Interactions of pancreatic lipase with bile salts and dodecyl sulfate

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Abstract  Sodium dodecyl sulfate (SDS) binds to pancreatic lipase in a cooperative manner up to about 200 moles/mole of protein. This binding results in a rapid and irreversible inactivation of lipase. Bile salts under certain conditions prevent SDS inactivation of lipase when both detergents are present together. Under these conditions bile salts also prevent the binding of SDS to lipase. This effect is parallel to and probably mediated by a decrease in the SDS monomer concentration as the result of the formation of mixed bile salt–SDS micelles.

Supplementary key words  dodecanoyl taurate  

It has been previously shown that pancreatic lipase is irreversibly inactivated in solutions of detergents of the alkyl sulfate type such as SDS (1). On the other hand lipase is stable in bile salt solution and bile salts prevent irreversible inactivation of lipase in the presence of SDS (1).

The present investigation was undertaken to study further the interaction between SDS and lipase and the mechanism by which the simultaneous presence of bile salts affords protection for lipase against SDS.

MATERIALS

Tributyrin used as substrate for lipase was a product of BDH Chemicals Ltd., Poole, England, and was purified by fractional distillation. Deoxycholic acid was a product of BDH Chemicals; it was >98% pure as judged by TLC. Synthesis of dodecanoyl taurate (DT) was analogous to that of conjugated bile salts (2), and was >99% pure as judged by TLC. Under the conditions of the experiments its critical micellar temperature was about 25°C, it was solubilized by heating in water to approximately 30°C in concentrated solution and it stayed in solution under experimental conditions. SDS was a product of Sigma (Sigma Chemical Company, St. Louis, Mo.), “lauryl sulfate sodium salt approx. 95%”. It was recrystallized three times from 95% ethanol (3). [³H]TDC and [³H]TC were synthesized in this laboratory (4). The radiopurity as revealed by TLC was greater than 97%. [³⁵S]SDS was obtained from the Radiochemical Center, Amersham, England. It had to be further purified by extraction into n-butanol to obtain a radiopurity on TLC of >99%. Porcine pancreatic lipase prepared as described by Verger et al. (5) was pure as judged by disc electrophoresis; it was further purified to remove colipase activity (6). Lipase concentrations were determined spectrophotometrically using E¹ cm = 13.3. The molecular weight of lipase was taken to be 52,000 (6). Pancreatic colipase was obtained from porcine pancreas as described by Erlanson, Fernlund, and Borgström (7). Dialysis tubing 23/32 in diameter (Union Carbide Corp., New York, N.Y.) was treated and used as previously described (8); we also used Spectrapor membranes (Spectrum Medical Industries Inc., Los Angeles, Calif.). The approximate molecular weight cutoffs for Spectrapor membranes 1–3 are given as 6000–8000, 12,000–14,000, and 3500, respectively. The 23/32 in Union Carbide tubing is intermediary between Spectrapor 1 and 3 as judged from the rate of dialysis of a bile salt solution.

Determination of lipase stability

In the experiments in which the effect of the different detergents or combinations of detergents on lipase activity was studied the procedure was as follows. Lipase, 0.1 nmole, was incubated overnight in a 1 ml solution of the detergents containing 150 mM NaCl, 2 mM Tris–maleate, pH 7.0, and 1 mM CaCl₂. For determination of lipase activity 100 µl aliquots of these solutions were added to incubation flasks containing 15 ml of the above buffered solution containing, in addition, 4 mM TDC, [³H]TDC, [³H]TC, [³⁵S]SDS, [³⁵S]S-bile sulfate. 

Abbreviations: CMC, critical micellar concentration; DT, dodecanoyl taurate; TDC, taurodeoxycholate; TC, taurocholate; SDS, sodium dodecyl sulfate; TLC, thin-layer chromatography; [³H]TDC, [³H]H-labeled taurodeoxycholate; [³H]TC, [³H]H-labeled taurocholate; [³⁵S]SDS, [³⁵S]S-labeled sodium dodecylsulfate.
Determination of lipase-detergent binding by equilibrium dialysis

Binding studies were performed by equilibrium dialysis using different experimental approaches. In the early experiments 5 ml glass vials containing 2 ml of buffer or detergent solution were used. One ml of the protein solution in buffer or detergent solution was added to a dialysis bag that was inserted into the vials and secured by a snap-on cap as described previously (8). At low detergent concentration, equilibrium was obtained in 24–48 hr. When the detergent concentration was above the CMC, equilibrium times of 10–14 days had to be used and in these experiments the detergent was applied on both sides of the membrane from the beginning.

