Tryptophan Octyl Ester in Detergent Micelles of Dodecylmaltoside: Fluorescence Properties and Quenching by Brominated Detergent Analogs

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ABSTRACT The fluorescence properties of tryptophan octyl ester (TOE), a hydrophobic model of Trp in proteins, were investigated in various mixed micelles of dodecylmaltoside (DM) and 7,8-dibromododecyl β-maltoside (BrDM) or 10,11-dibromoundecanoyl β-maltoside (BrUM). This study focuses on the mechanism via which these brominated detergents quench the fluorescence of TOE in a micellar system. The experiments were performed at a pH at which TOE is uncharged and almost completely bound to detergent micelles. TOE binding was monitored by its enhanced fluorescence in pure DM micelles or its quenched fluorescence in pure BrUM or BrDM micelles. In DM/BrUM and DM/BrDM mixed micelles, the fluorescence intensity of TOE decreased, as a nonlinear function of the molar fraction of brominated detergent, to almost zero in pure brominated detergent. The indole moiety of TOE is therefore highly accessible to the bromine atoms located on the detergent alkyl chain because quenching by bromines occurs by direct contact with the fluorophore. TOE is simultaneously poorly accessible to iodide (I^-), a water-soluble collisional quencher. TOE time-resolved fluorescence intensity decay is heterogeneous in pure DM micelles, with four lifetimes (from 0.2 to 4.4 ns) at the maximum emission wavelength. Such heterogeneity may arise from dipolar relaxation processes in a motionally restricted medium, as suggested by the time-dependent (nanoseconds) red shift (11 nm) of the TOE emission spectrum, and from the existence of various TOE conformations. Time-resolved quenching experiments for TOE in mixed micelles showed that the excited-state lifetime values decreased only slightly with increases in the proportion of BrDM or BrUM. In contrast, the relative amplitude of the component with the longest lifetime decreased significantly relative to that of the short-lived species. This is consistent with a mainly static mechanism for the quenching of TOE by brominated detergents. Molecular modeling of TOE (in vacuum and in water) suggested that the indole ring was stabilized by folding back upon the octyl chain, forming a hairpin conformation. Within micelles, the presence of such folded conformations, making it possible for the entire molecule to be located in the hydrophobic part of the micelle, is consistent with the results of fluorescence quenching experiments. TOE rotational correlation time values, in the nanosecond range, were consistent with a hindered rotation of the indole moiety and a rotation of the complete TOE molecule in the pure DM or mixed detergent micelles. These results, obtained with a simple micellar model system, provide a basis for the interpretation of fluorescence quenching by brominated detergents in more complex systems such as protein- or peptide-detergent complexes.

INTRODUCTION

Physicochemical studies of membrane proteins generally require the use of a detergent as a solubilizing agent. Many studies have focused on protein-detergent interactions, from both functional and structural points of view (as reviewed in Helenius and Simons, 1975; Tanford and Reynolds, 1976; Møller et al., 1986; Kühlbrandt, 1988), but many aspects of these interactions remain unclear. For example, the three-stage model of membrane solubilization, initially put forward for liposomes composed primarily of a single phospholipid (Helenius and Simons, 1975; Lichtenberg et al., 1983; Lichtenberg, 1985; Paternostre et al., 1988), is still being refined both for these simple systems (e.g., Vinson et al., 1989; Kragh-Hansen et al., 1998) and for more complex biological membranes (Kragh-Hansen et al., 1993, 1998). The structure of protein-detergent complexes in solution is also a matter of debate (e.g., Barber, 1989; Møller and le Maire, 1993; Haneskog et al., 1996; Gast et al., 1996). The idea that a monolayer of detergent covers the entire hydrophobic surface of the transmembrane sector of these proteins has emerged from various experimental approaches. However, the organization of detergent in protein-detergent crystals has been described in only a limited number of cases, from neutron diffraction studies (Roth et al., 1989, 1991; Pebay-Peyroula et al., 1995; Penel et al., 1998).

We synthesized brominated analogs of nonionic detergents to investigate structural aspects of protein-detergent interactions (de Foresta et al., 1996). DM was used as one of the parent detergents because it is widely used for membrane proteins as diverse as transporters, receptors, or proteins from the photosynthetic apparatus (as reviewed in de Foresta et al., 1996). Bromine atoms quench the fluorescence of the chromophores with which they are in contact,

Received for publication 25 November 1998 and in final form 23 August 1999.

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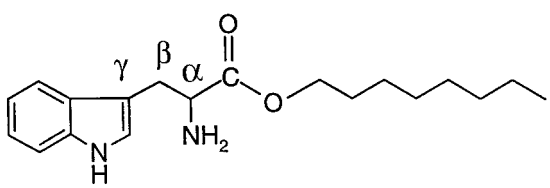
0006-3495/99/12/3071/14 $2.00

by a mechanism called heavy atom quenching (Berlman, 1973; Bolen and Holloway, 1990). This property has been the basis for numerous studies of lipid-protein interactions in membranes, using brominated phospholipids as quenchers (e.g., East and Lee, 1982; for recent examples, see also de Kroon et al., 1990; Ulbrandt et al., 1992; Tretyachenko-Ladokhina et al., 1993; Duché et al., 1994; Mishra and Palgunachari, 1996; Vécsey-Semjén et al., 1997). We used the same approach, but with brominated detergents rather than brominated phospholipids, to determine the extent of protein-detergent contact for a membrane transport protein, the sarcoplasmic reticulum (SR) Ca^{2+}-ATPase, in the membrane phase and in solubilized protein-detergent complexes (de Foresta et al., 1996). These brominated detergents were also used to detect conformational changes in solubilized mutants (with a single Trp) of another membrane protein, the lactose permease from *Escherichia coli* (Wang et al., 1997). Use of these detergents was also extended to the study of the interaction between detergent and peptides corresponding to transmembrane segments of the SR Ca^{2+}-ATPase (Soulié et al., 1998).

The quantitative interpretation of fluorescence quenching data obtained with brominated detergents relies on detailed knowledge about the mechanism of this quenching. In many studies of protein quenching by brominated phospholipids in a membrane environment, it has been assumed that quenching is static, i.e., fluorophores can only be quenched by quenchers in their immediate vicinity when fluorescence excitation occurs. As discussed by East and Lee (1982), the rationale for this assumption is that the excited state of the fluorophore (the Trp average lifetime in a protein is ~2–4 ns) is not long enough to allow the brominated phospholipids to exchange positions. In a micellar environment, however, the situation may be less clear cut because the motion and conformational changes of micellar detergent molecules are faster than those of phospholipid molecules in membranes. This may result in the collisional quenching mechanism making a significant contribution to total quenching. In addition, micelles provide an environment for the fluorophores that is less ordered than a membrane bilayer, so that the positions of the bromines are not as predictable as in a membrane phase.

In this study, we used a hydrophobic compound, tryptophan octyl ester (TOE) (Scheme 1), as a model for Trp, to analyze the characteristics of fluorescence quenching by brominated detergents in a micellar environment. Fluorescence emission spectra, quantum yield, fluorescence lifetimes, and correlation times were determined for TOE embedded in pure DM micelles and in mixed micelles consisting of DM and one of two brominated derivatives, 7,8-dibromododecyl maltoside (BrDM) (as characterized by de Foresta et al., 1996) or 10,11-dibromoundecanoyl maltoside (BrUM), at various molar ratios. We used BrUM, in addition to BrDM, to test whether the differences in the position of bromine on the detergent molecule would affect quenching, thereby enabling us to determine the mean fluorophore location in a micellar environment. Fluorescence data were compared with those for TOE in a membrane environment (Yeager and Feigenson, 1990; Abrams and London, 1992; Ladokhin and Holloway, 1995; Chattopadhyay et al., 1997). Additional topological information was obtained by determining the accessibility of TOE in micelles to the water-soluble collisional quencher I^-. Finally, molecular modeling of TOE was carried out to visualize the thermodynamically stable conformations of this molecule.



SCHEME 1 Neutral form of TOE.

MATERIALS AND METHODS

Solutions and chemicals

TOE, *N*-acetyltryptaphanamide (NATA), and KI were purchased from Sigma-Aldrich. DM was obtained from Calbiochem and was used as supplied. All other chemicals were of analytical grade.

Stock solutions of TOE (usually 2.5 mM) were made in ethanol. NATA was solubilized in water, at a concentration of ~2 mM. Stock solutions of DM, BrDM, and BrUM were made up in water at 20 and 200 mM. A 5 M stock solution of KI was made up in water, and 0.1 mM $Na_2S_2O_3$ was added to prevent oxidation (Eftink and Ghiron, 1976). Water was double-distilled, and buffers were filtered through Millex-HA filters (Millipore).

Synthesis of the brominated detergents

BrDM was synthesized as described by de Foresta et al. (1996). BrUM was synthesized by Dr. Anker (INSA, Lyon) by a similar procedure.

