Activation, Inhibition, and Destabilization of *Thermomyces lanuginosus* Lipase by Detergents†

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Received September 20, 2004; Revised Manuscript Received November 12, 2004

ABSTRACT: Lipases catalyze the hydrolysis of triglycerides and are activated at the water−lipid interface. Thus, their interaction with amphiphiles such as detergents is relevant for an understanding of their enzymatic mechanism. In this study, we have characterized the effect of nonionic, anionic, cationic, and zwitterionic detergents on the enzymatic activity and thermal stability of *Thermomyces lanuginosus* lipase (TlL). For all detergents, low concentrations enhance the activity of TlL toward *p*-nitrophenyl butyrate by more than an order of magnitude; at higher detergent concentrations, the activity declines, leveling off close to the value measured in the absence of detergent. Surprisingly, these phenomena mainly involve monomeric detergent, as activation and inhibition occur well below the cmc for the nonionic and zwitterionic detergents. For anionic and cationic detergents, activation straddles the monomer−micelle transition. The data can be fitted to a three state interaction model, comprising free TlL in the absence of detergent, an activated complex with TlL at low detergent concentrations, and an enzyme-inhibiting complex at higher concentrations. For detergents with the same headgroup, there is an excellent correspondence between carbon chain length and ability to activate and inhibit TlL. However, the headgroup and number of chains also modulate these effects, dividing the detergents overall into three broad groups with rising activation and inhibition ability, namely, anionic and cationic detergents, nonionic and single-chain zwitterionic detergents, and double-chain zwitterionic detergents. As expected, only anionic and cationic detergents lead to a significant decrease in lipase thermal stability. Since nonionic detergents activate TlL without destabilizing the protein, activation/inhibition and destabilization must be independent processes. We conclude that lipase−detergent interactions occur at many independent levels and are governed by a combination of general and structurally specific interactions. Furthermore, activation of TlL by detergents apparently does not involve the classical interfacial activation phenomenon as monomeric detergent molecules are in most cases responsible for the observed increase in activity.

Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) catalyze the hydrolysis of triglycerides. Triglycerides are found in an aggregated state in water, and most lipases are activated at the water−lipid interface: their activity is very low on monomeric substrate but increases dramatically above the critical aggregation concentration (*3*, *4*), a phenomenon called interfacial activation, which is reflected in the three-dimensional structure of the enzyme. Lipases share a common fold of the α/β-hydrolase type, and the structure usually contains a small α-helix or loop, referred to as the lid, which covers the active site pocket (*5*). This conformation is termed the closed conformation. When the lipase is adsorbed to an interface, the lid is displaced so that the active site becomes accessible to substrate (*6*). This conformation is termed the open conformation. The structures of the free and bound lipase are thought to represent the start and end conformations in the interfacial activation process (*7*). The active site is composed of a catalytic triad of Ser, Asp, and His, similar to what is found in serine proteases (*5*).

One of the best studied lipases is *Thermomyces lanuginosus* lipase (TlL).[1] Its three-dimensional structure is stabilized by three disulfide bonds and contains four tryptophans, one of which, Trp89, is located in the lid and has been shown to be important for efficient hydrolysis (*8*−*10*). TlL has a preference for hydrolyzing medium chain (C_{10}−C_{12}) triglycerides as compared to short (C_4) and long chain (C_{18}) ones (A. Svendsen, personal communication), but in general, it is capable of degrading a wide variety of substrates. The pH activity optimum is around 11; however, TlL displays

† This research was supported by the Danish Technical Research Council (J.E.M. and D.E.O.) and the Danish Natural Science Research Council (P.S.).

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[1] ANS, 8-anilino-1-naphthalene sulfonic acid; C_8E_4, tetraethylene glycol monooctyl ether; C_8E_5, pentaethylene glycol monooctyl ether; cmc, critical micelle concentration; DDM, *n*-dodecyl-β-D-maltoside; DHepPC, 1,2-diheptanoyl-*sn*-glycero-3-phosphocholine; DHexPC, 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine; DLS, dynamic light scattering; DM, *n*-decyl-β-D-maltoside; DOPC, 1,2-dioctanoyl-*sn*-glycero-3-phosphocholine; E600, diethyl *p*-nitrophenyl phosphate; HTAB, hexadecyl trimethylammonium bromide; LTAB, dodecyl trimethylammonium bromide; lyso-LPC, 1-lauroyl-2-hydroxy-*sn*-glycero-3-phosphocholine; lyso-MPC, 1-myristoyl-2-hydroxy-*sn*-glycero-3-phosphocholine; lyso-MPG, 1-myristoyl-2-hydroxy-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)]; NM, *n*-nonyl-β-D-maltoside; OG, *n*-octyl-β-D-glucoside; OM, *n*-octyl-β-D-maltoside; pNPB, *p*-nitrophenyl butyrate; SDeS, sodium decyl sulfate; SDS, sodium dodecyl sulfate; SDSulfonate, sodium dodecyl sulfonate; TlL, *Thermomyces lanuginosus* lipase; TlLW89m, TlL with mutations W117F, W221H, and W260H; TTAB, tetradecyl trimethylammonium bromide; UM, *n*-undecyl-β-D-maltoside.

10.1021/bi0479757 CCC: $30.25
Published on Web 01/11/2005

activity to below pH 7 (*11*). Furthermore, it has a high thermal stability with a melting temperature of 74 °C at pH 8 (*12*). Because of these properties, TlL has found important applications in the detergent industry as a facilitator of fat stain removal in washing powder (*13*). This means that the lipase has to be effective (i.e., active and stable) in a complex mixture of compounds, including anionic and nonionic detergents. Detergents are similar to the natural substrates of a lipase, in that they are capable of forming higher-order aggregates. Thus, from the view of both basic and applied science, it is important to understand the effect of detergents on the catalytic and structural properties of the lipase.

The impact of detergents on the enzymatic activity of TlL has been studied in only a few cases. SDS was found to enhance the activity of TlL toward *p*-nitrophenyl butyrate (pNPB) at premicellar concentrations of SDS peaking at around the critical micelle concentration (cmc) (*14*); above the cmc, the activity declined but was still significantly higher than in buffer alone, indicating that this aggressive detergent did not denature the lipase (*14*). This contrasts with the effect of SDS on conventional proteins, which unfold above the cmc (*15*−*18*). The nonionic detergent pentaethylene glycol monooctyl ether (C_8E_5) was found to have the opposite effect on TlL, as the activity toward pNPB decreased at submicellar concentrations approaching the cmc of C_8E_5 (*19*), while it increased dramatically above the cmc. Also, mixed micelles of the fatty acid *cis*-parinaric acid and the anionic detergent sodium taurodeoxycholate were shown to interact with TlL (*20*). In the case of C_8E_5 and *cis*-parinaric acid/sodium taurodeoxycholate, the microenvironment of Trp89, which is placed in the lid, was shown to change upon binding of detergent. Taken together, these studies indicate that the detergent−lipase interaction is very specific to the detergent used and involves the lid region of the lipase.

Here, we present a detailed study on the influence of detergents on the enzymatic activity, thermal stability, and structure of TlL. To study the influence of charge and hydrophobicity, we included both anionic and cationic detergents as well as different nonionic and zwitterionic detergents with different chain lengths. The mutant W89m, in which all Trp residues except Trp89 have been mutated to histidine or phenylalanine, is used to probe the microenvironment of the lid in kinetic fluorescence experiments. We find that the ability to activate and subsequently inhibit TlL activity with increasing detergent concentration is a general feature of all the detergents tested and that within each detergent group there is an excellent correlation between these abilities and detergent hydrophobicity. However, headgroups also modulate these effects, making anionic and cationic detergents the least effective activators and inhibitors relative to their cmc. This may reflect the more unspecific binding of this group of detergents, which also leads to significant thermal destabilization of TlL.

