room temperature. All lifetime data presented were satisfactorily fitted to singe exponential decays. In these experiments pyrene was excitated at 337 nm. The emission time decay of pyrene was collected at 394 nm. In citrate buffer, $\tau \approx 102$ ns, and in 1 mg/ml LDH-citrate buffer solution, $\tau \approx 146$ ns. The experimental error of all fluorescence measurements was ±5% or better as determined by some of the samples run in triplicate.

PGSE-NMR

Pulsed-field-gradient spin-echo (PGSE)-NMR (13) was used to measure the transverse self-diffusion coefficient of Tween 80. In the stimulated echo pulse sequence used here (14), three 90° pulses are separated in time by delays τ_1 and τ_2, and two magnetic gradient pulses are separated by time Δ. The echo amplitude A at time $\tau_1 + \tau_2$ is dependent on the diffusion coefficient D according to

$$A(\tau_1 + \tau_2) = 0.5A(0)\exp[-(\tau_2 - \tau_1)/T_1 - 2\tau_1/T_2 - (\gamma g\delta)^2 D(\Delta - \delta/3)]$$

where A(0) is the amplitude at time 0, T_1 and T_2 are the longitudinal and transverse relaxation times, respectively, and γ is the gyromagnetic ratio. g is the intensity and δ the duration of the gradient pulses. By recording 16 spectra varying g between 0.22 and 1.5 Tm^{-1}, keeping δ and Δ constant at 2 ms and 220 ms, respectively, D was separated from the transverse relaxation effect. PGSE-NMR measurements were performed on a Bruker AMX 300 spectrometer equipped with a wide-bore magnet and a dedicated diffusion probe head (Cryomagnet Systems, Indianapolis, IN,USA), and a gradient driver made by W.S. Woodward, University of North Carolina, Chapel Hill, NS,USA. NMR data were evaluated by the CORE-NMR (COmponent REsolved NMR) approach by Stilbs *et al.* (15). CORE-NMR is a global minimization procedure using all spectral information rather than single peak heights, which in addition to the possibility of resolving partly overlapping spectral peaks, gives an enhanced signal-to-noise ratio of about ten. Temperature was set to 25 ± 0.1°C by the temperature control program. Diffusion coefficients were measured and successfully fitted to a simple Gaussian process with an experimental error of ±1%.

RESULTS AND DISCUSSION

DSC Thermograms

To evaluate occasional phase transitions in the surfactant/buffer systems an investigation by DSC was performed of the relevant samples. Heating thermograms of the pure surfactant/buffer systems are presented in Fig. 1. In all thermograms the large ice melting endotherm appears at about 0°C. An endothermic peak is monitored between 10 and 30°C for the most concentrated system containing 256 mg/ml SDS. It reflects the melting of solid hydrated SDS that is precipitated below the Krafft point. The transition is also observed in the cooling process. Varying concentrations of Tween 80 in citrate buffer do not involve any phase transitions above the melting point of ice as monitored by DSC. However, when heating concentrated (pure) Tween, one exothermal peak at −43°C and one endothermal peak at −14°C is observed. The transitions occur at the same temperature as transitions of ice and/or PEG hydrates in aqueous PEG systems (data from this laboratory to be published elsewhere). Because no phase transitions are observed above the freezing point at the concentrations used in this study, both Tween and SDS form aqueous solutions where monomers or micelles are present from room temperature down to the freezing point of water (Tween) or the Krafft point (SDS). The LDH-Tween interaction described here at room temperature can be considered as representative for the interactions down to the freezing point.