Determination of BrUM cmc

The cmc of BrUM was determined by a spectrophotometric method, based on the blue shift in the absorption spectrum of methyl orange when it binds to detergent micelles (Benzonana, 1969). Fig. 1 shows plots of methyl orange absorbance changes $\Delta A_{414} - \Delta A_{484}$ (where the subscript refers to the wavelength) as a function of total detergent concentration for DM, BrDM, and BrUM. The cmc for each detergent is given by the x coordinate of the intersection of the two straight lines fitting the data. For BrUM, a cmc of 320 μM (±~10%) was obtained. This is slightly higher than the cmcs for DM (~170 μM) or BrDM (220 μM). The values obtained for DM and BrDM are consistent with results previously obtained by the same method (de Foresta et al., 1996).

Absorption measurements

Absorption spectra were recorded on an HP 8452A diode array spectrophotometer equipped with a thermostatically controlled sample holder. The sample was continuously stirred. The path length through the cuvette was 1 cm.

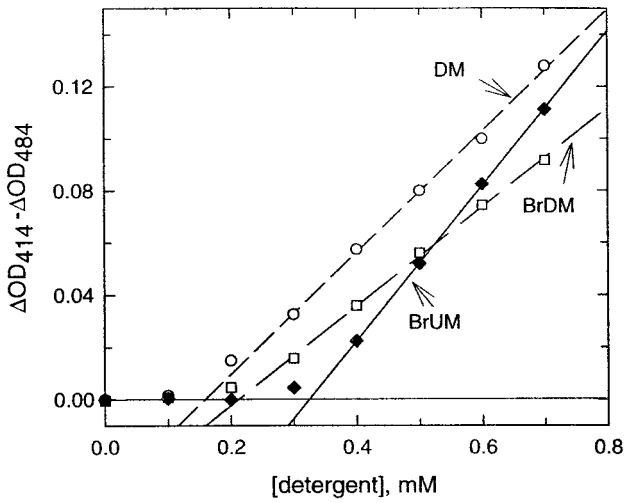


FIGURE 1 Spectrophotometric determination of the cmc of BrUM (♦) versus that of DM (○) and BrDM (□). Methyl orange (50 μM) was added to 2 ml of 50 mM Tes/Tris (pH 7.5), 100 mM KCl, and 1 mM $MgCl_2$ at 20°C, and the absorption spectrum of this solution, from 300 to 600 nm, was taken as a blank. Aliquots of detergent were then sequentially added under continuous stirring, and the differential absorption spectrum was recorded after each addition. The differential absorbance at 414 nm minus that at 484 nm was plotted as a function of the total concentration of detergent added. Straight lines were fitted to the almost linear initial and final parts of each plot: a short-dashed line for DM (○), a long-dashed line for BrDM (□), and a solid line for BrUM (♦). For clarity, only one fit (that for BrUM) is shown for the initial part of the plots. The other two (not shown) were also almost horizontal. Each cmc was determined as the x coordinate of the intersection of the fitted lines for each plot.

Steady-state fluorescence measurements

Fluorescence intensities were generally measured on a Spex Fluorolog spectrofluorometer. The temperature in the cuvette was controlled with a thermostat and the sample was continuously stirred. We used a standard quartz cuvette (1 × 1 cm). A few experiments were also performed with a Perkin-Elmer MPF-44A spectrofluorometer and an SLM 8000 spectrofluorometer, with which data were collected so as to correct for polarization artifacts.

Time-resolved fluorescence measurements

Fluorescence intensity and anisotropy decays were determined by the time-correlated single photon counting technique from the polarized components, $I_{vv}(t)$ and $I_{vh}(t)$, on the experimental setup of the SB1 window of the synchrotron radiation machine Super-ACO (Anneau de Collision d'Orsay), as previously described (Rouvière et al., 1997, and references therein). The excitation wavelength was selected using a double monochromator (Jobin Yvon UV-DH10, 4-nm bandwidth). A Hamamatsu MCP-PMT (model R3809U-02) was used. The time resolution was ~20 ps, and the data were accumulated in 2048 channels. Automatic sampling cycles were carried out, including a 30-s accumulation time for the instrument response function and a 90-s acquisition time for each polarized component, so that a total of (2–4) × 10^6 counts was reached for each fluorescence intensity decay. Fluorescence intensity and anisotropy decays were analyzed as sums of exponentials by the maximum entropy method (MEM) (Livesey and Brochon, 1987). Time-resolved emission spectra (TRES) were reconstructed in each set of experimental conditions from 10 individual decays at various emission wavelengths, from 305 nm to 395 nm (bandwidth 5 nm) with a 10-nm interval, according to a published procedure. Each decay accumulated 10^4 or more counts in the peak channel and was analyzed with the MEM program, using the negative amplitude option (Vincent et al., 1995). The spectral shift was quantified by calculating the barycenters in frequency and full widths at half-peak from the raw transient spectra.

Molecular modeling

All molecular mechanics calculations were performed using the CHARMM program (Brooks et al., 1983) with the potential function parameter set 22 (Mackerell et al., 1997). Nonbonded van der Waals and Coulombic electrostatic interactions were switched off between 11 and 15 Å, and the vacuum dielectric constant was used.

The TOE molecule was initially built by using the Chemnote two-dimensional molecular construction facility in the molecular modeling package QUANTA. TOE conformational analysis in a vacuum was performed on this initial model. Five dihedral angles (C_γ-C_β, C_β-C_α, C_α-CO, OC-O, O-CH_2) in the TOE molecule were changed in 30° steps, and the energy of each generated conformation was minimized. Thus a total of 12^5 conformations were tested. The conformations with minimal potential energy were selected and compared.

For modeling in water, TOE molecules in two conformations (folded and extended) were positioned in a periodic water box 36 × 30 × 25 $Å^3$ in size that had previously been equilibrated. Water molecules were modeled with the TIP3P potential function (Jorgensen et al., 1983). All water molecules closer than 2.6 Å to any TOE atom were eliminated. Both systems (folded and extended) involved 872 water molecules and one molecule of TOE. In the first energy minimization step, the TOE molecule was constrained and the water molecules were free; in the second step, the water molecules were constrained and the TOE molecule was free; and in the final step all molecules were free.

RESULTS

Binding of TOE to DM, BrDM, and BrUM micelles

We first monitored the binding of TOE (5 μM) to DM, BrUM, and BrDM micelles from the resulting fluorescence intensity changes at 335 nm (Fig. 2). In all cases, we waited a few minutes for equilibration of the TOE fluorescence signal, either in buffer alone (the signal exhibited a slight initial decrease) or after each addition of detergent. The binding of TOE to DM micelles led to a large (maximum eight times) increase in TOE fluorescence intensity (Fig. 2 *A*; see also Soulié et al., 1998), which was correlated with a blue shift in the emission spectrum. These changes may result from a significant decrease in the polarity (and/or mobility) of the TOE microenvironment upon binding to detergent micelles. In contrast, because of the quenching properties of the bromine atoms, the curves of binding of TOE to pure BrDM or pure BrUM micelles (Fig. 2 *B*) involve a significant decrease in fluorescence, resulting in a residual intensity 25–30% of that for TOE in buffer alone (i.e., only a few percent of that in DM; note the different scales used in Fig. 2, *A* and *B*). The shapes of the three binding curves are similar (except at lower detergent concentrations, where there is clearly a slight shift between the curves with BrDM and BrUM, because of the slightly different cmcs of these detergents; cf. Materials and Methods), showing that binding was similar in all cases. Subsequent experiments were performed at concentrations of 4 mM detergent; at this concentration, for all three detergents,

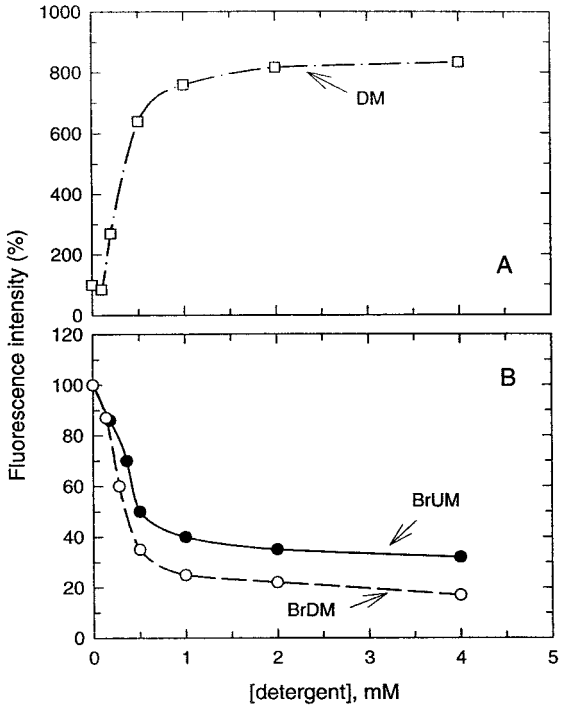


FIGURE 2 Binding of TOE to BrDM (*B*, ○) and BrUM micelles (*B*, ●), relative to that to DM (*A*, □). (*A*) TOE (5 μM) was added to 50 mM Tes/Tris (pH 7.5), 100 mM KCl at 20°C and allowed to equilibrate for ~4 min so that its fluorescence could stabilize. Aliquots of DM were then added sequentially with continuous stirring, at regular intervals of ~3–4 min, long enough to allow the signal to become stable between additions. Fluorescence intensity was continuously recorded with λ_{ex} and λ_{em} set at 290 and 335 nm, respectively, and bandwidths of 1.25 mm (~5 nm) at both wavelengths. The fluorescence intensities obtained after each DM addition, corrected using blank values (DM alone in buffer), were plotted (as a percentage of the initial value) as a function of final DM concentration (□ and – · –). (*B*) Similar experiments, but with either BrDM (○ and – – –) or BrUM (● and ——).