EXPERIMENTAL PROCEDURES

General. *n*-Alkyl-*β*-D-maltosides were from Anatrace (Maumee, OH); 1,2-diacyl-*sn*-glycero-3-phosphocholines, 1-acyl-2-hydroxy-*sn*-glycero-3-phosphocholines, and 1-myristoyl-2-hydroxy-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] were from Avanti Polar Lipids (Alabaster, AL); sodium decyl sulfate and sodium dodecyl sulfonate were from Lancaster (Eastgate, England). All other chemicals were from Sigma Chemical Co. (St. Louis, MO). TlL and TlLW89m (TlL with mutations W117F, W221H, and W260H) were purified as described (*13*, *21*). They contain a high mannose type carbohydrate branch at position Asn33 of 1−2 kDa. Protein concentrations were determined spectrophotometrically at 280 nm using molar extinction coefficients of 37 275 and 20 800 M^{-1} cm^{-1} for TlL and TlLW89m, respectively.

Data Analysis. Nonlinear least-squares regression analysis of enzymatic activity profiles and thermal scans was carried out with Kaleidagraph, version 3.5 (Synergy Software, Reading, PA).

Determination of Detergent cmc. The detergent cmc values under the buffer conditions used in this study were determined using the fluorescent probe *N*-phenyl-1-naphthylamine (NPN). The partitioning of NPN into micelles is associated with a dramatic increase in the fluorescence intensity of the probe (*22*). When plotting NPN fluorescence as a function of detergent concentration, the cmc was determined from the breakpoint of the curve. The fluorescence was measured on a SpectraMax Gemini XS (Molecular Devices, Sunnyvale, CA) fluorescence plate reader (E_m/E_x monochromator bandwidth 9 nm) at room temperature using excitation at 350 nm and emission at 435 nm. NPN was dissolved in absolute ethanol, and the final probe concentration in 50 mM TRIS, pH 8 was 10 μM (in the absence of pNPB) or 50 μM (in the presence of 0.36 mM pNPB). The higher NPN concentration was necessary to increase the signal-to-noise ratio in the presence of pNPB. The increased NPN concentration did not affect the cmc in the absence of pNPB as compared to 10 μM NPN (data not shown). The cmc values are listed in Table 1.

Enzymatic Activity Measurements. The enzymatic activity of TlL was measured on a UVIKON 943 A (Bio-Tek Kontron Instruments, Milano, Italy) spectrophotometer at 20 °C using pNPB as substrate. Hydrolysis of pNPB was monitored as the change in absorbance at 400 nm corresponding to the absorption maximum of the *p*-nitrophenolate anion. pNPB was dissolved in absolute ethanol to a concentration of 126 mM and used at a final concentration of 0.36 mM. The final enzyme concentration was 6 nM. All measurements were performed in 50 mM TRIS, pH 8 in a volume of 700 μL. Immediately after the addition of substrate to the sample, consisting of buffer and detergent, it was transferred to the cuvette, and subsequently the enzyme was added. The cuvette was capped with Parafilm, and the contents were mixed by gently being inverted 5 times. The time taken from addition of enzyme to start of measurement was 30 s. Generally, time profiles of 120 s were recorded, which were linear over the entire period. The effect of detergent on enzymatic activity was investigated by varying the detergent concentration from 0 to 45 mM. As mentioned, detergent was added prior to substrate and enzyme, and incubation of detergent and enzyme over time scales up to 1 h before addition of substrate did not change the activity levels (data not shown). The change in autohydrolysis of pNPB with detergent concentration was negligible. Consequently, time profiles of samples containing detergent and no enzyme were subtracted as blank for all spectra. The absorbance after 60 s was taken as a measure of the enzyme activity.

Table 1: Overview of Detergent Physical Properties and Apparent Activation and Inhibition Parameters of TlL[a]

detergent	chain length	cmc[b] [mM]	cmc[c] [mM]	C_{max}[d] [mM]	$C_{1/2}$[e] [mM]	A_{max} [ΔOD_{400}/min]	A_{min}[f] [ΔOD_{400}/min]
DHexPC	2 × 6	12.5	11.5	0.56	1.3	0.63	0.17
DHepPC	2 × 7	0.7	1.3	0.061	0.13	0.26	0.17
DOPC	2 × 8	0.1	0.1	0.00080	0.012	0.38	0.15
Lyso-LPC	12	0.5	0.5	0.061	0.16	0.34	0.20
Lyso-MPC	14	0.06	0.07	0.0058	0.019	0.15	0.12
Lyso-MPG	14	0.3	0.3	0.044	0.097	0.36	0.20
OG	8	19.3	19.3	2.5	4.7	0.90	0.16
OM	8	19.5	19.5	3.8	8.3	1.78	0.12
NM	9	7	6	0.75	2.3	0.89	0.13
DM	10	1.9	2	0.24	0.64	0.84	0.24
UM	11	0.45	0.45	0.066	0.14	0.69	0.21
DDM	12	0.12	0.07	0.0060	0.018	0.49	0.21
SDSulfonate	12	3	2.8	0.46	0.93	0.41	0.17
SDeS	10	22	22	10	19	0.37	0.14
SDS	12	2	1.6	3.3	6.4	0.48	0.066
LTAB	12	7	8	6.4	17	0.19	0.18
TTAB	14	0.8	0.8	4.5	11	0.63	0.27
HTAB	16	0.1	0.07	0.57	1.2	0.86	0.63

[a] The error on the cmc determinations is 10−15%. [b] Measured in 50 mM TRIS pH 8 at 25 °C. [c] Measured in 50 mM TRIS pH 8 and 0.36 mM pNPB at 25 °C. [d] The detergent concentration where TlL activity peaks (at the level A_{max}). Obtained by directly reading the values from the fitted curves. [e] The detergent concentration where TlL activity has declined halfway between A_{max} and A_{min}, the final plateau activity level. Obtained by calculating the activity midway between A_{max} and A_{min} and reading the corresponding detergent concentration from the fitted curves. [f] For comparison, TlL activity in the absence of detergent is ca. 0.05 ΔOD_{400}/min (see Figure 1).

The enzymatic activity profiles (TlL activity vs detergent concentration) were fitted to eq 3, which is derived from Scheme 1 (see Results). As explained in Results, we emphasize that eq 3 is used to obtain fitted curves to the data, from which the empirical parameters C_{max}, $C_{1/2}$, A_{max}, and A_{min} can be read directly by visual inspection. Eq 3 is obtained as follows: K_1 and K_2, the equilibrium constants describing the activation and the inhibition reaction, respectively, are defined as

$$K_1 = \frac{[E][D]}{[E:D]}; K_2 = \frac{[D]^n[E:D]}{[E:D_{n+1}]} \quad (1)$$

The measured total enzymatic activity, A_Σ, has three contributions

$$A_\Sigma = A_E f_E + A_{E:D} f_{E:D} + A_{E:D_{n+1}} f_{E:D_{n+1}} \quad (2)$$

where A_E, $A_{E:D}$, and $A_{E:D_{n+1}}$ are the activity of the detergent-free enzyme, the activated enzyme−detergent complex, and the inhibited enzyme−detergent complex, respectively, and f_E, $f_{E:D}$, and $f_{E:D_{n+1}}$ are the mole fractions of the free enzyme, the activated enzyme−detergent complex, and the inhibited enzyme−detergent complex, respectively. By combining eqs 1 and 2, the final model is obtained

$$A_\Sigma = \frac{A_E\frac{K_1}{[D]} + A_{E:D} + A_{E:D_{n+1}}\frac{[D]^n}{K_2}}{\frac{K_1}{[D]} + 1 + \frac{[D]^n}{K_2}} \quad (3)$$

where E denotes enzyme, D denotes detergent, E:D denotes the activated enzyme−detergent complex, $E:D_{n+1}$ denotes the inhibited enzyme−detergent complex, n is an integer, and K_1 and K_2 are the equilibrium constants describing the activation and the inhibition reaction, respectively. Because of the two opposing reactions (i.e., activation and inhibition), unrestrained fitting of the data to eq 3 led to unrealistically large errors for the fitted parameters. Therefore, $A_{E:D}$ was defined as twice the highest ordinate value of the fitted plot without any associated error, as used in other studies on detergent inhibition (*17*). $A_{E:D}$ was then locked to this value, and the fitting was repeated to obtain values for A_E, $A_{E:D_{n+1}}$, K_1, and K_2. n was increased from 1 in steps of 1 until the quality of the fit did not improve significantly. When comparing values of $A_{E:D}$ for the different detergents, it is assumed that any systematic deviation between the locked value and the actual value will be overall similar for the different detergents. While the locking is an arbitrary decision, the absolute values of $A_{E:D}$ and the other parameters obtained from the fit are not central to this study.