Micropolarity

The intensity ratio of the first to third vibronic peaks (I_1/I_3) in the emission spectrum of pyrene is a measure of the

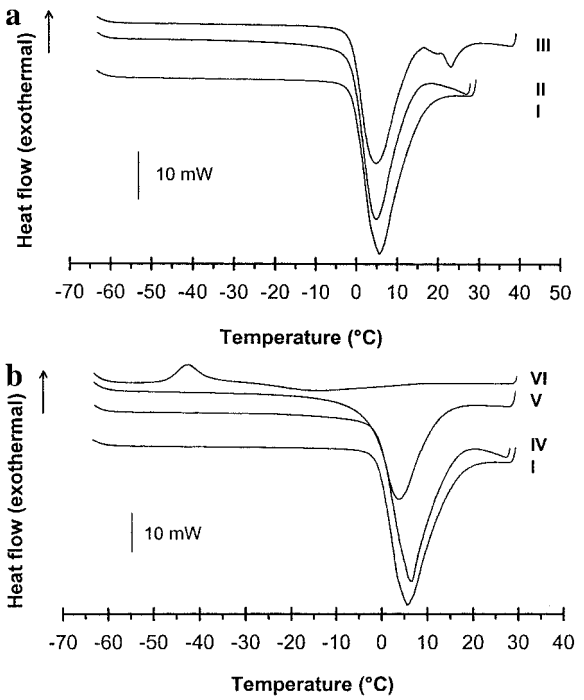

Fig. 1. Heating thermograms of SDS (a) and Tween 80 (b) solutions in 10 mM sodium citrate buffer, pH 6.5. (I) sodium citrate buffer; (II) 5.59 mg/ml SDS; (III) 256 mg/ml SDS; (IV) 0.186 mg/ml Tween 80; (V) 269 mg/ml Tween 80; and (VI) concentrated (pure) Tween 80.

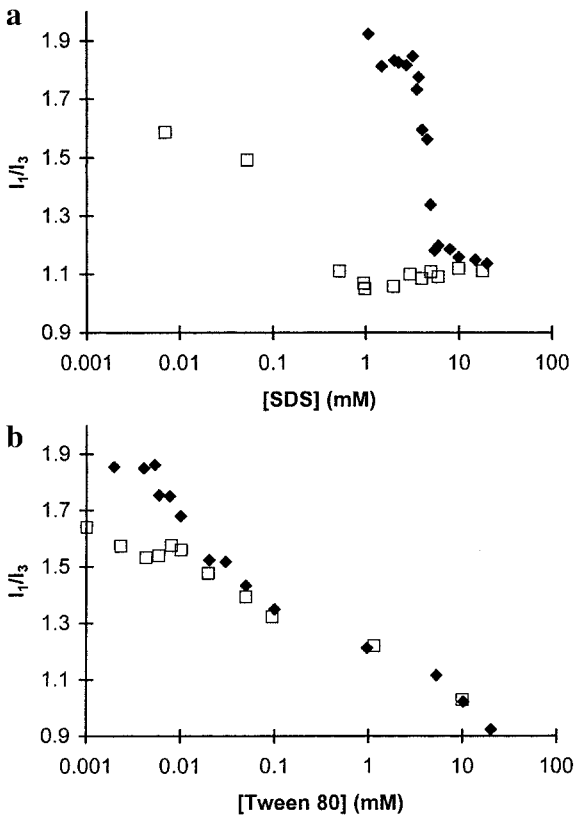

Fig. 2. The hydrophobic index, I_1/I_3, of pyrene in SDS (a) and Tween 80 (b) solutions in 10 mM sodium citrate buffer, pH 6.5, in absence (♦) and in presence (□) of 1 mg/ml LDH.

micropolarity at the solubilization site of the probe as explained in the methods section. The interaction between LDH and SDS, monitored by the fluorescence methods used here, is presented as reference.

The micropolarity-indices, I_1/I_3, of the systems with SDS or Tween are presented in Fig. 2. I_1/I_3 of SDS in pure buffer is seen to be at about 1.8 at SDS concentrations below and up to the cmc (Fig. 2a) suggesting pyrene to be in an aqueous environment. The cmc of SDS I_1/I_3 steeply decreases due to formation of micelles and a quantitative solubilization of the ensemble of pyrene molecules therein. I_1/I_3 is about 1.2 inside the hydrophobic micelle population. The cmc of SDS in this citrate buffer is about 5 mM, whereas in water cmc is 7 mM determined by the same method (16). When LDH is present at 1 mg/ml, I_1/I_3 is about 1.6 and with addition of SDS the ratio drops to some 1.1 throughout the higher SDS concentration range investigated. Hydrophobic zones are thus formed in solution from the very first addition of SDS at these concentrations, pointing to a strong LDH-SDS interaction.