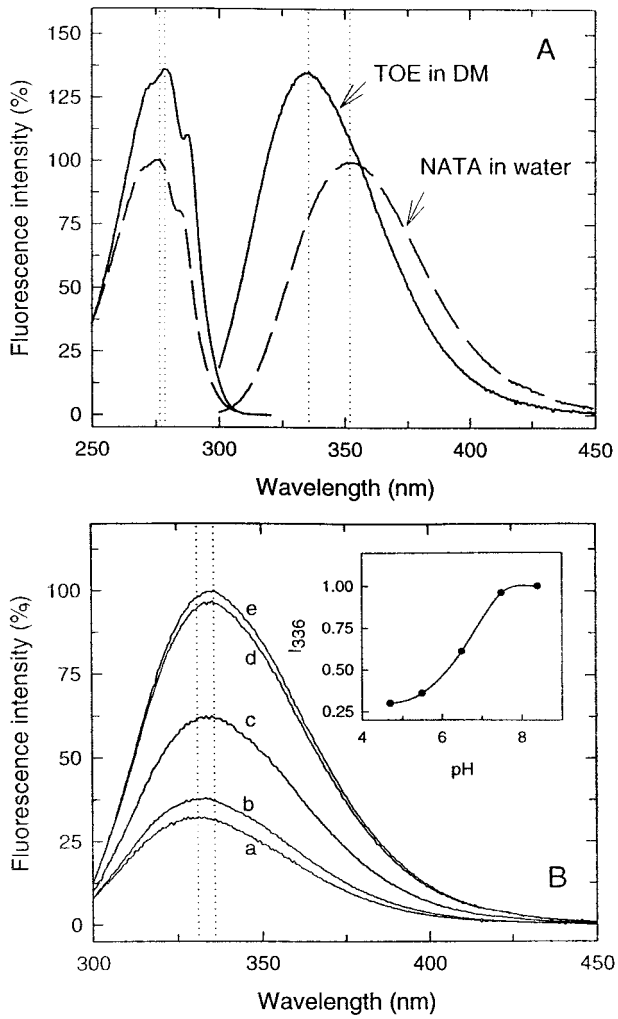


FIGURE 3 Fluorescence excitation and emission spectra of TOE in DM: effect of pH. (*A*) Normalized excitation (*left*) and emission (*right*) spectra of TOE (——) in 50 mM K_2HPO_4/KH_2PO_4 buffer, pH 7.5, containing 4 mM DM, and of NATA (– – –) in pure water. In both cases, the temperature used was 20°C. For excitation spectra, λ_{em} was 335 for TOE and 350 nm for NATA, and bandwidths were 0.5 mm (~2 nm) at excitation and 1.25 mm (~5 nm) at emission. For emission spectra, λ_{ex} was 280 nm in both cases, and the bandwidths were 1.25 mm at the excitation and 0.5 mm at the emission. Spectra were corrected using buffer blanks, and excitation spectra were also corrected for the spectrum of the lamp. The excitation and emission spectra for NATA, recorded at a concentration of ~5 μM, were both normalized to 100%. For TOE, both spectra were recorded at a concentration of 10 μM and were normalized as follows. The maximum emission intensity of TOE was set at the percentage of the maximum intensity for NATA, for solutions of similar fluorophore absorbance at 280 nm. The excitation spectrum of TOE was normalized such that its maximum intensity was equal to that of the emission spectrum. Vertical dotted lines show the various λ_{max} positions. (*B*) TOE (5 μM) was added to 100 mM KH_2PO_4/K_2HPO_4 buffer containing 4 mM DM, at various pH values at 20°C. Emission spectra were recorded after 4 min of equilibration, with λ_{ex} set at 280 nm, and bandwidths of 1.25 mm and 0.5 mm at excitation and emission, respectively. They were corrected using blanks (buffer plus detergent). pH values were as follows: (*a*) 4.7; (*b*) 5.5; (*c*) 6.5; (*d*) 7.5; (*e*) 8.4. Vertical dotted lines show λ_{max} positions for pH 4.7 and 8.4. *Inset*: Fluorescence intensity at 336 nm as a function of pH.

almost all of the observed TOE fluorescence was due to TOE incorporated into the detergent micelles. In addition, the number of micelles was in a large excess over the number of TOE molecules (~7, for an aggregation number of 110 for DM; Møller and le Maire, 1993).

Steady-state fluorescence of TOE in DM micelles: pH effect

We compared the fluorescence excitation and emission spectra of TOE in DM micelles with those of NATA, a hydrophilic Trp model compound, in water. The excitation spectra of TOE and NATA (Fig. 3 *A*, *left*) were similar, except that that of TOE is slightly red shifted (~2 nm). Both spectra have a main peak (at ~278 nm for TOE) and a shoulder (at ~288 nm for TOE) characteristic of the absorption of the fluorescent indole moiety. The excitation spectra resembled the absorption spectra of TOE in DM and NATA in water (not shown), respectively (in particular, the same slight shift between the absorption spectra was observed). The maximum of the emission spectrum of TOE in

DM was close to 335 nm (Fig. 3 *A*, *right*) and was strongly blue shifted, by more than 15 nm, with respect to that of NATA in water (λ_{max} ~352 nm), as previously described (Soulié et al., 1998). The TOE quantum yield value was also slightly higher (~30% higher) than that of NATA. The fluorescence emission of the indole moiety is known to be very sensitive to the polarity of its environment (Callis and Burgess, 1997). Our data therefore show that the environment of TOE in DM is significantly less polar than that of NATA in water, consistent with the fluorescent indole moiety of TOE being partially buried in the DM micelles. This is also consistent with the slight difference in λ_{max} of the excitation spectra.

Fig. 3 *B* shows how the fluorescence emission spectrum of TOE changes with pH, in the presence of DM. Depending on pH, TOE may exist as a deprotonated, neutral form, or as a protonated, cationic form. Its location within detergent micelles and its intrinsic fluorescence properties may depend on its ionization state. TOE fluorescence intensity in 4 mM DM at λ_{max} was three times higher at pH 8.4 than at pH 4.7 (Fig. 3 *B*), and this increase in fluorescence intensity was correlated with a slight red shift in the emission spectra (λ_{max} varying from ~331 to ~336 nm). A plot of TOE fluorescence intensity at 336 nm as a function of pH yields an apparent pK_a value of 6.7 (Fig. 3 *B*, *inset*). Subsequent experiments were all performed at pH 7.5; under these conditions, the fluorescence of TOE is mainly due (at least 95%) to its neutral form.