In later experiments we used Lucite cells with shallow compartments of equal volume (≈1.2 ml) separated by the membrane (9). The cells were rotated at a rate of about 10 rpm and equilibrium was obtained after 24–48 hr for detergent concentrations below the CMC. The figures given are generally means from two chambers; the reproducibility of the method was ±10% (8).

For binding studies above the CMC we also used a three-chambered equilibrium dialysis cell (9). As used by Robinson and Tanford (9) the middle chamber contained the detergent solution from the beginning; the other two chambers contained buffer and protein solutions, respectively. In these experiments the binding was calculated from the difference in detergent concentration between the two outer chambers, the concentration in the “buffer chamber” at equilibrium being equal to the free detergent concentration in equilibrium with the lipase. Detergent concentrations were based on radioactivity determination of 10–200 μl aliquots of the different solutions.

Gel filtration experiments

Gel filtration experiments were performed to determine micellar size and relative micellar content of TDC and SDS in mixed micellar solution. Sephadex G-100 in K9/30 columns (Pharmacia Fine Chemicals, Uppsala, Sweden) was used (bed volumes = 18 ml). The columns were pre-equilibrated with labeled detergent solutions slightly above the CMC: 1 mM [3H]TDC or 1 mM [35S]SDS, or a mixture of both detergents. A volume of 0.5 ml of a micellar solution that was 10 mM in TDC, in SDS, or in mixture was applied and the column was eluted with the solution used for equilibration. Detergent concentrations in the eluted fractions (≈0.6 ml/fraction) were determined by radioactivity. The void volume was 6.8 ml as determined with blue dextran; the total volume (using 3H2O) was 18.7 ml. The Kav for the detergents alone or in mixture was calculated as described (8).

Determination of CMC for TDC and SDS alone or in mixtures

Determination of CMC for single detergent solutions or solutions of detergent mixtures was carried out with the spectral shift method using Rhodamine 6 G as described by Carey and Small (10). The CMC was calculated from the extrapolated intercept as given by these authors. A Unicam S.P. 800 instrument was used to record the spectra.

This method does not give information as to the

![Fig. 1](image)

**Fig. 1.** Initial rates of dialysis of SDS (ordinate) vs starting concentrations of SDS (abscissa). Buffer: 2 mM Tris-maleate, 150 mM NaCl, pH 7.0. A spectrapor membrane 3 was used.

![Fig. 2](image)

**Fig. 2.** Activity of pancreatic lipase after incubation for 18 hr in different concentrations of SDS alone ○ --- ○, or in the presence of 3 mM ● --- ●, 6 mM ▲ --- ▲, and 12 mM TDC ■ --- ■.
RESULTS

Effect of preincubation with SDS, TDC, and TC on the activity of pancreatic lipase

Pancreatic lipase incubated in 150 mM NaCl pH 7.0 for 24 hr at 25°C in the presence of 1 mM, or higher concentrations of SDS, was completely and irreversibly inactivated. The inactivation occurred rapidly, and its rate was dependent on detergent concentration. Fifty percent inactivation was obtained in 150 min with 1 mM SDS, and in ca. 2 min with 3 mM SDS. With 0.2 mM SDS half the activity was lost in 24 hr. Lipase activity could not be restored by later dilution in 4 mM TDC or by dialysis against 4 mM TDC for a long period of time. In the presence of bile salts in the concentration range tested (TDC 0.2-12 mM, TC 0.8-16 mM) lipase was stable. The protective effect of TDC or TC on lipase incubated with SDS under certain conditions is shown in Figs. 2-4.