The probing of very weak hydrophobic macromolecule-surfactant interactions is not a trivial task by any method, and, as illustrated below, must often include several—in this case fluorescence—methods to give a fairly accurate picture of the interaction.

The pure Tween system shows a high micropolarity (1.9) at concentrations below 0.01 mM (Fig. 2b), indicating that no micelles are present. At 0.01 mM Tween I_1/I_3 starts to decrease due to micellar growth and this growth seems to be somewhat continuous on the logarithmic scale. The reason is that larger micelles better screen pyrene from water, thereby lowering I_1/I_3. The cmc of Tween 80 in water is 0.011 mM according to the literature (17). In the presence of LDH I_1/I_3 is below 1.7 at the lowest Tween concentration. It then decreases slightly down to 1.5 just below 0.01 mM Tween, and then follows the curve of the pure Tween system. This complex behavior can explain the distribution of pyrene to hydrophobic regions on LDH at low Tween concentrations. As more Tween is added, some surfactant is adsorbed to these hydrophobic regions of the protein as monitored by the initial small decrease in I_1/I_3. In the absence of any LDH-Tween interaction I_1/I_3 would be unchanged. As the cmc of Tween is approached in solution, micelles start to form and pyrene is redistributed to the micellar population, thus changing I_1/I_3 towards that of free micelles.

Lifetime of Pyrene

The lifetime of pyrene in the SDS and LDH/SDS systems, shown in Fig. 3a, also reflects the micropolarity results. The pyrene lifetime in pure SDS in citrate buffer is 130 ns below the cmc. At the cmc, 5–6 mM SDS, the average lifetime of the pyrene ensemble steeply rises to 165 ns as a consequence of micelle formation and solubilization of pyrene. The longer lifetime of pyrene distributed to micelles is largely an effect of less collisional quenching of pyrene by dissolved oxygen inside micelles (5). In the presence of 1 mg/ml LDH the pyrene lifetime is 146 ns, and when SDS is added to the protein solution the pyrene lifetime is 235 ns. A maximum of 330 ns is reached close to 1 mM SDS followed by a drop to asymptotic values at higher SDS concentrations. Pyrene might in this system be distributed to hydrophobic zones made up by mixed LDH-SDS aggregates. The maximum suggests that pyrene is effectively shielded from oxygen, but also

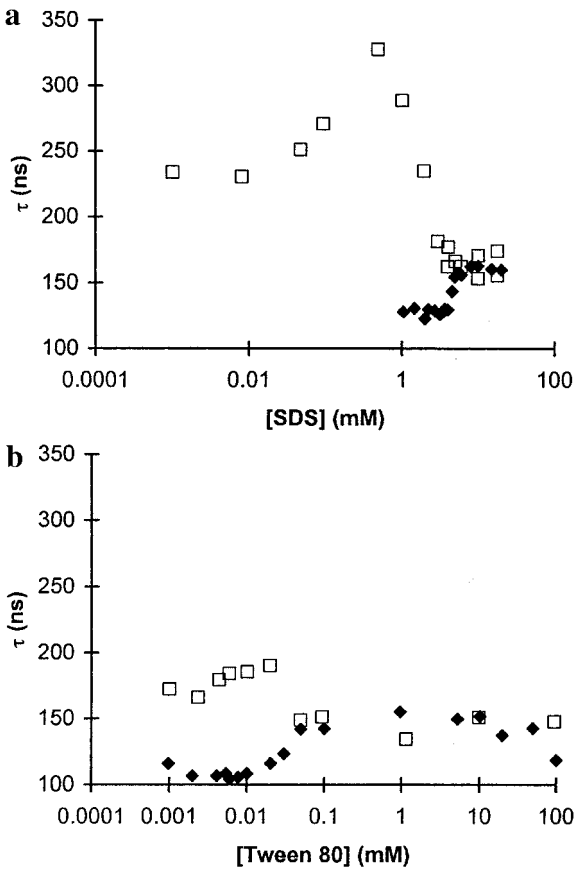

Fig. 3. The lifetime of pyrene in SDS (a) and Tween 80 (b) solutions in 10 mM sodium citrate buffer, pH 6.5, in absence (♦) and in presence (□) of 1 mg/ml LDH.