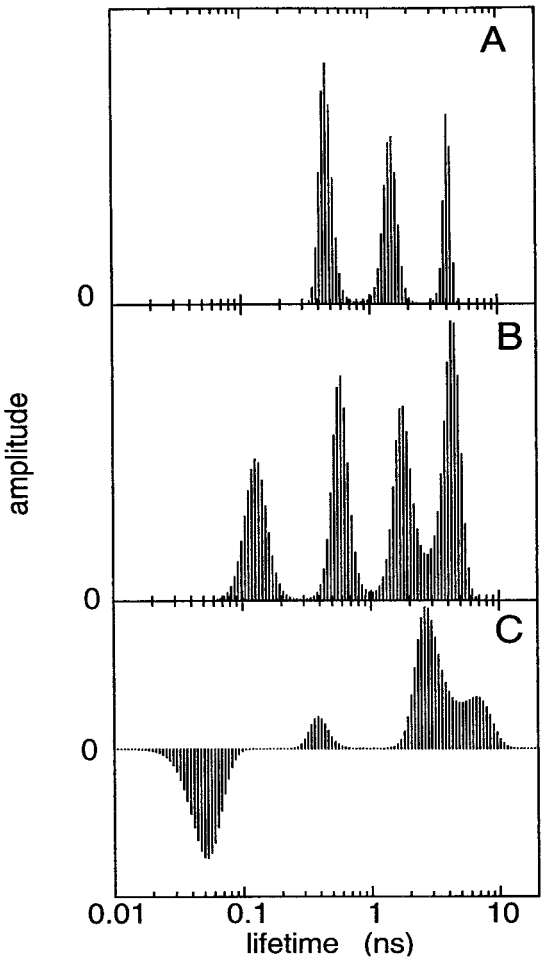


FIGURE 4 MEM recovered lifetime distributions of TOE in DM micelles at three emission wavelengths. TOE concentration was 5 μM in 50 mM phosphate buffer (pH 7.5, 20°C) containing 4 mM DM. λ_{ex} = 280 nm. (*A*) Emission wavelength: 305 nm; $\alpha_1 = 0.44$; $\alpha_2 = 0.34$; $\alpha_3 = 0.22$; $\tau_1 = 0.47$ ns; $\tau_2 = 1.46$ ns; $\tau_3 = 4.04$ ns; $\chi^2 = 1.16$. (*B*) Emission wavelength: 335 nm; $\alpha_1 = 0.20$; $\alpha_2 = 0.24$; $\alpha_3 = 0.25$; $\alpha_4 = 0.32$; $\tau_1 = 0.13$ ns; $\tau_2 = 0.56$ ns; $\tau_3 = 1.83$ ns; $\tau_4 = 4.28$ ns; $\chi^2 = 1.03$. (*C*) Emission wavelength: 375 nm; $\alpha_1 = 1.33$; $\alpha_2 = 0.24$; $\alpha_3 = 1.53$; $\alpha_4 = 0.56$; $\tau_1 = 0.05$ ns; $\tau_2 = 0.40$ ns; $\tau_3 = 2.96$ ns; $\tau_4 = 6.88$ ns; $\chi^2 = 1.04$.

Time-resolved fluorescence decays of TOE in DM micelles: dipolar relaxation and conformations

In pure DM, the fluorescence emission decay of TOE is not monoexponential, irrespective of the emission wavelength used. Up to four lifetime populations were detected (Fig. 4). Emission heterogeneity may arise from the existence of various ground-state conformations, as suggested for Trp in buffer (Szabo and Rayner, 1980), or from dipolar relaxation around the excited state (for a recent review, see Callis, 1997). In principle, the measurement of time-resolved fluorescence emission decays at various emission wavelengths should make it possible to discriminate between these possibilities. For ground-state conformations, lifetime values should be constant throughout the emission spectrum with only relative amplitudes changing. For dipolar relaxation, characteristic features of spectral relaxation should be observed, such as time constants associated with negative amplitudes at the red edge of the fluorescence emission spectrum, as observed for indole in protic solvents (Vincent et al., 1995). Two time constants should be obtained for a single excited state subjected to a single dipolar relaxation process. The four time constants obtained for TOE at the maximum emission wavelength (335 nm; see Fig. 4 *B*) indicate that the underlying mechanisms are complex. In addition, increasing λ_{em} from 305 to 375 nm changed lifetime constants and their respective amplitudes; three lifetime components were detected at the blue edge of the spectra (Fig. 4 *A*), four at the emission maximum (Fig. 4 *B*), and then two or three (with positive amplitudes) at the red edge (Fig. 4 *C*). A fast component with a negative amplitude was also detected in this red-edge region. Such a complex pattern precludes the assignment of each time constant to a particular excited state and to a characteristic emission spectrum. Nevertheless, the existence of a negative component suggests that a dynamic process (such as dipolar relaxation) occurs in the excited state(s).

Reconstruction (from the fluorescence decay at various emission wavelengths) of the TOE transient emission spectra at various times showed a shift toward longer wavelengths: the barycenter of the emission spectra shifted by 11 nm within a few nanoseconds (Fig. 5 *A*). The full width at half-maximum of the emission spectrum (not shown) in-

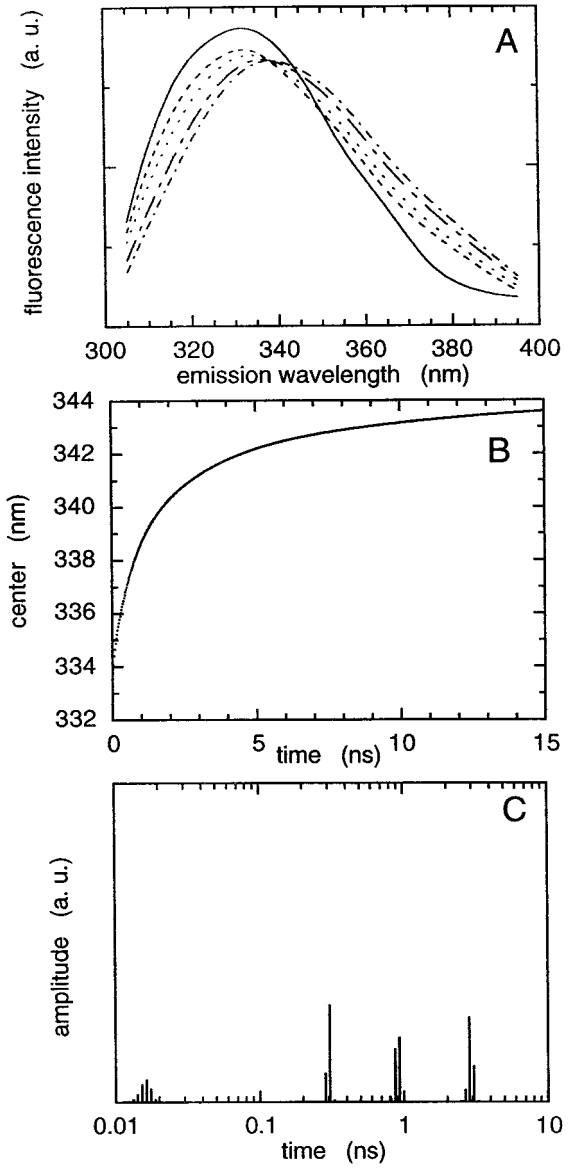


FIGURE 5 Time-resolved emission spectra of TOE in DM micelles. The sample used was as in Fig. 4. The emission spectra were reconstructed at various times (see Materials and Methods). (*A*) Solid line: emission spectrum at $t = 0$. From left to right: emission spectra at $t = 640$ ps; 920 ps; 2600 ps; 6400 ps. The spectra were normalized to a constant area. (*B*) Displacement of the barycenter of the emission spectrum as a function of time. (*C*) Distribution of relaxation time constants τ_{iR}. The kinetics of the displacement of the barycenter are characterized by a function $C'(t)$ defined as $C'(t) = (\bar{\nu}_0 - \bar{\nu}_\infty)C(t)$, where $\bar{\nu}_0$ and $\bar{\nu}_\infty$ are the barycenter values in frequency at times 0 and ∞, respectively and $C(t)$ is the (normalized) shift function (Vincent et al., 1995), which is analyzed as a sum of exponentials: $C = \Sigma a_i \exp(-t/\tau_{iR})$. Values for a_i and τ_{iR} are as follows: $a_1 = 0.15$, $\tau_{1R} = 16$ ps; $a_2 = 0.28$, $\tau_{2R} = 300$ ps; $a_3 = 0.28$, $\tau_{3R} = 910$ ps; $a_4 = 0.29$, $\tau_{4R} = 2888$ ps; $(\bar{\nu}_0 - \bar{\nu}_\infty) = 962$ cm^{-1}.

creased by ~6 nm in the same amount of time, demonstrating a heterogeneity of dipolar interactions, as observed for indole dissolved in isobutanol (Vincent et al., 1995). The kinetics of the barycenter shift were analyzed as a sum of exponentials using MEM (Fig. 5 *B*); three major time constants were obtained (0.3, 1, and 3 ns) in equal proportions along with a very small minor constant of 20 ps (Fig. 5 *C*).

These results suggest that the indole ring in DM micelles is sensitive to dipolar relaxation. The blue shift of steady-state emission, relative to the emission of TOE or NATA in bulk water, is then probably due to the slower motion of the surrounding dipoles in the hydrated region of the micelle as compared to the motion of dipoles in bulk water, which occurs in less than a picosecond (Vajda et al., 1995), rather than to their being located in the pure hydrophobic core of the micelle. This, however, does not rule out the coexistence of several conformers, as suggested below.

Steady-state fluorescence quenching of TOE in DM/BrDM and DM/BrUM mixed micelles

The steady-state fluorescence emission spectra of TOE incorporated into DM or various mixed micelles of DM/BrDM or DM/BrUM were compared (data not shown). TOE fluorescence was almost totally quenched in pure BrUM and pure BrDM (see the quenching curves in Fig. 6). However, there was no significant shift in the TOE emission maximum, if relative fluorescence intensity changed from 100% to ~40%. A red-shifted component, with a spectrum similar to that of TOE in buffer alone, was detected at a lower relative fluorescence (20% or below), i.e., with a brominated detergent molar fraction of 0.4 or more. This minor component presumably arose from TOE molecules in a more polar environment, possibly including some TOE molecules not bound to the micelles.