Affinity of pNPB for Detergent Micelles. The binding constant of pNPB for SDS and TTAB micelles was determined by following the change in absorbance of pNPB at 280 nm on a UVIKON 943 A (Bio-Tek Kontron Instruments, Milano, Italy) spectrophotometer. 0.36 mM pNPB was titrated with increasing concentrations of detergent in 50 mM TRIS, pH 8 at 20 °C. The absorbance of buffer and detergent was subtracted as blank. The binding constant was determined from a simple rectangular hyperbolic binding model.

Enzymatic Activity Measurements in the Presence of E600. Enzymatic activity experiments in the presence of the inhibitor E600 were performed as described previously with the following modifications. E600 was added from a 100 mM stock solution in 2-propanol to give a final concentration of 3 mM. The 2-propanol trace (3%) did not have any significant effect on the enzymatic activity in the absence of E600 (data not shown). The samples containing buffer, protein, inhibitor, and detergent were incubated for 5 h at room temperature. pNPB (0.36 mM) was added immediately prior to measurement.

Dynamic Light Scattering and Pyrene Fluorescence Experiments. Dynamic Light Scattering. TlL (final concentration 20 μM) was mixed with SDS or NM below and above the cmc in 50 mM TRIS, pH 8 and measured at 25 °C on a DynaPro99 molecular sizing instrument (Protein Solutions,

High Wycombe, England) equipped with a Protein Solutions temperature control unit. All samples were filtered twice through a 100 nm filter. Dynamics version 5.25.44 was used for data acquisition, and Dynals version 1.51 was used for data analysis using standard settings.

Pyrene Fluorescence. TlL (final concentration 1 μM) was mixed with SDS, OM, or LTAB at concentrations below and above the cmc in 50 mM TRIS, pH 8. Pyrene from a 100 μM stock solution in ethanol was added to a final concentration of 1 μM. Pyrene was excited at 335 nm, and fluorescence emission spectra (350−450 nm) were recorded at 25 °C on a PerkinElmer LS55 spectrometer (PerkinElmer A/S, Hvidovre, Denmark) using 2.5 nm excitation and emission slit widths. Fluorescence spectra in the absence of pyrene were recorded as blanks and subtracted. The ratio of the intensities of the third (I_3) and first (I_1) emission bands, I_3/I_1, was used as a measure of the polarity of the probes environment (*23*). In water, I_3/I_1 is ∼0.55, and with decreasing polarity, I_3/I_1 increases (*24*).

Stopped-Flow Fluorescence Measurements. Stopped-flow fluorescence experiments were carried out on an SX18MV stopped-flow microanalyzer (Applied Photophysics, Leatherhead, UK) in a thermostatically controlled sample-handling unit at 25 °C. The reaction was followed by excitation at 280 nm with a 320 nm cutoff filter. All measurements were performed in 50 mM TRIS, pH 8. Enzyme solution was mixed 1:10 (v/v) with detergent solution, yielding a final enzyme concentration of 2 μM. Control spectra were recorded using buffer and detergent solutions only, and no change in signal was observed. Data were analyzed using the software provided by the manufacturer.

Far-UV CD Measurements. Far-UV CD wavelength and temperature scans were recorded on a Jasco J-715 spectropolarimeter (Jasco Spectroscopic Co. Ltd., Hachioji City, Japan) in a 1 mm path-length cuvette at 25 °C. All measurements were performed in 10 mM TRIS, pH 8. The enzyme concentration was 10 μM. Thermal scans were performed with a scan-rate of 60 °C/h. The contribution of buffer and detergent on the measured ellipticity was subtracted as blank. The thermal scans were fitted to the following equation, assuming a linear dependence of the pre- and post-transition baselines on temperature, to obtain T_m, the midpoint of denaturation (*25*)

$$\theta = \frac{\alpha_N + \beta_N T + (\alpha_D + \beta_D T)e^{(-\Delta H_{vH}/R\cdot(1/T-1/T_m))}}{1 + e^{(-\Delta H_{vH}/R\cdot(1/T-1/T_m))}} \quad (4)$$

where θ is the observed ellipticity, α_N and α_D are the ellipticities of the native and denatured state, respectively, at 298 K, β_N and β_D are the slopes of the native and denatured state baselines, respectively, T is the temperature, ΔH_{vH} is the van't Hoff enthalpy change of unfolding, and R is the gas constant.

RESULTS

Dual Effect of Neutral Detergents on TlL Activity Involves Detergent Monomers. To study the influence of chemical composition and physical properties of detergents on TlL enzymatic activity, we used detergents with different chain length, headgroup size, and charge, and consequently, cmc. TlL activity was measured by recording absorbance time profiles at different detergent concentrations using pNPB as substrate. Although TlL has a preference for medium chain substrates, pNPB was chosen for two reasons. First, earlier studies on detergent effects on TlL activity often used pNPB (*19*, *26*, *27*), making it possible to compare our data with previously published results. Second, its short (C_4) chain length makes it unable to form micelles on its own or form mixed micelles with monomeric detergent molecules well below the detergent cmc. Table 1 lists the cmc values of the detergents used measured in the presence and absence of pNPB, confirming that pNPB has a very insignificant effect on detergent cmc thereby reducing detergent−substrate interactions and thus simplifying our system.

The activity of TlL in the nonionic detergents octyl maltoside and octyl glucoside (Figure 1A) shows the general features of the TlL−detergent interactions. Two distinct activity regimes are seen. Initially, there is a steep rise to a level that is several orders of magnitude higher than in buffer. This is followed by a decline to a plateau that is similar to that without detergent. The arrows indicate the cmc values of the two detergents (determined in the presence of pNPB), making the important point that all these phenomena occur at submicellar concentrations and therefore cannot involve bona fide micelles. On the basis of this observation, we have carried out a systematic survey of the effect of several different classes of neutral detergents, namely, alkyl maltosides (chain length 8−12 carbon atoms), octyl glucoside (chain length eight carbon atoms), phosphatidylcholines (chain length six to eight carbon atoms; above a length of eight carbon atoms, phosphatidylcholines increasingly form double-layer vesicles rather than micelles), lysophosphatidylcholines (chain length 12 and 14 carbon atoms), and a lysophosphatidylglycerol (14 carbon atoms; strictly speaking an anionic detergent) on TlL enzymatic activity. In all cases, we see the same steep rise and decline in enzymatic activity, which is completed well below the cmc.