that the rigid less fluid environment in mixed protein-SDS clusters increases the pyrene lifetime, an effect further illustrated below. Higher viscosity always increases the quantum yield of a fluorophore due to less nonradiative energy loss (18). As more SDS is added micelles are formed in the system and the lifetime approaches that of pyrene in free SDS micelles.

The lifetimes of pyrene in the Tween and Tween/LDH solutions are presented in Fig. 3b. The cmc of the pure Tween system is not distinct but is starting at about 0.01 mM Tween as detected by increasing lifetimes beyond this concentration. In the presence of LDH, pyrene shows a small increase in the average lifetime at lower Tween concentrations when some Tween molecules are adsorbed to LDH. At 0.01-0.1 mM Tween the pyrene lifetime suddenly drops to values similar to that of the pure Tween system indicating a redistribution of pyrene from LDH to Tween micelles. Pyrene hence indicates the weak LDH-Tween interaction both by its steady state spectrum and its lifetime. The interaction is only detectable below the cmc of Tween where the pyrene population is distributed to the protein-Tween surroundings.

Microviscosity as Monitored by P3P

The probe P3P is particularly suitable for microviscosity studies of surfactant and polymer/surfactant systems (12,19,20). The monomer to excimer intensity ratio, I_M/I_E, has proven to be a qualitative index of the steric hindrance to formation of excimers imposed by the surroundings of the probe (12), an index often denoted by microviscosity. P3P is very hydrophobic and quantitatively dissolves to hydrophobic zones if there are any present in solution. P3P moves more freely in hydrophobically adhered clusters of small molecules than in hydrophobically adhered clusters of polymers (20), as these entangle the big P3P molecule and oppose excimer formation. I_M/I_E is thus particularly sensitive to covalently attached macromolecule segments.

Both I_1/I_3 and I_M/I_E will depend on the protein concentration. In this study the same concentration of LDH is used in all measurements, and the conclusion is based on relative changes when the surfactant concentration is varied. The interaction pattern might change with different protein concentration. Vasilescu *et. al.* (10) has shown that the interaction between BSA and a surfactant starts at a higher amphiphilic concentration when the concentration of the protein increases.

The ratio I_M/I_E of the SDS and LDH/SDS systems is presented in Fig. 4a. It is evident that the microviscosity I_M/I_E remains fairly unchanged for the pure SDS/buffer system throughout the composition interval investigated. In the presence of LDH on the other hand, a maximum is observed just below 1 mM SDS, coinciding with the maximum in pyrene lifetime. The maximum in I_M/I_E is obtained where SDS has denatured LDH enough to dissolve P3P in the protein-