Fig. 6 *A* shows TOE fluorescence quenching as a function of the molar fraction of the brominated detergent in mixed micelles of DM/BrUM or DM/BrDM. Despite the different locations of bromine atoms in BrUM (C10-C11) and BrDM (C7-C8), the two curves are similar: 1) the residual fluorescence is very low both in pure BrUM and in pure BrDM, 6% and 3%, respectively (this slight difference is also evident from the plateau values of the binding curves of TOE to these detergents); 2) both curves have a high curvature. These results show that the indole moiety of TOE is highly accessible to the brominated alkyl chains of BrUM and BrDM in the (mixed) detergent micelles.

We fitted to the data a model first described by London and Feigenson (1981) and used by East and Lee (1982). This model was originally designed to describe the quenching of membrane fluorophores (e.g., protein Trp) by spin-labeled or brominated phospholipids. This model considers two populations of fluorophores: one completely inaccessible to the quencher, responsible for residual fluorescence, F_{min} (e.g., Trp embedded in protein); and another, in which each fluorophore has n neighbors (phospholipids) and the fluorescence of which is completely quenched if one (or more) of these sites is occupied by a modified phospholipid. Phospholipids do not change positions during the lifetime of the fluorophore. If X is the molar ratio of quenchers in the membrane, $(1 - X)^n$ is the probability that none of the n

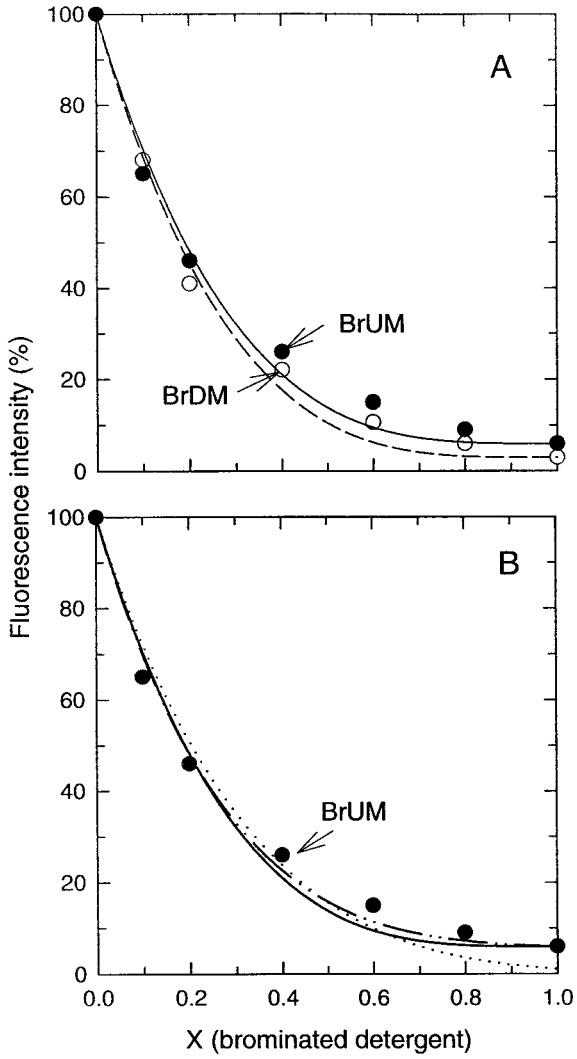


FIGURE 6 Quenching of steady-state TOE fluorescence by BrUM, relative to that by BrDM. (*A*) The quenching of TOE fluorescence in BrUM/DM mixed micelles (●). Data previously obtained in BrDM/DM mixed micelles are also shown (○, Soulié et al., 1998). BrUM and DM were added to 50 mM Tes/Tris (pH 7.5), 100 mM KCl (20°C), at a given molar ratio and a final total detergent concentration of 4 mM. TOE (5 μM) was then added. The fluorescence intensity, after ~100 s of equilibration and corrected for the blank due to detergent alone, was plotted as a function of the molar fraction, X, of BrUM (or BrDM) in the detergent mixture (X = [BrUM]/([BrUM] + [DM])). λ_{ex} = 290 nm, λ_{em} = 335 nm, with bandwidths of 10 nm at both wavelengths. The function $F/F_0 = (1 - F_{min}/F_0)(1 - X)^n + F_{min}/F_0$ (—— and – – – for BrUM and BrDM, respectively) was fitted to the data, according to a model described by East and Lee (1982) for the quenching of fluorophores in membranes containing various ratios of brominated and nonbrominated phosphatidylcholine. (*B*) Comparison of various fits for TOE in BrUM. The data and the continuous line fit (obtained from the equation given in *A*, with the constraint F_{min}/F_0 = 6%; resulting regression coefficient r = 0.994) are redrawn from *A*. Two other functions were also fitted to the data: $F/F_0 = (1 - pX)^n$ (with the constraint n = 6; then p = 0.53 and r = 0.991) (·····), and $F/F_0 = (1 - f)(1 - pX)^n + f$ (with the constraints n = 6 and f = 5.7%; f is chosen so that the intensity at X = 1 is equal to the experimental value, 6%; then p = 0.62 with r = 0.996) (– · · –).

sites is occupied by a quencher. The fluorescence is therefore given by

$$F/F_0 = (1 - F_{min}/F_0)(1 - X)^n + F_{min}/F_0 \quad (1)$$

This model fits the data well, giving n = 3.6 in BrUM and 3.7 in BrDM (Fig. 6 *A*, *solid* and *dashed lines,* respectively), which, for static quenching, would indicate that several detergent chains were simultaneously in contact with the indole moiety of TOE.

However, this model deals only with the "lateral" accessibility of the fluorophores to bromine atoms. The "transverse" accessibility of the fluorophore (i.e., along an axis perpendicular to the plane of the membrane, or to the surface of the micelle) can be assessed by assuming that the probability (p) of quenching by occupation of a neighboring site is not exactly 1 but is less than 1, depending on the relative depths within the micelle of the fluorophore and bromine atoms. Thus the probability that one neighbor acts as a quencher is pX, and the probability that none of the n neighbors of a fluorophore act as quenchers is $(1 - pX)^n$. The fluorescence is therefore described by the function

$$F/F_0 = (1 - pX)^n \quad (2)$$

If this function is applied to the data in Fig. 6 *A*, n tends to be high (up to 300) if no constraint is imposed, and this has no physical meaning. A reasonable maximum for n was set as a constraint ($n \leq 6$, corresponding to the maximum number of phospholipids surrounding another phospholipid in a hexagonal lattice; London and Feigenson, 1981), and the fit for TOE in BrUM, illustrated by the dotted line in Fig. 6 *B*, was obtained. This fit better follows the slope of the curve at high molar ratio (compare *solid* and *dotted lines*), but the final intensity is too low. If a population of totally inaccessible fluorophores (a fraction f of the total population) is included in this model, fluorescence is given by

$$F/F_0 = (1 - f)(1 - pX)^n + f \quad (3)$$

This model fit the data slightly better than the other (Fig. 6 *B*, *dash-dotted* line, corresponding to the following parameters: n = 6, p = 0.62, f = 5.7%; see details in figure legend). If our assumptions are correct, then, if TOE does indeed have six detergent neighbors, only ~60% of the brominated ones have their bromines sufficiently close to the indole to quench its fluorescence emission. Using BrDM and BrUM, in which the bromines are at different positions in the chain, it was not possible to test more elaborate models, such as the parallax method (Chattopadhyay and London, 1987; Abrams and London, 1992) or the distribution analysis method (Ladokhin and Holloway, 1995, and references therein) used for membrane fluorophores, because the mean depths of the detergent bromines in the micelles are not known and because the extent of quenching does not differ significantly between BrUM and BrDM. Note that none of the models takes into account the asymmetry of TOE, although the various conformations (see

below) may differ in sensitivity to quenching by bromine atoms, depending on the orientation of the indole ring with respect to the C-Br bonds.