Dual Effect of Anionic and Cationic Detergents Involves Both Monomers and Micelles. In addition, we studied the effect of anionic and cationic detergents using alkyl sulfates (chain length 10 and 12 carbon atoms), alkyl sulfonate (chain length 12 carbon atoms), and alkyl trimethylammonium bromides (chain length 12, 14, and 16 carbon atoms). The activity profiles of SDS (anionic) and TTAB (cationic) are shown in Figure 1B,C, respectively. Just as with the nonionic and zwitterionic detergents, we observe both activation and inhibition. However, there is an important difference as compared to the neutral detergents. The concentration range in which the anionic and cationic detergents activate and inhibit TlL overlaps with the transition from monomer to micelle (see Figure 1B,C and Table 1). Activation starts in the presence of detergent monomers, but maximal activation as well as inhibition occur in the presence of micelles. This could be expected to affect TlL activity since pNPB's low water solubility (∼1.2 mM in 10 mM TRIS, pH 8 (*26*)) may encourage it to partition into the water−micelle interface or the hydrocarbon part of the micelle to some extent, as is the case with phospholipid vesicles (*26*). However, as we show in the following, substrate dilution by micellar incorporation can only account for part of the decrease in activity.

The cmc of SDS is reduced from 2.0 ± 0.2 mM in the absence of pNPB to 1.6 ± 0.2 mM in the presence of 0.36 mM pNPB (Table 1), suggesting some interaction between

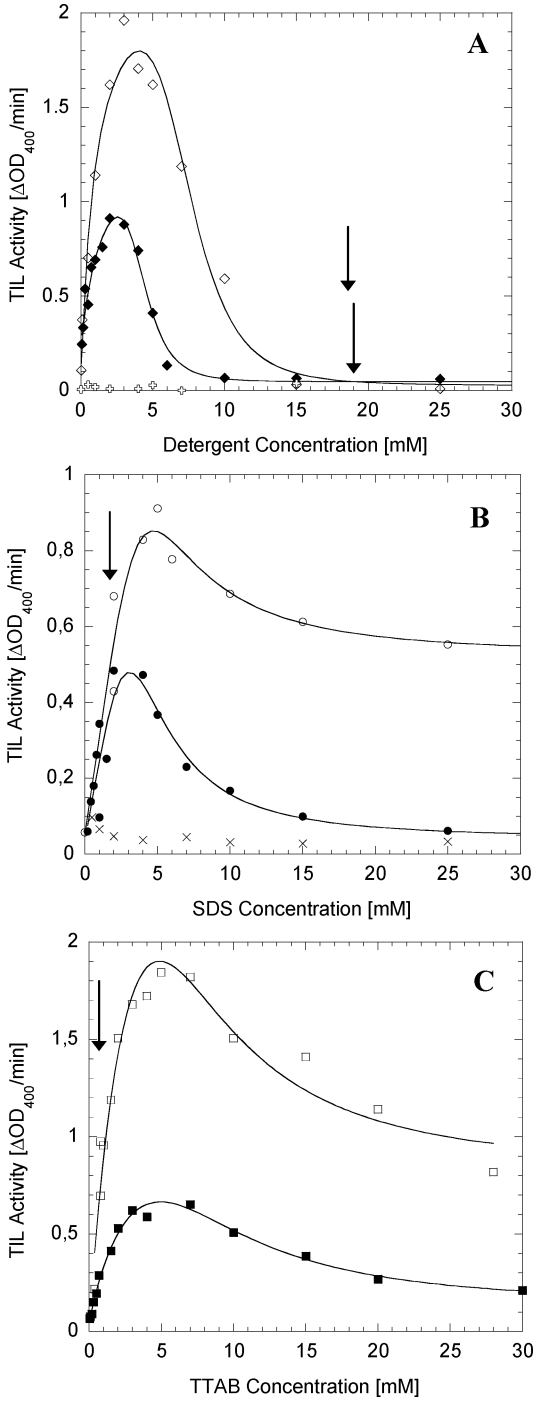

FIGURE 1: Representative examples of TlL enzymatic activity in detergents. **(A)** ◆: OG; ◇: OM; and ✧: OG in 3 mM E600. **(B)** ●: SDS; ○: SDS at [pNPB]/[SDS] = 0.079; and ×: SDS in 3 mM E600. **(C)** ■: TTAB and □: TTAB at [pNPB]/ [TTAB] = 0.071. The solid lines are the result of the best fit to eq 3. Experiments in 50 mM TRIS, pH 8, 6 nM TlL, and 0.36 mM pNPB (except ○ and □ in panels B and C, respectively). See Experimental Procedures for details on activity assay. The activity of TlL in the absence of detergent is ca. 0.05 ΔOD_{400}/min. The arrows indicate the cmc values of the detergents in buffer and 0.36 mM pNPB at 25 °C (from Table 1).

substrate and detergent. In the SDS experiment, the substrate concentration was kept constant while the SDS concentration was increased, meaning that fewer substrate molecules are present per micelle if they partition into these. Absorption titration experiments suggest that pNPB binds to SDS micelles with a binding constant of around 3 mM (data not shown). To investigate the possible effect of substrate dilution, we repeated the activity experiment at a constant [pNPB]/[SDS] ratio of 0.079. Under these conditions, the molar ratio of pNPB in SDS micelles remains essentially constant above 3−4 mM SDS. The decline in activity from 5 mM SDS onward is still clearly observed, although the activity only drops around 50% so that the activity at 48 mM SDS is approximately 10-fold higher than in buffer alone (see Figure 1B). Consequently, the decrease in activity with SDS concentration is genuine, although the SDS inhibition only leads to a decline of a factor of 2, while the dilution effect accounts for a factor of 5. In addition, the absolute activity level increases by a factor of 2, indicating that partitioning of substrate into SDS micelles at constant substrate concentration dilutes out substrate and leads to an underestimation of the actual degree of activation.

We could not determine the affinity of pNPB for TTAB by absorption titration. Instead, we use the dissociation constant of 1.9 mM for HTAB (*28*) as a reasonable estimate. When we carry out activity measurements at a constant [pNPB]/[SDS] ratio of 0.071,[2] the molar ratio of pNPB in TTAB micelles is essentially constant above 3 mM TTAB, nonetheless, a decline in activity is still observed as in the case of SDS (see Figure 1C).

Throughout the concentration range of both cationic and anionic detergents, the activity profile remains significantly higher than in buffer, indicating that the detergents are not able to denature TlL (see Figure 1B,C). This robustness toward ionic detergents is atypical for globular proteins but not for proteins designed for amphiphilic environments (e.g., bacterial outer membrane proteins (*29*) (J. E. Mogensen, P. Sehgal, and D. E. Otzen, unpublished results)).

Derivation of a Model Describing the General Detergent Effects. We now attempt a quantitative analysis of the data to be able to compare the effect of the different detergents on TlL enzymatic activity. Since two transitions are involved (Figure 1), a minimalist interpretation of the data invokes the existence of three states. Detergent monomers at low concentrations bind to and activate the enzyme, and at higher concentrations, an inhibited enzyme−detergent complex forms.

$$E + D \underset{K_1}{\rightleftharpoons} E{:}D \overset{n\,D}{\underset{K_2}{\rightleftharpoons}} E{:}D_{n+1} \qquad \text{(Scheme 1)}$$

Here, E denotes enzyme, D is a detergent molecule, E:D is the activated enzyme−detergent complex, and $E{:}D_{n+1}$ is the inhibited enzyme−detergent complex. K_1 and K_2 are the equilibrium constants describing the activation and the inhibition reaction, respectively. For simplicity, we use a model that mathematically implies that only one detergent molecule binds to the free enzyme, and a larger number of

[2] In the TTAB activity experiment with constant substrate/detergent ratio, it was necessary to dilute the pNPB stock concentration from 126 to 10 mM to avoid precipitation in the samples. The profile shown in Figure 1C is scaled by a factor representing the difference in activity between using a constant [pNPB] of 0.028 mM (10 mM stock) and the standard 0.36 mM (126 mM stock), although we generally do not use the absolute activity levels but rather the detergent concentrations for our conclusions.