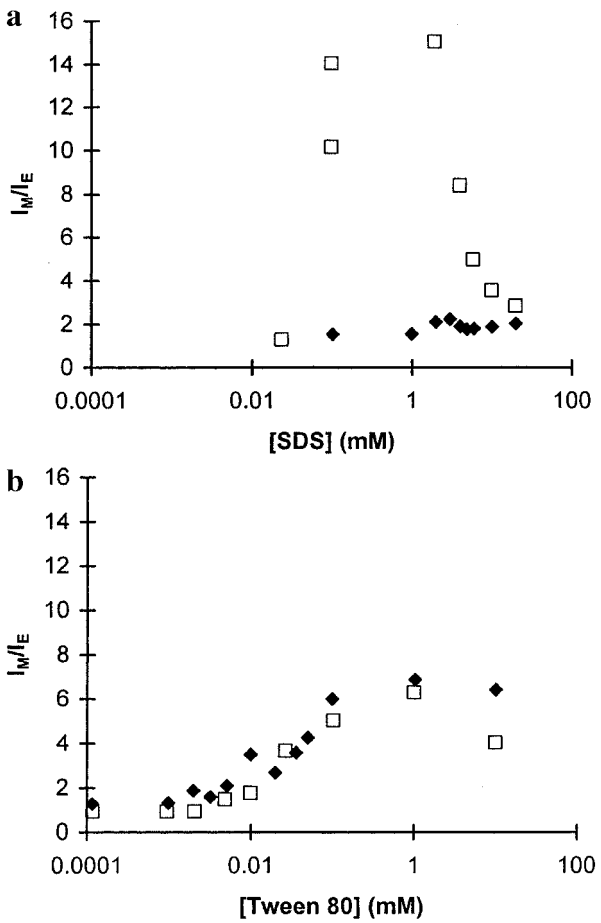

Fig. 4. The microviscosity index, I_M/I_E, of P3P in SDS (a) and Tween 80 (b) solutions in 10 mM sodium citrate buffer, pH 6.5, in absence (♦) and in presence (□) of 1 mg/ml LDH.

surfactant aggregate, but where the protein segment content is high enough to effectively hinder excimer formation. Addition of SDS beyond the maximum further denatures LDH with more SDS adsorbed to the protein. The aggregates become more fluid as relatively more low-molecular weight SDS molecules constitute the mixed LDH-SDS micelles and I_M/I_E is decreasing. P3P is also distributed to free micelles in the solution at higher SDS concentrations, resulting in lower I_M/I_E-values. This observation is similar to the aggregation-deaggregation process of cellulose ether/SDS systems also monitored by P3P (20).

In the Tween systems (Fig. 4b) no interaction between LDH and Tween is detectable. The I_M/I_E variation with concentration of both the Tween and LDH/Tween systems are insignificantly different. Both curves show the continuous micelle growth beyond the cmc in accordance with previously shown lifetime and microviscosity data. This is not too surprising if the protein-Tween interaction is very weak. To significantly decrease the ability of P3P to form excimers, LDH must be denatured enough to host at least part of the P3P molecule.

LDH Fluorescence

One of the prerequisites using free dissolved probes in fluorescence spectroscopy is knowledge of the probe solubilization site(s) in the case of microheterogeneous solutions as e.g., the protein/surfactant systems studied here. In the case of proteins, one has the advantage to use the intrinsic protein fluorescence largely originating in the tryptophan (Trp) residues. Protein fluorescence is, however, complex. Even single-Trp proteins show multiexponential fluorescence decays (18), and proteins with several Trp residues give steady state spectra and lifetimes that are averages of the residues, which all have different chemical environments depending on their ternary position in the protein. Trp emission around 350 nm is also sensitive to the hydrophobicity. Trp residues at the surface of a globular protein often show emission maxima at a longer wavelength than Trp residues in the core of the protein. The emission maximum can therefore change if denaturation takes place, an effect depending on the Trp positions in the native protein (18). The Trp emission intensity often changes at denaturation due to increased molecular mobility and collisional quenching. The wavelength of the emission-maximum of LDH and its emission intensity, when mixed with SDS or Tween 80, are given in Fig. 5.

It can be seen from Fig. 5a that the fluorescence intensity decreases upon addition of SDS down to an asymptotic level at and above 5 mM SDS. The wavelength of the emission maximum passes through a minimum of 342 nm at about 1 mM SDS and then increases again up to 354–356 nm at and above 5 mM SDS. These measurements are in some sense a fingerprint of the denaturation of LDH by SDS. The minimum in Fig. 5a coincides with the maximum in microviscosity and further strengthens the picture of a partly denatured protein, the hydrophobic parts of which are covered with SDS. Further addition of SDS denatures the protein completely and exposes the inner Trp residues, again increasing the overall emission wavelength.