Time-resolved fluorescence study of quenching of TOE in DM/BrDM and DM/BrUM mixed micelles

TOE quenching by brominated detergents was explored in greater detail by measuring time-resolved intensity decays at 335 nm (i.e., close to the maximum emission wavelength in DM) at various molar fractions of BrDM or BrUM in the mixed micelles. The effects observed with the two brominated detergents were similar (Fig. 7, *left* and *right panels*). In both cases, the number of lifetimes (four at this wavelength) was constant regardless of quenching. Only a slight decrease in the four lifetime values was observed if the molar fraction of brominated detergent was increased: for the two longest lifetimes (accounting for 90% of steady-state intensity I in pure DM, as calculated by $I_i = \alpha_i\tau_i/\Sigma\alpha_i\tau_i$ and $I = \Sigma I_i$), the maximum decrease was ~25%, i.e., much lower than the maximum decrease in steady-state fluorescence intensity (~95%). In contrast, the normalized amplitudes (α_i) for these lifetimes were much lower (15 times lower for the longer lifetime), resulting in a decrease in steady-state intensity. A lack of variation in lifetime is characteristic of a static quenching mechanism. Thus quenching is mainly static (i.e., resulting from the bromine atom-indole contacts existing at the time of excitation of the indole). Only a small fraction of the quenching is of dynamic origin (i.e., resulting from the diffusion and collision of the quencher with the indole moiety during its lifetime), and this dynamic component mainly occurs at low or intermediate molar fractions of BrDM or BrUM. The selective effect of quenching on the time components with the longest values is consistent with various TOE conformations differing in sensitivity to quenching by the brominated detergent.

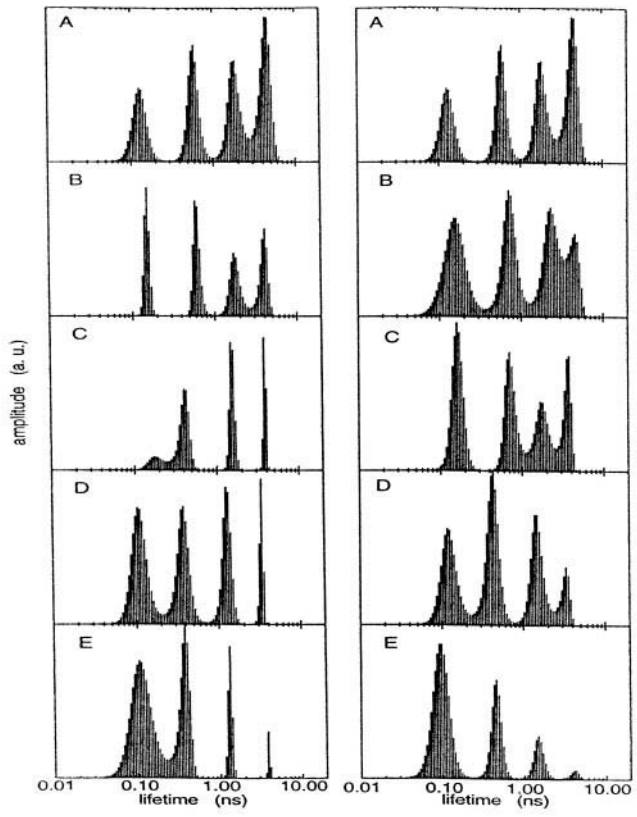


FIGURE 7 MEM recovered lifetime distributions of TOE in mixed micelles of DM and BrDM (*left panels*) or BrUM (*right panels*), at various molar fractions (X) of the brominated detergent. TOE concentration was 5–15 μM in 50 mM phosphate buffer (pH 7.5, 20°C), containing various mixtures of DM and either BrDM (*left*) or BrUM (*right*), at a constant total detergent concentration of 4 mM. (*A*) $X = 0$, (*B*) $X = 0.1$, (*C*) $X = 0.2$, and TOE = 5 μM in all three cases; (*D*) $X = 0.4$ and TOE = 10 μM; (*E*) $X = 1$ and TOE = 15 μM. λ_{ex} = 280 nm, λ_{em} = 335 nm, emission bandwidth = 10 nm. The experiment was performed and analyzed as described in Materials and Methods.

Quenching of TOE in DM by I^-: steady-state and time-resolved experiments

We assessed the quenching of TOE, in DM micelles, by the water-soluble quencher I^- and compared the results with those obtained for NATA in buffer alone. Steady-state fluorescence intensities for both fluorophores were determined at various KI concentrations, in the absence of KCl, or in its presence, such that ionic strength was constant ([KI] + [KCl] = 0.2 M). The data were analyzed using the Stern-Volmer equation: $F_0/F = 1 + K_{SV}[Q]$, where K_{SV} is the Stern-Volmer quenching constant and [Q] is the quencher concentration ([I^-] in this study). In all cases, the data were well fitted by a straight line, typical of a simple collisional quenching mechanism (Eftink, 1991). The accessibility to the quencher was evaluated by calculating k_q, the bimolecular quenching constant, defined as $K_{SV} = k_q\langle\tau_0\rangle$, where $\langle\tau_0\rangle$ is the mean lifetime, in the absence of quencher, of each fluorophore (Table 1). NATA in buffer was strongly quenched by I^-, whereas TOE in DM was only poorly quenched, as shown by K_{SV} values (values at constant ionic strength are indicated in Table 1). Ionic strength itself had a slight effect on quenching by I^-: for TOE in micelles, K_{SV} was 1.6 M^{-1} at constant ionic strength and 2 M^{-1} if ionic strength varied. The differences in TOE and NATA quenching by I^- are clearly due to the lower accessibility to I^- of TOE in DM than of NATA in buffer, estimated at 20–30% from the ratio of the k_q values (obtained in both steady-state and time-resolved experiments for TOE; see below).

The quenching by I^- of TOE in DM was also analyzed by determining time-resolved intensity decays at various I^- concentrations and at constant ionic strength. Under these conditions, Stern-Volmer plots of mean lifetime and of the two components with the longest lifetimes ($\langle\tau_0\rangle/\langle\tau\rangle$ or τ_{0i}/τ_i as a function of [KI]) were also linear (data not shown), consistent with a collisional mechanism. The bimolecular rate constant, derived from the mean lifetime plot, was

TABLE 1 Parameters of TOE and NATA fluorescence quenching by KI, from steady-state and time-resolved measurements*

Sample	ϕ	$\langle\tau\rangle$ (ns)	K_{sv} (M^{-1})	k_q ($M^{-1}\,s^{-1}$)
NATA	0.10[#]	3[¶]	11	3.7×10^9
TOE	0.13[§]	2	1.6	0.8×10^9
				1.1×10^9[‖]

ϕ, quantum yield; $\langle\tau\rangle = \Sigma\alpha_i\tau_i$, mean lifetime; K_{sv}, Stern-Volmer quenching constant; k_q, bimolecular quenching constant.

*Steady-state quenching experiments were performed with 5 μM NATA (without detergent) or 5 μM TOE with 4 mM DM, in 10 mM phosphate (K_2HPO_4/KH_2PO_4) buffer, pH 7.5 (20°C) at constant ionic strength ([KCl] + [KI] = 0.2 M). Fluorescence intensities were recorded with λ_{ex} set at 290 nm and λ_{em} at 350 nm or 335 nm, for NATA and TOE, respectively, and were corrected using blanks and for slight dilution effects. For time-resolved experiments, conditions were similar, except that the concentration of TOE was 10 μM.

[#]NATA in water, from Rouvière et al. (1997).

[§]TOE in DM, with NATA as a reference (this work).

[¶]From Gallay et al. (1993).

[‖]Obtained from the Stern-Volmer plot of TOE mean lifetime.

consistent with that obtained in steady-state experiments (see Table 1). The values for the two components with the longest lifetimes differed slightly ($k_q = 0.23 \times 10^9$ and $0.56 \times 10^9\ M^{-1}\ s^{-1}$), suggesting differences in the accessibilities of the various indole excited states.

Rotational mobility of TOE in detergent micelles

As the intensity decays used to evaluate lifetime distributions were actually reconstructed from their two polarized components, we were also able to determine rotational correlation times (θ) for TOE in micelles (see Materials and Methods). The results obtained for TOE in DM and for one particular molar fraction ($X = 0.4$) of BrDM or BrUM in mixed micelles (at which relative steady-state TOE fluorescent intensity is significant, ~25%) are presented in Fig. 8. In pure DM, two main rotational correlation times, ~1.5 and 6 ns, were obtained. These values are one order of magnitude higher than that expected for TOE in buffer alone (subnanosecond range), indicating restricted motion due to insertion into the micelle. Assuming that $\theta = \eta V/kT$, where V is the spherical volume of the rotor, η is the viscosity of the medium, $\eta = 1$ cP for water at 20°C, $M_w = 353$ for TOE, and taking the density of TOE to be $d \approx 1$ g/cm^3, a correlation time of ~0.14 ns was obtained for TOE in water. Both measured correlation times are significantly lower than expected for rotation of the whole micelle (a similar estimation, with $M_w = 511$ for DM and an aggregation number $n = 110$ (Møller and le Maire, 1993), yields a correlation time of $\theta \approx 20$ ns, or more if the hydration of the micelle is taken into account). This therefore indicates nanosecond rotation of TOE (or its indole moiety) within the micelle. Similar correlation times were also observed for TOE in mixed micelles, although the values obtained were slightly lower in BrUM. The rotational behavior of TOE therefore follows the same trends in all three detergents.