Fig. 2 shows the effect on lipase activity of preincubation with different concentrations of SDS in the presence of 0, 3, 6, and 12 mM TDC. SDS at concentrations of 1 mM or greater resulted in an irreversible inactivation of lipase (see above). However, in the presence of 3 mM TDC and 3 mM SDS, or 6 mM TDC and 6 mM SDS, lipase was completely protected. At 3 mM TDC the SDS concentration had to be increased to 9 mM to obtain complete inactivation. Fig. 3 shows the effect of TDC concentration on the activity of lipase preincubated with different concentrations of SDS in the presence of 0, 3, 6, and 12 mM TDC. SDS at concentrations of 1 mM or greater resulted in an irreversible inactivation of lipase (see above). However, in the presence of 3 mM TDC and 3 mM SDS, or 6 mM TDC and 6 mM SDS, lipase was completely protected. At 3 mM TDC the SDS concentration had to be increased to 9 mM to obtain complete inactivation. Fig. 3 shows the effect of TDC concentration on the activity of lipase preincubated at four different concentrations of SDS. It is seen that complete protection for lipase could be obtained at all levels of SDS tested and that the relationship between SDS and TDC was nearly

![Graph](https://example.com/graph1.png)

**Fig. 3.** Activity of pancreatic lipase after incubation for 18 hr in different concentrations of TDC in the presence of 1 mM V — V, 3 mM ○ — ○, 6 mM ▲ — ▲, and 9 mM SDS ■ — ■.

Relative monomer concentrations of the two detergents when present in mixtures. To obtain approximate values for the effect of TDC on the SDS monomer concentration, we measured the rate of dialysis of SDS alone and in mixture with TDC. Determination of CMC by equilibrium dialysis was first attempted by Yang and Foster (11) and further elaborated by Abu-Hamdiyyah and Mysels (12) for SDS. The assumption made is that the dialysis membrane allows the free passage of the monomeric detergent ions but prevents passage of the micelles. In the dialyzed solution the micelles remain in rapid equilibrium with the monomers that have the same concentrations as in the dialyze. Abu-Hamdiyyah and Mysels (12) showed that dialysis also continued above CMC, indicating an increase in monomer concentration with total concentration even above the CMC. We used this method for TDC and SDS solutions with the Lucite cells described above and with Spectrapor 3 membranes.

We have measured the initial rates of dialysis and related these values to the total detergent concentrations. It appears from Fig. 1 that the initial rate of dialysis for SDS solutions is directly related to total detergent concentration at low detergent concentrations. In accord with the findings of Abu-Hamdiyyah and Mysels (12), the rate of dialysis continued to increase even when the total concentration of SDS was above the CMC. In this paper the effect of TDC on the initial rate of dialysis of SDS was used to indicate changes in SDS monomer concentration. Six mM [35S]SDS alone or in the presence of 3, 6, or 12 mM TDC was used for these experiments.

![Graph](https://example.com/graph2.png)

**Fig. 4.** Activity of pancreatic lipase after incubation for 18 hr in different concentrations of TC and 3 mM SDS.
Binding of SDS to pancreatic lipase alone or in combination with TDC by equilibrium dialysis

The binding of SDS to lipase was determined at different concentrations of SDS with 36 nmoles of lipase per ml in the inner solution using the dialysis bag procedure. The results are in Fig. 5, which shows a plot of moles of SDS bound per mole of lipase vs log concentration of unbound (free) SDS. Binding of SDS to lipase started at a concentration of free SDS on the order of 0.3 mM and increased markedly with an increase in free SDS concentration; a maximum binding of approximately 200 moles/mole of lipase was reached at 3.2 mM SDS. Further increase in free SDS did not lead to any increase in binding; the observed decrease was most probably due to the difficulty in reaching equilibrium at high SDS concentrations. The binding of 200 moles of SDS per mole of lipase corresponds to 1.1 g/g of protein, using 52,000 for the molecular weight of lipase.

We have also varied the concentration of SDS at a constant concentration of TDC (3 mM). The results in Fig. 5 show that the presence of TDC causes a shift of the binding curve to the right, i.e., higher concentrations of SDS are needed to obtain a similar extent of binding. The binding studies were repeated in two experiments with 3 and 6 mM SDS in the presence of 3 and 6 mM TDC. The results are shown in Fig. 6. The presence of TDC decreased and finally almost completely prevented the binding of SDS to lipase.

In the above experiments binding was measured when both TDC and SDS were present. Experiments were also carried out in which lipase was first equilibrated with 3 mM [35S]SDS for 16 hr. The solution was then dialyzed against 3 mM [35S]SDS, 9 mM TDC in the middle compartment of the dialysis cell (with 3 mM [35S]SDS also in the lateral compartments). The results showed that the SDS previously equilibrated with lipase (approximately 100 moles of SDS bound per mole of lipase) was largely displaced from the lipase by the presence of TDC (14 moles of SDS bound per mole of lipase at equilibrium). The binding of SDS to lipase therefore is reversible.