The SDS-LDH interaction pattern outlined above is similar to that of SDS in other protein systems (5,9,10) as well as SDS mixed with nonionic polymers (5,20). In summary, the

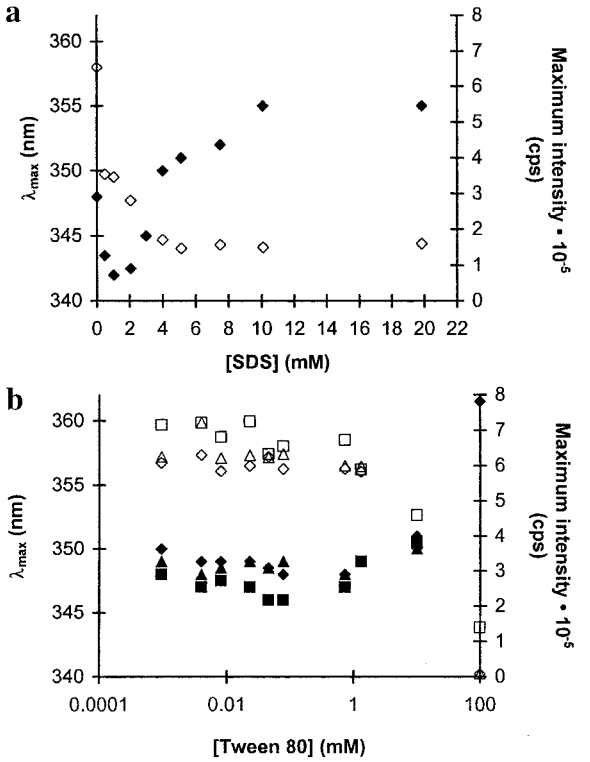

Fig. 5. The emission fluorescence of tryptophan in 1 mg/ml LDH in SDS (a) and Tween 80 (b) solutions in 10 mM sodium citrate buffer, pH 6.5. Filled symbols correspond to wavelength of the maximum and open symbols to the maximum intensity at 21°C (◆, ◇), at 10°C (▲, △) and at 4°C (■, □).

combination of fluorescent methods above gives a quite detailed picture of the denaturation of LDH by SDS including the concentration region required to totally denature LDH, which seems to be 5 mM SDS at 1 mg LDH/ml solution.

Both the protein emission intensity and the wavelength maximum of LDH mixed with Tween (Fig. 5b) remain fairly unchanged over the concentration region investigated. At the highest Tween concentrations, however, the intensity decreases and the wavelength maximum increases. This is an artifact due to the weak Tween absorption at 285 nm resulting in an inner filter effect. The conclusion from these measurements must be that the interaction is too weak to denature LDH. To evaluate the temperature dependence of the LDH-Tween interaction, the protein fluorescence was measured also at lower temperatures (Fig. 5b). The wavelength maximum shows a small decrease when the temperature is lowered. This might indicate a small change in the conformation of the protein. As the temperature decreases the kinetic fluctuations of the solution is reduced, there is less collisional quenching, and hence, the fluorescence intensity increases as seen in Fig. 5b. The observations show that the interaction pattern between LDH and Tween does not change significantly as the temperature decreases from 21 to 4°C.

PGSE-NMR

To verify and quantify the micellar growth of Tween, and the weak Tween-LDH interaction, NMR self-diffusion measurements were performed. Pulsed field gradient spin-echo NMR (PGSE-NMR) data on the Tween/D_2O, Tween/D_2O-