detergent molecules bind to the E:D complex. The derivation of the corresponding equation is described in the Experimental Procedures. All detergent data fit well to the model (cfr. eq 3, Figure 1, and Table 1). Nevertheless, although the absence of micelles greatly simplifies matters,[3] we caution against too blithe a reading of the fitted parameters for several reasons. First, the model, while simple, is only a proposal that cannot be proved definitively. Second, the value of n—and with it the magnitude and dimensionality of K_2—cannot be determined with high precision (generally, a value of 3 gave good fits, but $n = 2$ also led to satisfactory fits in many cases). Third, the fitting is not mathematically stable because of the presence of two opposing actions (activation vs inhibition), and data only converted to a realistic solution if the activity level of the activated complex was restrained (typically to a value twice the maximum value measured). Instead, we use the fitted data curves to extract four empirical parameters, namely, C_{max}, the detergent concentration where TlL activity peaks (at the level A_{max}) and $C_{1/2}$, the detergent concentration where the activity has declined halfway between A_{max} and A_{min}, the final plateau activity level. The two C values are obtained by directly reading their values from the fitted curves; in the case of $C_{1/2}$, this is done by calculating the activity midway between A_{max} and A_{min} and reading the corresponding detergent concentration from the fitted curves. These parameters are summarized in Table 1. As will be shown next, the C_{max} and $C_{1/2}$ values are the key parameters for interpreting detergent−lipase interactions in our system.

Analysis of the Correlation between Detergent Properties and Their Activation and Inhibition of TlL. Having obtained the empirical parameters describing the activation and inhibition of TlL by different detergents (Table 1), we now turn to an analysis of the correlation with their biophysical properties. The simplest parameter to employ is the number of carbon atoms in the alkyl chain attached to the hydrophilic headgroup. Within each detergent group, the log of both C_{max} and $C_{1/2}$ shows an excellent correlation with the alkyl chain, in the sense that the longer the alkyl chain, the lower the detergent concentration at which activation and inhibition occur (Figure 2A,B). However, it is also apparent that there is large variation from one detergent group to another. To be able to compare detergents from different groups, we employed the cmc value, which as a first approximation is an expression of detergent hydrophobicity. Double logarithmic plots of the two C values versus cmc show a good overall linear correlation (correlation coefficient $R = 0.84$ for C_{max} and 0.86 for $C_{1/2}$ using 18 points) (Figure 2C,D; stippled lines). However, while the correlation with cmc is excellent within each detergent class, significant variation from one class to another remains. Consequently, hydrophobicity is not the sole parameter governing the lipase−detergent interaction (see Discussion for further analysis).

We were unable to distinguish any clear trends in the absolute activity levels. This may in part reflect small variations in concentrations of lipase batch stocks used throughout our experiments, but it does not affect our conclusions with regard to C_{max} and $C_{1/2}$, as the detergent concentration remains vastly in excess of protein (typically at least 1000-fold).

E600 Inhibition of TlL Coincides with Detergent Activation. To examine whether binding of detergents induces TlL to form the open conformation, we measured TlL enzymatic activity in the presence of 3 mM E600 as a function of SDS and OG concentration. E600 inhibits lipases by covalently binding to the active site serine (*30*). Binding can only take place if the enzyme is in the open conformation (i.e., when the lid is displaced), as the lid in the closed conformation effectively prevents access of E600 to the active site. TlL activity in the presence of E600 gradually declines as a function of SDS concentration (Figure 1B). This clearly indicates that SDS binding, initially as monomers, causes a conformational change of the lid thereby activating the lipase. In OG, a similar trend is observed although the activity seems to be constant as a function of OG concentration indicating that very small amounts of OG are sufficient to completely shift the lipase population to the open conformation (Figure 1A).

DLS and Pyrene Fluorescence Experiments Give No Evidence for Premicellar Aggregates in Complex with TlL. Since the observed activation of TlL occurs below cmc in most cases (see Table 1), we investigated whether premicellar aggregates could be detected in complex with TlL. Dynamic light scattering (DLS) was used to measure increases in size of TlL below and above the cmc for selected detergents, as enzyme-bound premicellar aggregates are expected to increase the hydrodynamic radius of the protein (data not shown). The hydrodynamic radius, r_H, of TlL measured from several independent measurements was 2.9 ± 0.5 nm. This is slightly larger than the 2.3 nm average radius predicted from the crystal structure of TlL (*31*); however, the glycan at position Asn33 probably contributes to the measured radius as well as the water molecules and salts that are bound to the surface of the protein in solution. In the presence of 10 mM SDS and 20 mM NM (i.e., above the cmc), we measure an r_H of 4.7 ± 2.3 and 2.9 ± 0.5 nm, respectively. This shows that the SDS micelles bind to TlL but not the NM micelles. Control experiments in the absence of protein showed r_H values of 2.7−2.9 nm, indicating that the SDS and NM micelles have similar sizes as compared to TlL. Measurements in 0.5 mM SDS and 0.7 mM NM (i.e., below the cmc) gave consistent r_H values of 2.9 ± 0.5 nm. This indicates that although the enzyme is in the activated state, no premicellar aggregates are present on the surface of the protein.

To confirm the results of the DLS experiments, we studied pyrene fluorescence in the presence of TlL mixed with SDS, OM, and LTAB below and above the cmc (data not shown). Pyrene fluorescence is very sensitive to the polarity of its environment (*23*). We reasoned that it would partition into premicelles on the surface of TlL resulting in fluorescence changes as earlier studies have shown that pyrene can partition into very small micelle-like structures, called hemimicelles, with very low (<5) aggregation numbers (*24*, *32*). For pyrene in buffer, we found $I_3/I_1 = 0.56$ in agreement with published values. For pyrene in micellar SDS (10 mM), OM (30 mM), and LTAB (10 mM), we obtained I_3/I_1 values of 0.86, 0.80, and 0.61, respectively, showing that pyrene partitions into the detergent micelles thereby experiencing a

[3] Although micelles are involved for the ionic detergents, we assume that the general mechanisms are the same, as the activity curves display the same features, and we only use parameters extracted directly from the fitted activity curves.