It was observed in these experiments that the addition of 9 mM TDC to the middle compartment of a three-compartment cell (with 3 mM [35S]SDS in all three compartments) resulted in the rapid initial i-
Fig. 7. Effect of TDC on the equilibrium dialysis of SDS. Two compartment cells were used with a Spectrapor 2 membrane. At the start both compartments contained 3 mM [35S]SDS in buffer, pH 7.0, and with 9 mM TDC in one of the compartments. 20 μl samples were taken from both compartments at different times and assayed for radioactivity. (●) represents SDS concentration in the side in which 3 mM SDS was alone from the beginning and (▲) represents SDS concentration in the side that contained both 3 mM SDS and 9 mM TDC.

crease in SDS concentration in this compartment. This phenomenon was separately investigated and the results are given in Fig. 7. The most probable explanation is that the presence of TDC decreased the monomer concentration of SDS, resulting in a rapid flow of monomeric SDS into this compartment. This effect would be counteracted by a flow of TDC in the other direction until equilibrium was finally reached.

When 3 or 6 mM [3H]TDC was equilibrated with lipase in a three-chamber dialysis cell (lipase in one of the lateral cells) and SDS was then added to all compartments in a final concentration of 3 or 6 mM, respectively, no binding of TDC to lipase could be measured.

In the dialysis experiments with 6 mM SDS, the remaining lipase activity (given in Fig. 6) was measured and plotted as a function of moles of SDS bound per mole lipase. The results are seen in Fig. 8 and indicate that the remaining lipase activity is inversely related to the number of SDS moles bound per mole of lipase. The curve indicates that 6 mM TDC did not fully protect the enzyme from inactivation (as shown in Fig. 3). This is most probably explained by loss of enzyme activity during the long incubation time in the dialysis experiments.

**Gel filtration experiments**

The radioactivity peaks for [3H]TDC, [35S]SDS, and a 1:1 mixture of the detergents were eluted from the column with $K_{av}$ values of 0.50, 0.21 and 0.33. The values for TDC and SDS micelles were similar to those previously reported for these detergents and correspond to a hydrated radius of the equivalent sphere of the micelles of 20 Å and 35 Å, respectively (13). The corresponding value for the 1:1 TDC–SDS micelles was 28 Å.

The elution volumes for the peaks of SDS and TDC when applied to the column in a 1:1 ratio were identical (Fig. 9) and lay between the values for each of the detergents alone. The ratio SDS to TDC was largely constant and the same as that in the applied mixture. The results indicate that mixed SDS–TDC micelles are present in the solution with approximately the same ratio between the two detergents as that in the mixture applied to the column.

**The CMC for SDS and TDC in mixtures**

The CMC values determined for SDS, TDC, and a mixture of both by the spectral shift method are given in Table 1. The results indicate that the CMC for the mixtures is lower than for the individual components.

When 6 mM [35S]SDS was dialyzed alone or in the presence of different concentrations of TDC, the initial rate of dialysis of SDS decreased with an increase in TDC concentration; this is shown in Fig. 10. At a concentration of 6 mM SDS and 9 mM TDC in the same solution, the rate of dialysis of SDS was decreased to approximately one-fourth that of a pure SDS solution. As was discussed in the experimental section, the rate of dialysis is related to the monomer concentration of SDS. The most likely explanation is that the simultaneous presence of TDC decreases the monomer concentration of SDS through the formation of

![Fig. 8. Lipase activity remaining after dialysis for 6 days as a function of the moles of SDS bound per mole of lipase. The initial concentration of SDS was 6 mM. (Same experiments as in Fig. 5.) The activity is related to the amount of lipase added to the dialysis bag at the start of the experiment. The figures 1-6 adjacent to the curve represent the concentrations of TDC.](image-url)
mixed SDS-TDC micelles. Similar experiments with labeled TDC likewise indicated a decrease in its monomer concentration in the presence of SDS.

**DISCUSSION**

It was mentioned in a previous paper that incubation of pancreatic lipase with SDS leads to irreversible inactivation and that TDC protects lipase from such inactivation (1). The quantitative relationship for these interactions has now been further studied. It has to be emphasized that the interactions studied are related to the irreversible inactivation of lipase when incubated with SDS and they have nothing to do with the acute detergent effects on lipase activity in the presence of substrate. The results show that lipase is rapidly, completely, and irreversibly inactivated by SDS, the rate of inactivation being related to the SDS concentration. The simultaneous presence of bile salt will prevent this inactivation, and certain stoichiometric relationships between bile salts and SDS are indicated.