buffer, and LDH/Tween/D_2O-buffer systems are presented in Fig. 6. The lowest Tween concentration investigated here is 0.1 mM, and due to sensitivity reasons that is from the late part of the cmc region and up. The pure Tween system in both D_2O and D_2O-buffer shows overlapping diffusion coefficient curves, indicating that the buffer does not significantly alter the cmc or shape/size of the surfactant micelles. The diffusion coefficients for these systems first decrease rapidly and then slowly at higher surfactant concentrations. The last part of the cmc region is hence monitored followed by a region with micelle growth. Tween micellar aggregate size of some 76,000 g/mol (an aggregation number of 58) have been reported (21), which is in agreement with a micelle diffusion coefficient in the 10^{-11} m^2s^{-1} range. As a reference, the self-diffusion coefficient of pure SDS micelles with an aggregate size of some 18 000 g/mol (aggregation number of 60–70) is about $1{\cdot}10^{-10}$ m^2s^{-1}. In the presence of LDH, the drop of the diffusion coefficient curve, the cmc, comes at a slightly higher Tween concentration. This is a strong indication of some LDH-Tween interaction. A reasonable explanation is that Tween interacts with LDH in such a way that the effective free Tween concentration in solution able to form micelles decreases, leading to a slight increase in the cmc. Once micelles are formed in the ternary LDH/Tween/buffer system, the diffusion coefficients become similar to the binary Tween/solvent systems. Unfortunately, the low sensitivity of NMR does not permit measurements at lower Tween concentrations. The interaction pattern follows the normally accepted model for surfactant-protein interaction with an onset of interaction below the normal cmc of free surfactant and micellar-like systems at higher surfactant concentrations (5).

If Tween is bound to LDH the diffusion should decrease, if fast exchange conditions are present. Here, when adding LDH the apparent Tween diffusion coefficient is increased. That might be an effect of the fact that the LDH-Tween interaction moves the cmc to higher surfactant concentrations, and the experiment thus monitors the drop of the curve at cmc. Furthermore, LDH is in a suspension and the signal from any bound Tween to a solid LDH particle might be reduced compared to the signal from free Tween molecules.

The results suggest only a weak Tween-LDH association, because the effect of added protein on the Tween diffusion coefficient is small but significant. Other methods used to study similar nonionic surfactant/protein systems also detect a very weak interaction. Microcalorimetry measurements for example, on the system Human Growth Hormone/Tween 80 by Bam *et al.* (22), monitored a binding enthalpy as weak as 240 J/mol surfactant. Because the LDH-Tween association is very weak and detectable only at about the cmc, any calculation of the amount Tween bound to the protein from these PGSE-NMR data would be very ambiguous.

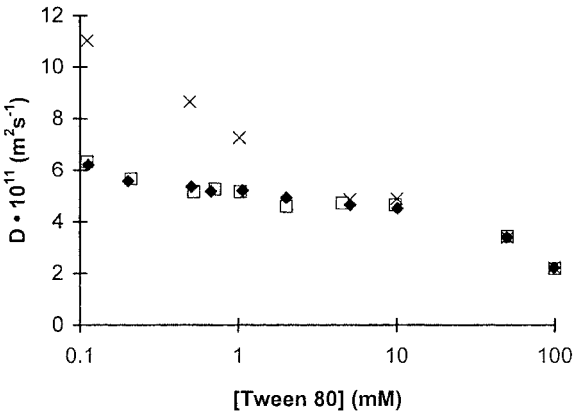

Fig. 6. The observed diffusion coefficient in Tween 80 solutions in D_2O (♦), in 10 mM sodium citrate buffer in D_2O, pH 6.5 (□), and in sodium citrate buffer in D_2O with 1 mg/ml LDH (×).

CONCLUSIONS

A hydrophobic interaction between LDH and Tween 80 is monitored at room temperature by fluorescence and NMR spectroscopy at and below the cmc of Tween. This interaction occurs at hydrophobic zones of the protein, but it is not strong enough to denature LDH. Further, the cmc of Tween is slightly altered in the presence of LDH. To understand such a weak interaction, a strong protein-surfactant interaction showing a very clear pattern may be used for comparison. The strong denaturation of LDH by SDS helps this interpretation.

The study verifies pyrene as a probe to monitor relative micellar growth, with reference to diffusion coefficients monitored by PGSE-NMR, and the probe P3P as a microrigidity-sensor for charged macromolecule-amphiphile interactions. From DSC experiments, it is obvious that no phase transitions are influencing the system between room temperature and the freezing point, at the concentrations used.

The combination of these techniques provides a useful approach to detect even very weak protein-surfactant interactions. Knowledge about the interactions between LDH and Tween at temperatures in which the system is in liquid state is crucial for understanding the protection mechanism of LDH by Tween at freeze-thawing—a study under current investigation at this laboratory.

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