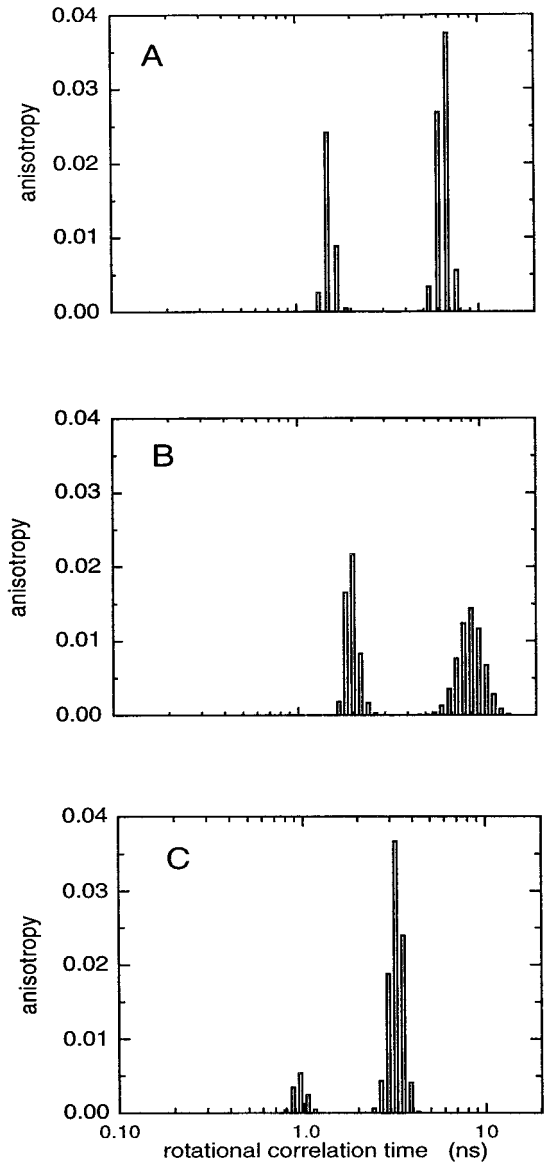


FIGURE 8 Rotational correlation time distribution recovered by MEM of TOE in pure DM (*A*) or in mixed micelles of DM and BrDM (*B*) or BrUM (*C*) at a molar fraction $X = 0.4$ of BrDM or BrUM. The data were collected as described in Fig. 7. (*A*) $\theta_1 = 1.5$ ns, $\beta_1 = 0.036$; $\theta_2 = 6.5$ ns, $\beta_2 = 0.074$; (*B*) $\theta_1 = 2$ ns, $\beta_1 = 0.050$; $\theta_2 = 8.9$ ns, $\beta_2 = 0.062$; (*C*) $\theta_1 = 1.1$ ns, $\beta_1 = 0.012$; $\theta_2 = 3.1$ ns, $\beta_2 = 0.088$.

Molecular modeling of TOE

Conformational analysis of TOE was performed by energy minimization, both in vacuum and in a water environment. In vacuum, the most stable conformations (i.e., those with the minimum potential energy) were all folded. In the most stable folded conformation ($E_{POT} \approx -25.1$ kcal/mol) the indole ring was located close to the octyl chain (Fig. 9). This conformation was stabilized by van der Waals ($E_{VDW} \approx$

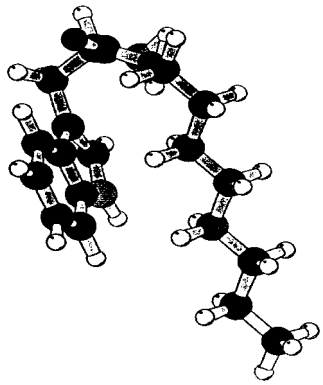


FIGURE 9 Folded TOE conformations obtained by energy minimization. Energy minimization was performed as described in Materials and Methods.

−7.9 kcal/mol) and electrostatic ($E_{ELE} \approx -22.7$ kcal/mol) interactions that overcame the energetically unfavorable stereochemical constraints ($E_{angle} \approx 2.4$ kcal/mol, and $E_{dihe} \approx 2.7$ kcal/mol). The other energetically less favorable models of TOE ($E_{POT} \approx -24.9$ kcal/mol, $E_{POT} \approx -23.05$ kcal/mol, respectively) were also folded conformations differing from the most stable conformation only by rotation of the indole ring around the C_β-C_γ bond. Extended (unfolded) conformations were not energetically favorable in vacuum. If an extended conformation was given as the starting model, it was immediately converted to a folded conformation.

Extended conformers did occur in water because they were stabilized by electrostatic and van der Waals interactions between the solvent molecules and the polar moiety of the TOE molecule (ester bond and amino group). Such interactions were weaker for folded conformations. Nevertheless, there was a difference in potential energy of ~5 kcal/mol between the extended and folded conformations of TOE in the presence of solvent, again favoring the folded conformation. The occurrence, in DM micelles, of such a folded conformation is consistent with the quenching data and would allow both the hydrophobic chain and the indole moiety to be simultaneously embedded in the hydrophobic core of the micelle.

DISCUSSION

The aim of this study was to characterize the quenching of TOE fluorescence by two DM analogs, BrDM and BrUM, which are brominated at positions 7–8 and 10–11 of their acyl chains, respectively, in mixed micelles (DM/BrDM or DM/BrUM). Such brominated detergents were originally synthesized for investigation of the topology of protein-detergent and peptide-detergent complexes (de Foresta et al., 1996; for applications, see Wang et al., 1997, and Soulié et al., 1998). As in previous studies (e.g., Ladokhin and Holloway, 1995; Chattopadhyay et al., 1997), we used TOE as a suitable hydrophobic model compound for Trp residues in membrane proteins.

The binding of TOE to the various detergent micelles was studied by fluorescence enhancement, in DM, or by fluorescence quenching in BrDM or BrUM. Note that the molecular mass of DM micelles is 56,000–72,000 D, according to the aggregation numbers (110–140) reported in the literature (Møller and le Maire, 1993, and references therein; Dupuy et al., 1997). TOE bound similarly to the micelles of all three detergents, binding being almost total at ~1 mM detergent (for 5 μM TOE) (under these conditions, most of the detergent is in a micellar form, because the cmc values were from 170 to 320 μM). The presence of bromine atoms therefore had no effect on the affinity (or partitioning) of TOE for the detergent micelles. TOE bound to these detergents with a slightly lower affinity than that with which it bound to various phosphatidylcholine lipid vesicles, for which binding was total, at similar TOE concentrations, for 100–200 μM lipid (Ladokhin and Holloway, 1995; Chattopadhyay et al., 1997, and our unpublished observations) (in this case, all of the lipids are in the condensed membrane phase because their micellar concentrations are very low). The rigid sugar headgroup of DM (which has a molecular area of 50 Å^2; Møller and le Maire, 1993) may be a more efficient barrier to TOE binding than the more flexible PC headgroups. Small-angle x-ray and neutron scattering measurements have shown that DM micelles possess a well-defined crown of sugar heads and solvation water molecules surrounding an aliphatic core (Dupuy et al., 1997). The enhancement of TOE fluorescence (measured at the λ_{max} of the bound probe) is greater upon binding to DM micelles than upon binding to lipid vesicles (six to eight times higher fluorescence, as shown in this work and in Soulié et al. (1998), versus three times higher, respectively). The apparent pK_a value for TOE was slightly lower in micelles than in lipid membranes (6.7 versus 7.5; Chattopadhyay et al., 1997).

Steady-state fluorescence experiments provided preliminary information about the microenvironment of the indole moiety of TOE in detergent micelles. The ~15-nm blue shift of the maximum emission wavelength of TOE in DM, relative to that of NATA in buffer alone, suggests that the indole moiety is located in an environment significantly less polar than bulk water (e.g., for Trp, λ_{max} changes from ~305 nm in hexane to 355 nm in water) and/or more rigid (the maximum emission wavelength is 334 nm in glycerol at room temperature, whereas it is blue-shifted to 307 nm at 77 K; Meech et al., 1983). NATA was considered a better reference than TOE in water because TOE is likely to form small aggregates in water because of its hydrophobic chain. The quantum yield value of TOE in DM was similar to that in DOPC at pH 7 and to that of Trp in solution (0.14) (Ladokhin and Holloway, 1995; Szabo and Rayner, 1980).