The results of the present equilibrium dialysis studies show that SDS starts to bind to lipase at monomeric concentrations below CMC and that a cooperative interaction takes place above the CMC that leads to a saturation value of around 200 moles of SDS/mole of protein. The binding isotherm in this respect is similar to that described for lysophosphatidylcholine by Haberland and Reynolds (14). It is well documented that SDS binds in a cooperative manner to many proteins and that this binding is parallel to a conformational change of the protein as indicated by changes in their circular dichroic spectra (15). The results of other experiments, which will be published separately, indeed show that lipase also undergoes conformational changes in SDS solution. These changes obviously result in irreversible loss of lipase catalytic activity and this has been used in the present investigation to indicate the effect of SDS on lipase. Addition of TDC to the SDS solution decreased the extent of binding of SDS to lipase, and at equimolar concentrations of SDS and TDC the binding of SDS to lipase was reduced to 5-10%.

In another series of experiments it was shown that

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**TABLE 1.** CMC for SDS and TDC alone and in mixtures determined by the spectral shift method

<table>
<thead>
<tr>
<th>Ratio SDS:TDC</th>
<th>CMC mM</th>
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<tbody>
<tr>
<td>1:1</td>
<td>0.49</td>
</tr>
<tr>
<td>0:1</td>
<td>1.18</td>
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<tr>
<td>1:1</td>
<td>0.38</td>
</tr>
<tr>
<td>2:1</td>
<td>0.34</td>
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Details of experimental conditions are in the text. The solutions were 150 mM in NaCl, 1 mM in CaCl₂, and 2 mM in Tris-maleate, pH 7.0.

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the presence of TDC shifted the binding curve for SDS to the right, i.e., in the presence of TDC higher concentrations of SDS were needed for binding of SDS to lipase to occur. It was also found that the inactivation of lipase was related to the extent of binding of SDS to lipase. It therefore seems reasonable to conclude that TDC protects lipase from SDS inactivation by preventing the binding of SDS to the protein. The question then is why does TDC prevent binding of SDS?

One obvious possibility is that bile salts can compete favorably with SDS in binding to a specific site on lipase that is important for its natural conformation. This possibility is probably not due to the present lipase that is important for its natural conformation.

It was found that the inactivation of SDS is decreased when in mixture with TDC may be explained by an initial decrease in monomer concentration even though this conclusion may not be completely justified by the experimental results.

Three different lines of evidence indicate that the monomer concentration of SDS is lowered by the presence of TDC. (1) The CMC for the mixture measured by the spectral shift method is lower and, if mixed micelles are formed, thermodynamics requires that the CMC of each amphiphile be lower. (2) The dialysis experiments in which a rapid inflow of SDS takes place into the compartment to which TDC is added can best be explained by an initial decrease in monomer concentration as mixed micelles are formed. (3) The finding that the initial rate of dialysis of SDS is decreased when in mixture with TDC may be the result of a lowered monomer concentration even though this conclusion may not be completely justified by the experimental results.

The initial binding of SDS to lipase may take place from SDS monomers below the CMC. When the concentration of SDS is increased over the CMC, the cooperative binding of SDS to lipase results from the same forces that lead to the formation of micelles. Lipase directs the binding of monomeric amphiphiles and governs the final structure of the complex. The formation of mixed TDC–SDS micelles decreases the monomer concentration of the amphiphiles and prevents binding to lipase. Another way of stating this is that the TDC–SDS interactions due to the formation of mixed micelles are more favorable than the SDS–lipase interaction that otherwise would lead to a cooperative binding of SDS to lipase.

The finding that a detergent such as DT that is closely related to SDS chemically does not denature lipase is of interest. It is also of interest that DT in mixtures with SDS does not prevent lipase denaturation, nor does it decrease the monomer concentration of SDS to the same extent as bile salts. In this respect bile salts may have unique properties but the experience with other types of detergents so far is limited.

This work was supported by the Swedish Medical Research Council (grant B75-13X-71).

Manuscript received 4 February 1976 and accepted 20 May 1976.

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