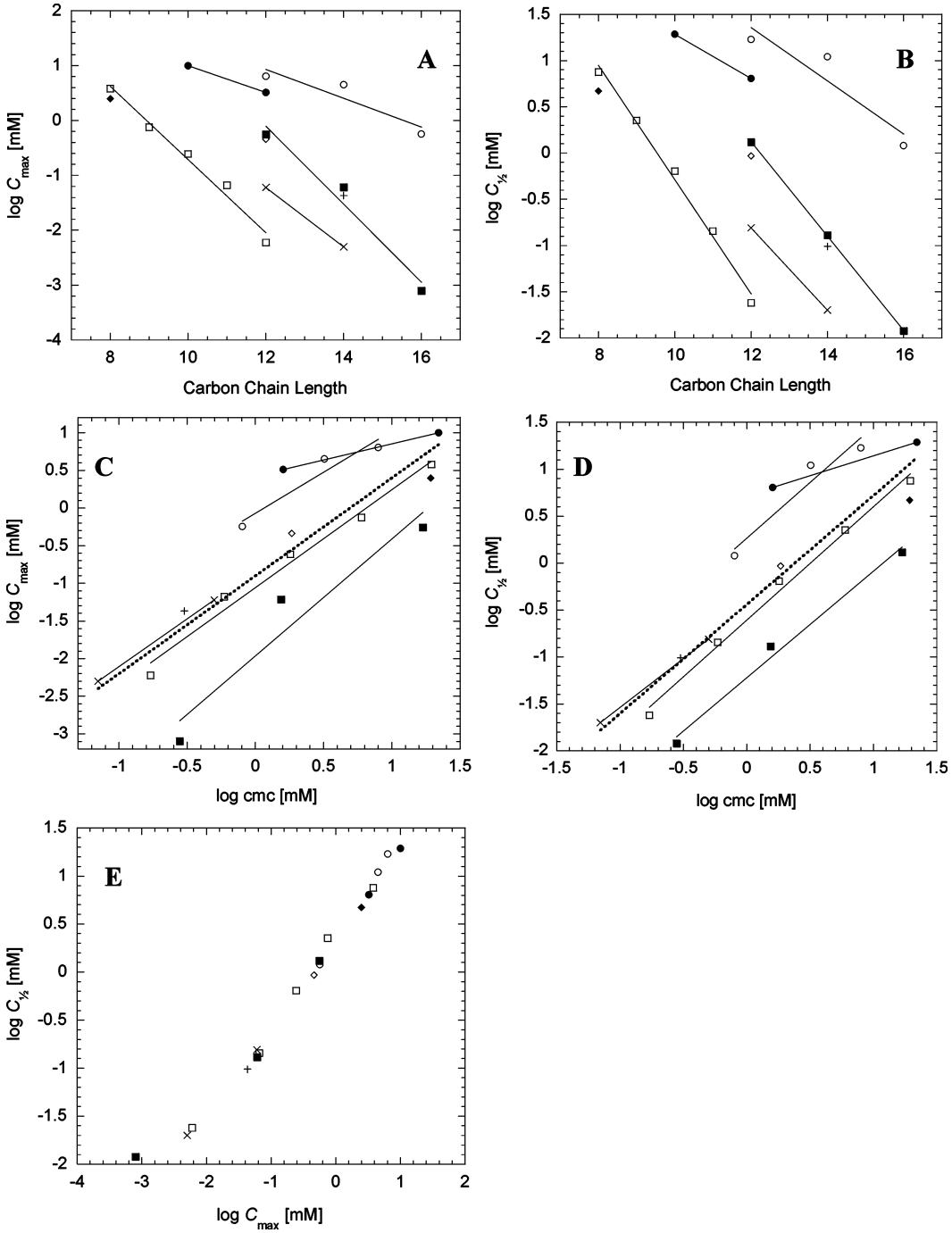

FIGURE 2: Correlation between detergent properties and their activation and inhibition of TlL. **(**A**)** C_{max} and **(**B**)** $C_{1/2}$ vs number of carbon atoms in the alkyl/acyl tail(s) of the detergents (for diacylphosphatidylcholines, the number of carbon atoms in the two tails has been added together). The solid lines are the best linear fits within each detergent class except for SDSulfonate, OG, and lyso-MPG, which are solitary representatives. **(**C**)** C_{max} and **(**D**)** $C_{1/2}$ vs cmc of the detergents. The solid lines are the best linear fits within each detergent class except for SDSulfonate, OG, and lyso-MPG; the stippled lines represent the best linear fits to all the points ($R = 0.84$ for C_{max} and 0.86 for $C_{1/2}$ using 18 points). **(**E**)** $C_{1/2}$ vs C_{max} for all detergents. The detergents are ●: alkyl sulfates; ○: alkyl trimethylammonium bromides; ■: diacylphosphatidylcholines; ×: lysophosphatidylcholines; +: lyso-MPG; □: alkyl maltosides; ◆: OG; and ◇: SDSulfonate.

less polar environment. For pyrene in monomeric SDS (0.5 mM), OM (3 mM), and LTAB (4 mM), the I_3/I_1 values yielded 0.55, 0.57, and 0.57, respectively. The presence of TlL did not alter any of these values, indicating that no premicelles exist in complex with TlL in accordance with the results of the DLS experiments.

SDS Binds in the Vicinity of Trp89 in the Enzyme's Lid. We turned to stopped-flow Trp-fluorescence experiments to further investigate possible conformational changes associated with detergent binding. TlL has 4 Trp in positions 89, 117, 221, and 260 scattered around the enzyme. Earlier fluorescence studies have shown that TlL binds to vesicles and micelles via the lid region (*20*, *27*), as the fluorescence changes arise mainly from a change in the environment of Trp89, which is part of the lid covering the active site pocket. We used both wild-type TlL and the mutant TlLW89m, in which all Trp residues were mutated to histidine or phenylalanine except Trp89, to be able to monitor lid regional as

well as global fluorescence changes upon detergent binding. The kinetic experiments were supported by equilibrium fluorescence experiments, which showed the same end-state detergent effects (data not shown).

In OG, OM, and DDM, no fluorescence signals could be observed for wild-type TlL or TlLW89m in the concentration range of 0−5 mM, indicating that no significant structural transitions occur or that the microenvironment of the Trp are not affected by these detergents. However, when TlLW89m is mixed with SDS, relaxation profiles (see Figure 3A) are obtained between 2 and 5 mM SDS; at higher and lower detergent concentration, no signal change is detected. The kinetic traces are best-fitted to three relaxation phases, in which the observed rate constants for the two fast phases show a bell-shaped dependency on [SDS], whereas the third and slowest rate does not depend on detergent concentration (Figure 3B). The variation in amplitude is slightly more complex (Figure 3C). Wild-type TlL shows very similar results (data not shown), suggesting that the fluorescence changes observed for TlL arise almost exclusively from the lid tryptophan.

We were unable to obtain proper kinetic data on the binding of TTAB to TlL. Complex kinetic traces were observed between 1 and 20 mM TTAB with particularly large amplitudes at 1 and 2 mM detergent (data not shown). Below 1 mM and above 20 mM TTAB, no signals could be detected (data not shown). Visual inspection revealed that TTAB precipitates TlL around 1−20 mM, and consequently, the phases observed can be attributed to this phenomenon. TlL has a p*I* of 5.0, giving it an overall negative charge at pH 8. The precipitation must be driven by electrostatic interactions since no absorption or fluorescence signals were obtained at pH 4, where the protein is positively charged (data not shown). In addition, diluting out the positively charged TTAB molecules with the nonionic detergent DDM restricted the concentration range of precipitation (data not shown). Most likely the negatively charged protein acts as a mortar to bind cationic micelles together at sufficiently high protein concentrations. Similar precipitation at intermediate protein/detergent concentrations is observed for, for example, cationic antimicrobial peptides in complex with SDS (D. E. Otzen, unpublished results). At the low protein concentrations used in the activity measurements (6 nM, ca. 300 times lower than the stopped-flow experiments), no precipitation was observed.

Anionic and Cationic Detergents Decrease the Thermal Stability of TlL. The thermal stability of TlL in various detergents was examined by CD spectroscopy to investigate if there is any correlation between the activity in detergent and the stability of the enzyme. As shown in the inset of Figure 4, the T_m of TlL in buffer is 74.0 ± 0.1 °C, which agrees well with the value of 74.4 °C found by Zhu et al. using fluorescence spectroscopy (*12*). In Figure 4, T_m is plotted as a function of detergent concentration for SDS, TTAB, DDM, OG, OM, and DHexPC. SDS has a destabilizing effect on TlL, as T_m decreases from 74.0 to 45.6 °C between 0 and 5 mM SDS, after which it does not decrease further. Since a cooperative transition is seen even at high detergent concentration, the thermal scans confirm that SDS cannot denature TlL, although it is significantly destabilized. This agrees with the activity experiments that show enzyme activity over the same SDS concentrations. Additionally, far-