Time-resolved fluorescence measurements demonstrated the complexity of TOE photophysics in detergent micelles. Four main lifetimes were required to describe TOE intensity decay at the maximum emission wavelength, and the lifetimes obtained strongly depended upon the emission wavelength. The estimated time constants describing TOE fluo-

rescence decay as a function of the emission wavelength probably did not refer to pure emission components. They were more likely to be composite values, depending on the excited-state lifetime constants of conformers and on the kinetics of excited-state reactions, probably dipolar relaxation. Dipolar relaxation probably occurred, as shown by the time-dependent spectral red shift and the observation of components with negative amplitude at the red edge of fluorescence emission spectra. This clearly indicated that the indole moiety was located in a partially polar microenvironment of restricted mobility (dipolar relaxation occurs on a subpicosecond time scale in buffer). Such dipolar relaxation may correspond to that of the hydration water molecules in the detergent headgroups region of the micelles. A similar interpretation has also been suggested for the wavelength-specific fluorescence effects observed for a fluorescent phospholipid (NBD-PE) inserted into various detergent micelles (Rawat et al., 1997).

TOE energy minimization (in vacuum and in a water box) suggested that all stable conformations were "folded," with the indole ring located close to the octyl chain. In such conformations, the amino and carboxylate groups are both located at one end of the folded molecule, creating a polar extremity. Such conformations may be oriented in the micelles such that these groups are in contact with the maltoside polar head moieties, with the indole ring located deeper in the acyl chain region (Fig. 10). This suggested that conformation differs from the recently suggested major rotamer of TOE (rotamer I) corresponding to an unfolded conformation of TOE (with a *trans* configuration for the indole ring and the octyl chain; Chattopadhyay et al., 1997). In some of our proposed rotamers, the indole ring was close to the ester group (in *cis* position), which effectively quenches the indole fluorescence (Cowgill, 1963, 1967). This may account for the presence of short-lived components in the fluorescence decay. The presence of various conformations (differing, in particular, in the efficiency of indole fluorescence quenching by the two substituents on the C_α atom) may result in differences in lifetime (Petrich et al., 1983). Measurements of the fluorescence of a related compound, tryptophan ethyl ester, as a function of pH, has demonstrated that the ammonium group has a stronger quenching effect than the uncharged amino group, because the charge transfer efficiency from the excited indole to the positively charged group is higher (Petrich et al., 1983). In TOE, this effect would account for the increase in fluorescence intensity at high pH.

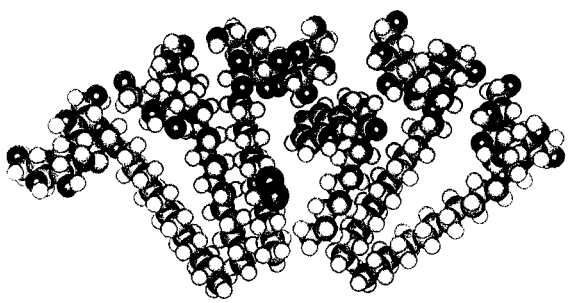


FIGURE 10 Molecular model of part of a DM/BrDM mixed micelle with one incorporated TOE molecule. The various atoms, C, N, O, Br, and H, are indicated by a gray scale.

The rotational correlation times obtained for TOE in DM micelles are consistent with the rotation of the molecules within the detergent micelles, but at a rate at least 10 times slower than that of the rotation of monomeric TOE in bulk water. The two correlation times observed for TOE in micelles probably describe two modes of rotation of TOE, that of the whole TOE molecule and that of the indole moiety (which is probably hindered). For comparison, 3,3′-diethyloxadicarbocyanine iodide, a fluorescent dye used to study dynamics in model systems, because of its appropriate photophysical characteristics (in particular, a single lifetime in buffer), has a rotational correlation time of 0.2 ns in aqueous solution, and a similar 10-fold increase in θ (up to 2.2 ns) in cholate micelles (Das, 1996). However, because of the low aggregation number of cholate, the last rotational correlation time may correspond to the rotation of the whole probe/detergent micelle. Similarly, a fluorescent lysophospholipid dye, *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-monomyristoylphosphatidylethanolamine (N-NBD-MPE), has a single rotational correlation time of ~0.2 ns in solution (below its own cmc), and two correlation times (0.73 and 6.5 ns) in the presence of micellar taurodeoxycholate (at a concentration of 5 mM). These results have been interpreted as reflecting both local probe motion and the rotation of the whole micellar aggregate (Delong and Nichols, 1996). Our results are consistent with those of these experimental studies. However, we did not observe Brownian rotational motion of the whole micelle, because of the fast rotation of the TOE molecule within these highly dynamic detergent assemblies.

The slight differences in the apparent correlation times observed for intermediate molar fractions of brominated detergents in the micelles may be due to slight differences in micelle microviscosity and to changes in the distribution of lifetime such that the rotation of slightly different mixtures of conformers is observed.

TOE fluorescence quenching was studied in mixed micelles of DM/BrDM or DM/BrUM at various molar fractions of brominated detergent (from 0 to 1). In interpreting the results, we assumed that the various mixed micelles of detergent did not differ significantly from pure DM micelles in size, shape, and, most importantly, chain disorder. We made this assumption because the bromines only weakly perturb detergent properties (de Foresta et al., 1996); in particular, the detergent cmc is only slightly higher for BrDM and BrUM than for DM. In addition, most of the quenching is observed at a low molar fraction (X) of brominated detergent (more than 50% of TOE fluorescence is quenched for $X = 0.2$; see Fig. 6), under conditions in which the mixed micelles should closely resemble those of pure DM.

TOE fluorescence was almost totally quenched in pure brominated detergent micelles, a first indication of its accessibility to the brominated chains. In addition, the fitting of preexisting models to the quenching curves (Eq. 1; see London and Feigenson, 1981; East and Lee, 1982) gave high n values (close to 4), n being a parameter reflecting the simultaneous accessibility to several brominated chains under conditions of static quenching (which was shown to be predominant). Thus the indole moiety may be partly surrounded by brominated chains. This suggests that the indole moiety is not located at the surface of the micelle (with only the hydrophobic octyl chain anchoring TOE in the micelle), but rather at a position below the detergent headgroups, consistent with the folded "hairpin" conformations suggested by modeling. Such a location is also consistent with the results of quenching experiments with I^-, a soluble collisional quencher, demonstrating significantly lower accessibility to TOE in DM than to NATA in water. These data are also supported by recent quenching experiments (Tortech and de Foresta, unpublished results) performed with another soluble quencher, acrylamide, which is considered to be a perfectly neutral quenching probe (Eftink and Ghiron, 1976). These experiments showed a similar restricted accessibility of TOE in DM.

These quenching results were reminiscent of those obtained for TOE in biological membranes. Significant quenching is observed with various brominated phosphatidylcholines: for instance, the relative residual fluorescence is 0.26 and 0.17 in pure 1-palmitoyl-2(9,10-dibromostearoyl)-*sn*-glycero-3-phosphocholine (BrPC) and 1,2-bis-(9,10-dibromostearoyl)-*sn*-glycero-3-phosphocholine (di-BrPC), respectively (Yeager and Feigenson, 1990). The mean depth of TOE is thought to be 11–12 Å from the bilayer center (i.e., within the hydrophobic core), based on studies with phospholipids brominated at various positions (Abrams and London, 1992; Ladokhin and Holloway, 1995).

In our experiments, the lack of a significant difference between the quenching effects of BrDM and BrUM may be due, apart from or in addition to large fluctuations in TOE position, to significant disorder of the brominated acyl chains and/or peculiar folding of the BrUM chains such that the bromines come close to the headgroup region of the micelle. With respect to the quenching mechanism of TOE by the brominated detergents, the decrease in steady-state fluorescence intensity (Fig. 6) was not due to a parallel decrease in lifetimes τ_i (see Fig. 7), but rather to a lower contribution to fluorescence intensity, of excited-state species with long lifetimes. Quenching was therefore mainly static, and the models for interpreting fluorescence quenching in membrane systems are essentially valid. We can therefore use such quenching curves to evaluate the accessibility of Trp to the detergent alkyl chains. We suggest that this model system, TOE in micelles, could be used as a valuable reference system for evaluating the accessibility of Trp in peptide-detergent or protein-detergent complexes.

CONCLUSION

Our results provide evidence that TOE is embedded in dodecylmaltoside (and analogs) micelles and suggest that it is probably in a folded conformation (see Figs. 9 and 10). In DM micelles, the indole moiety was sensitive to dipolar relaxation, in a pico/nanosecond time range, in a microenvironment of lower mobility than bulk water. The indole moiety was strongly quenched in mixed micelles containing, in addition to DM, analogs of DM with brominated alkyl chains. The mechanism of quenching was mainly static, as shown by the small differences in TOE lifetime values. These results, obtained with a simple model system, provide an initial basis for the interpretation of further studies of peptide-detergent and protein-detergent interactions. Such studies will be continued by investigating the differences in quenching obtained for fluorophores with Trp located at various positions within the micelles, such as the synthetic peptides studied by Ren et al. (1997).

We thank the technical staff of the Laboratoire pour l'Utilisation du Rayonnement Electromagnétique (LURE) for running the synchrotron ring during the beam sessions. We also thank Dr. J. Smith, in whose laboratory the molecular modeling was performed.

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