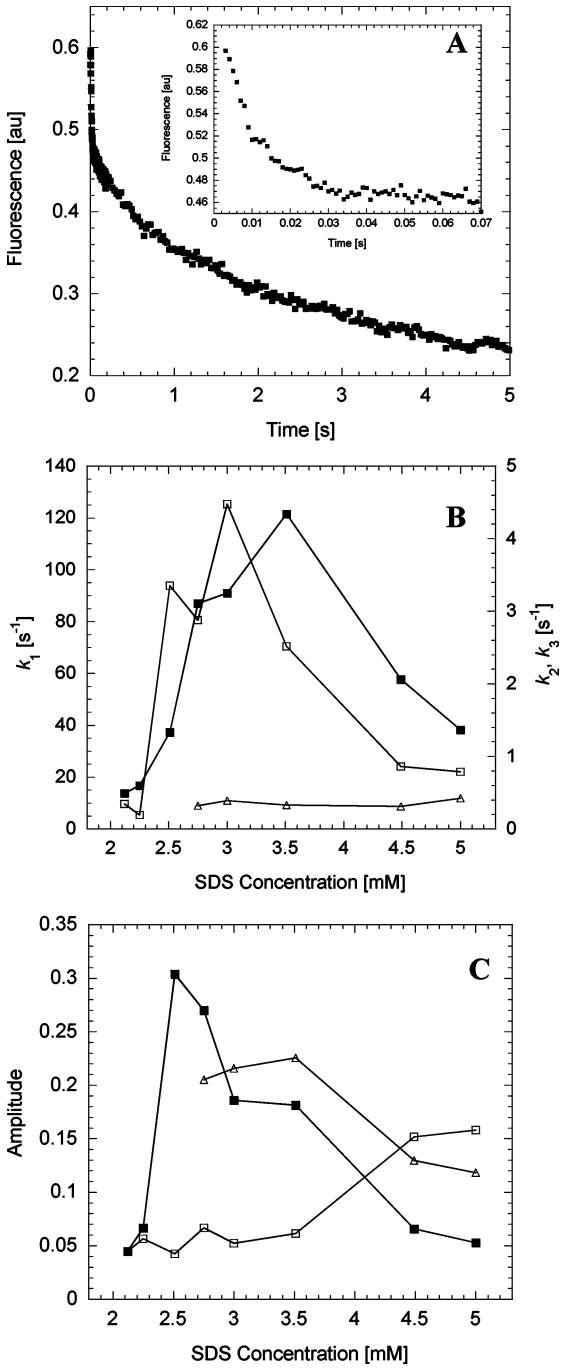

FIGURE 3: Example of a raw fluorescence time profile and fitted rate constants and amplitudes for TlLW89m as a function of SDS concentration from stopped-flow fluorescence experiments. **(**A**)** Fluorescence time profile in 3.5 mM SDS; the inset shows the fast phase only (k_1). **(**B**)** ■: k_1; □: k_2; and △: k_3 vs SDS concentration. **(**C**)** Amplitudes associated with ■: k_1; □: k_2; and △: k_3 vs SDS concentration. The solid lines are drawn only to guide the eye. Experiments in 50 mM TRIS, pH 8, and 2 μM enzyme at 25 °C.

UV CD wavelength scans show that no change in secondary structure occurs (data not shown). The thermal stability of TlL in DDM, OG, OM, and DHexPC is around 72 °C, i.e., the enzyme is not significantly affected by the presence of nonionic and zwitterionic detergents. In TTAB, the thermal stability could not be determined at pH 8 due to precipitation, so thermal scans were performed at pH 4 instead. The change in pH does not destabilize TlL much as T_m at pH 4 is 70.0

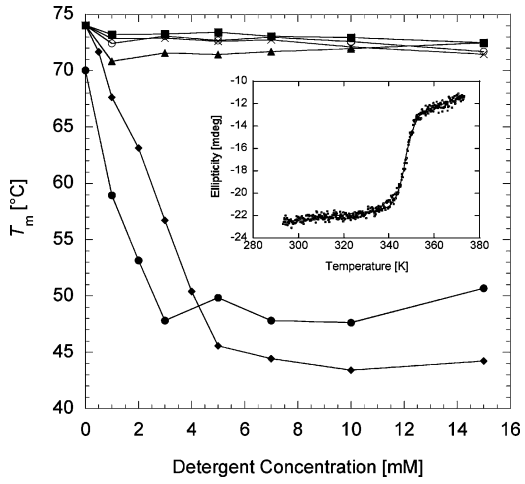

FIGURE 4: Thermal stability as monitored by CD of TlL in various detergents: ◆: SDS; ●: TTAB; ▲: DDM; ×: OM; ○: OG; and ■: DHexPC. Experimental conditions are as described for the inset figure except for TTAB, which is at pH 4. The solid lines are drawn only to guide the eye. Inset*:* representative thermal scan of 10 μM TlL in 10 mM TRIS, pH 8, at 220 nm. The solid line is the result of the best fit to eq 4. T_m yields 74.0 ± 0.1 °C.

°C. Like SDS, TTAB has a destabilizing effect on TlL as T_m decreases to approximately 47.5 °C at 3 mM TTAB, after which it does not decrease further. Thus, the ionic detergents SDS and TTAB destabilize TlL to similar extents, while the neutral detergents do not affect stability.

DISCUSSION

In this study, we have investigated the effect of detergents on the enzymatic activity and thermal stability of *T. lanuginosus* lipase. In all cases, we see activation at low detergent concentrations followed by inhibition at higher detergent concentrations. Similar activation/inhibition effects on lipases have been reported earlier on a more preliminary level (*14*, *30*, *33*−*36*). A priori, detergents are expected to influence lipase activity because of their close resemblance to lipase substrates, both in terms of structure and in terms of complex aggregation behavior. To simplify things, we have therefore used a water-soluble substrate that does not form aggregates by itself below its solubility limit and that does not appreciably lower the cmc values of detergents. In the case of the nonionic and zwitterionic detergents, activation and inhibition takes place well below the cmc, which is not appreciably affected by the substrate, confirming only insignificant substrate−detergent interactions that would otherwise complicate the analysis. Activation and inhibition of charged detergents straddles the monomer−micelle transition in most cases. Here, solubilization of substrate is clearly an issue, but activation and inhibition remain as genuine phenomena even when substrate dilution effects are taken into account. We do not attempt to extrapolate our data to other systems with substrates that interact more extensively with detergents or other amphiphiles or lipids.

Influence of Hydrophobicity on Detergent−*Lipase Interactions.* When using cmc values as an indicator of hydrophobicity, we see a clear correlation with detergent ability to activate and inhibit TlL. However, the significant variation between detergent classes indicates that polar and electrostatic interactions also play a large role.[4] On the basis of Figure 2C,D, the detergents can be grouped in three classes in increasing order of activation and inhibition potency, namely, (1) the anionic and cationic detergents, (2) the nonionic and zwitterionic detergents (which include lysophosphatidylglycerol, strictly speaking an anionic detergent but very similar in structure to lyso-LPC and lyso-MPC), and (3) the diacylphosphatidylcholines. How can this be rationalized? Hydrophobic moieties such as alkyl chains are highly likely to bind to the active site of TlL, given the hydrophobic nature of the substrate. The fact that TlL's natural substrates are triglycerides suggests that group 3 detergents can exploit a certain degree of substrate mimicry, enabling them to perform more strongly than lysophosphatidylcholines (relative to their cmc). However, detergent binding, even for this group of substrate-like detergents, cannot be identical to substrate binding, otherwise we would only see inhibition, not activation. Group 2 detergents are more efficient inhibitors than group 1, possibly because electrostatic repulsions make it difficult to bind as many charged detergent molecules as noncharged detergent molecules in the confined region around the active site. In support of this, the parameter *n* in Scheme 1, which may be interpreted as a qualitative measure of the number of detergent molecules binding and inhibiting the initially formed enzyme−detergent complex, is larger for the nonionic detergents (data not shown).

Strong Correlation between Activation and Inhibition. The two *C* values follow each other remarkably closely (Figure 2E), irrespective of the detergent class. This indicates that the two phenomena, activation and inhibition, involve the same kind of detergent−lipase interactions. Together with the observations on the three detergent groups mentioned previously, a minimalist explanation is that activation occurs when a detergent molecule binds in the region of the active site, facilitating substrate binding by either inducing a conformational change in the enzyme (lid opening) or by binding directly to the substrate. The latter is less likely, as there is no evidence from our measured cmc values of substrate−detergent interactions; in addition, this would lead to more variation between detergent classes (unless interaction occurred entirely via the alkyl chains). Inhibition then occurs when additional detergent molecules bind in the active site region, blocking substrate access. The possibility that detergent molecules can bind in the active site causing inhibition is substantiated by two crystal structures of TlL in complex with detergent-like molecules: the S146A mutant with oleic acid bound (*20*) and wild-type TlL with 1,2-dodecyl-*sn*-glycero-3-phosphocholine bound (*37*). In both cases, the amphiphilic molecule is bound in the active site pocket, and the enzyme is in the open conformation. In the

[4] Thus, cmc values alone do not paint the whole picture. This is not necessarily surprising. SDS and TTAB have similar cmc values (1.6 vs 0.8 mM under our conditions), but SDS is a much more potent solubilizer and emulsifier in general (*1*). A more comprehensive parameter to quantify the combination of hydrophobic and polar/ionic constituents in detergents is the hydrophilic-to-lipophilic balance (HLB value). HLB values are a direct expression of the ratio of the hydrophilic and lipophilic moieties in a detergent and vary from 0 (very hydrophobic) to 40 (very hydrophilic) (*2*). SDS has the highest value (*40*), whereas TTAB only scores around 12. On the other hand, it is not clear that HLB values would improve the correlation with *C* values since the alkyl sulfates and cationic detergents actually lie close to each other in the cmc plots (Figure 2C). Further, HLB values for many of the detergents used in this study are not available, precluding a further analysis.

case of oleic acid, the alkyl chain is positioned right above the active site serine and wrapped around Trp89 of the lid. In the wild-type complex, one of the acyl chains is also wrapped around Trp89.

Detergents Bind to TlL as Monomers and Induce the Open Conformation. To confirm that detergent binding indeed induces a conformational change in the lid of the enzyme, we titrated TlL with SDS and OG in the presence of the covalent inhibitor E600 and measured the activity. We found that the activity declines as a function of detergent concentration showing that the presence of low mM concentrations of SDS and OG induce the open conformation in the lipase. How can the lid be displaced and the lipase activated in the absence of bulk micelles? In principle, detergent molecules may not necessarily bind as a bulk micelle, but monomers could bind individually on hot spots on the protein surface and form premicellar aggregates below the cmc of the detergent (*38*). This has previously been suggested for TlL in the presence of submicellar concentrations of pentaoxyethylene octyl ether (*19*). In our system, the activity profiles (Figure 1) show that the activity increase takes place over a very narrow concentration interval; thus, the detergent monomers might be envisaged to bind in a cooperative fashion probably to the active site region forming a protein−premicelle complex that represents the activated state. However, we do not find any evidence of premicellar aggregates as judged from DLS and pyrene fluorescence experiments. Furthermore, the *n* values determined from the fit of the activity profiles generally yield only two to three detergent molecules, which also is not compatible with a premicellar aggregate. It is more likely that activation occurs at the monomer level; thus, our data support a model where activation of the lipase by detergents does not occur via the classical interfacial activation pathway but rather via a noninterfacial mechanism without the presence of a well-defined interface. This has also been suggested for other lipases (e.g., the pancreatic lipase related protein 2 from horse (*39*) and the fungal lipases from *Rhizopus delemar* and *Geotrichum candidum* (*40*)). In the case of porcine pancreatic lipase, a noninterfacial activation mechanism was also suggested; however, this situation cannot be compared directly with TlL since pancreatic lipase is part of a more complex system involving a colipase as well as the lipase proper and a whole micelle that shows no interaction with the active site region of the lipase (*41*).

Effect of Detergent on Protein Stability Is Linked to Detergent Charge. We found a clear correlation between detergent charge and thermal stability of TlL as SDS and TTAB significantly destabilize the protein whereas the neutral detergents had no impact on stability. This is as predicted in that nonionic detergents normally are regarded as mild detergents and that they do not interact extensively with the protein surface, whereas ionic detergents, in particular SDS, are more aggressive (*42*). Nevertheless, SDS and TTAB cannot denature TlL at room temperature as we see cooperative unfolding transitions in CD thermal scans even at 50 mM detergent, which is significantly higher than the SDS concentration used in SDS−PAGE. This in turn forms the basis for TlL's applicability in the detergent industry where anionic detergents are widespread. The fact that TlL is much more robust toward detergents than globular proteins such as S6 and CI2, which unfold above the cmc of SDS and LTAB (*16*, *17*), shows how the lipase is designed to cope with detergent-like compounds.

Binding of SDS Micelles Lead to Rapid Conformational Changes that Probably Are Related to Global Destabilization Rather than Activation. Stopped-flow fluorescence experiments identify several rapid relaxation phases when TlL is mixed with SDS between 2 and 5 mM (the onset of inactivation). Since the concentration at which signals appear coincides with the cmc of SDS, the process monitored must be caused by micelles binding to TlL. Essentially identical signals are seen for wild-type TlL and TlLW89m, indicating that Trp89 is the major residue affected by detergent binding. Trp89 has the highest quantum yield (65%) of the four Trp residues (*43*) and also the highest solvent accessibility (*44*). Trp117, Trp221, and Trp260 are all significantly buried in the native state of TlL and presumably remain so in the activated state (*44*). The fact that we observe several relaxation phases suggest that multiple protein conformational changes occur; alternatively, a conformational change could be accompanied by regrouping of micellar structures around Trp89, causing polarity changes in its microenvironment. What structural changes are being monitored? Since TlL remains active in SDS at concentrations well above 5 mM, the process we are monitoring is not denaturation. The signals disappear around 5 mM when inhibition sets in (see Figure 1B). This could indicate that we are in fact measuring TlL activation. However, we disfavor this for two reasons: first, activation starts well below 2 mM, the concentration at which the relaxation signals first appear. Second, we do not see relaxation signals for other activating detergents such as OG and OM. It is possible that the conformational change is linked to the global destabilization monitored by thermal scans, although it is not clear why stopped-flow fluorescence signals first appear at the cmc of SDS while destabilization commences before the cmc. The fluorescence signal associated with this change is probably subsequently eclipsed by other micelles bound to TlL at higher SDS concentrations (witness the gradual decline in amplitude in Figure 3C at higher SDS concentrations), as the protein becomes increasingly inhibited without affecting its stability.

Destabilization and Activation/Inhibition Are Independent Processes. Is the enhancement of activity coupled to the decrease in stability? In SDS and TTAB, the increase in enzymatic activity of TlL coincides with the decrease in stability at low detergent concentrations (i.e., when T_m reaches a plateau, the activity starts to drop). If the destabilized protein is more flexible, it might accommodate the substrate molecules better, leading to a higher catalytic turnover, and this could be the reason for the increased activity observed in SDS and TTAB. The data observed for the neutral detergents, however, make this connection unlikely as these detergents do not destabilize TlL but cause activation. Rather, the two phenomena are uncoupled processes: (1) all detergents show a specific interaction with the active site region enhancing enzymatic activity and (2) in addition, SDS and TTAB unspecifically bind to the protein surface, and their charge leads to a weakening of the interactions in the native state. The interfacial activation process, for which the protein is evolutionarily designed, hinders denaturation, and the net result is only a destabilization of the protein. Although the thermal stability drops by around 30 °C to give a melting temperature around 45 °C, the native state remains the

predominant species at 25 °C where the activity measurements are performed. At this temperature, the denatured state is populated to such a low extent that it will not affect the overall activity level.

ACKNOWLEDGMENT

We thank Peter Westh, Kim Borch, and in particular, Allan Svendsen for constructive discussions and Svend Kaasgaard and Allan Svendsen for the gift of purified wild-type and mutant TlL, respectively.

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BI0479757