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CHARACTERIZATION OF MIXED MICELLES OF PHOSPHOLIPIDS OF VARIOUS CLASSES AND A SYNTHETIC, HOMOGENEOUS ANALOGUE OF THE NONIONIC DETERGENT TRITON X-100 CONTAINING NINE OXYETHYLENE GROUPS

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Summary

The synthesis and high-pressure liquid chromatographic purification of the homogeneous nonionic surfactant *p*-(1,1,3,3-tetramethylbutyl)phenoxynonaoxyethylene glycol (OPE-9) in quantities suitable for membrane solubilization studies is reported. Micelles of OPE-9 and mixed micelles of OPE-9 with dimyristoyl and dipalmitoyl phosphatidylcholine as well as phosphatidylserine, phosphatidylethanolamine, lysophosphatidylcholine, sphingomyelin, and palmitic acid were characterized by column chromatography on 6% agarose. It was found that at 28°C OPE-9 micelles have a Stokes' radius of 32 Å, giving a molecular weight for a spherical micelle of about half that of micelles of the polydisperse nonionic surfactant Triton X-100 under the same conditions. The micelle size is temperature dependent: at 40°C the OPE-9 micelles have a Stokes' radius of 44 Å, giving a molecular weight for a spherical micelle of about twice that of the OPE-9 micelles at 28°C. The size of the mixed micelles varies linearly (as measured by K_{av}) with the mole fraction of phospholipid. The mixed micelle size was found to be relatively independent of the absolute concentration of surfactant over a four-fold range if the mole fraction of phospholipid is kept constant. The usefulness of the OPE-9/phospholipid mixed micelle system for lipolytic enzyme substrates and membrane-related studies is considered.

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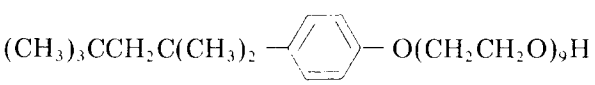
Abbreviation: OPE-9, *p*-(1,1,3,3-tetramethylbutyl)phenoxynonaoxyethylene glycol.

Introduction

Mixed micelles of the nonionic surfactant Triton X-100 and phospholipid serve as effective substrates for studies of phospholipase A_2 action [1], and their formation plays a major role in the solubilization and activation of membrane-bound proteins [2—4]. Unfortunately, Triton X-100 is a highly polydisperse surfactant: *p*-(1,1,3,3-tetramethylbutyl)phenoxypolyoxyethylene glycol, with an average of 9.5 oxyethylene units per monomer. Some heterogeneity in the alkyl region of the molecule has also been suggested [5]. Because of this polydispersity and heterogeneity, certain questions in using the Triton X-100-containing systems remain unanswered [5—7]. Homogeneous nonionic surfactants of the octylphenoxypolyoxyethylene type are not commercially available, so we prepared a homogeneous analogue of Triton X-100 that, hopefully, would have similar properties to the polydisperse Triton X-100, but would allow precise quantitative conclusions. For this purpose, we have synthesized the oligomer with nine oxyethylene units, *p*-(1,1,3,3-tetramethylbutyl)phenoxynonaoxyethylene glycol (OPE-9) *, which is shown in Structure I, and we have examined its interaction with various phospholipids.



I

This report concerns the characterization of OPE-9 micelles and mixed micelles with various phopholipids by gel chromatographic techniques. We have also recently used ^1H NMR techniques to probe phospholipid conformation in mixed micelles with Triton X-100 [8] and we also found that the conformation of phospholipids is similar in OPE-9 micelles (Roberts, M.F. and Dennis, E.A., unpublished).

Experimental Procedure

Materials

Ethyl acetate and acetic acid (Mallinckrodt) were distilled before use. *p*-(1,1,3,3-Tetramethylbutyl)phenol (Eastman) was recrystallized three times from *n*-heptane (Mallinckrodt) to a constant m.p. (85—86°C) and to a single peak on gas chromatography. 1,2-Bis(2-chloroethoxy)ethane (Eastman) was distilled before use, b.p. 99—100°C (2 Torr). Hexaoxyethylene glycol (Columbia Organic Chemicals) was used without purification. Blue dextran 2000 (Pharmacia), AMP (Sigma), and Bio-Gel A-5m (Bio-Rad) which is a 6% agarose gel, 100—200 mesh, were used as obtained. Yeast alcohol dehydrogenase (Worthington), *Escherichia coli* β-galactosidase (Boehringer), bovine serum albumin (Mann), enolase (Boehringer), ovalbumin (Mann), bovine brain sphingomyelin (Calbiochem), and dimyristoyl and dipalmitoyl phosphatidyl-

* *p*(1,1,3,3-tetramethylbutyl)phenoxypolyoxyethylene glycol will be abbreviated OPE-*n*, where *n* refers to the number of oxyethylene groups in the molecule.

choline (Calbiochem) were used without further purification. Palmitic acid (Eastman) containing trace amounts of [1-^{14}C]palmitic acid (gift of Dr. John Elovson) was employed. Phosphatidylserine was purified from bovine brain [9]. Phosphatidylethanolamine, prepared by transesterification of egg phosphatidylcholine, was obtained from Avanti Biochemicals. Lysophosphatidylcholine was prepared by phospholipase A_2 treatment of egg phosphatidylcholine followed by alumina chromatography. All other chemicals were of reagent grade and distilled water was used routinely in the experiments.

Gas chromatography

Gas chromatography analyses were performed on a Varian Series 2100 gas chromatograph, temperature programmed from 100 to 280°C at 20°C · min^{-1}. The column used was a 4 ft. glass column packed with 1% SE 30 on Gas Chrom Q, and samples were dissolved in $CHCl_3$ prior to injection.

High-pressure liquid chromatography

High-pressure liquid chromatography was performed with a Waters Associates chromatographic pump (Model M-6000) equipped with a Waters Associates injector (Model U6K), and a preparative Porasil column (3/8 in. × 4 ft.). Samples containing up to 0.8 ml of a 50% (w/w) solution of OPE-9 in redistilled ethyl acetate were employed with this system. A linear gradient of ethyl acetate/acetic acid/water (265 : 6 : 4, v/v) against ethyl acetate/acetic acid/water (225 : 28 : 22, v/v) was used to isolate OPE-9 [10]. The concentration of the detergent in the eluate was followed by absorption at 280 nm using an Isco ultraviolet analyzer (Model UA-5) equipped with an Isco Type 6 optical unit and an Isco high pressure micro-flow cell (series no. 0080-011-03) with a 5 mm path length. The temperature was ambient. Columns were washed between runs with a 1 : 1 mixture of ethyl acetate and acetic acid.

Thin-layer chromatography

Thin-layer chromatography was carried out on pre-coated silica gel G plates (E. Merck) with the solvent system [10] ethyl acetate/acetic acid/water (30 : 5 : 4, v/v). The spots were visualized with iodine vapor.

OPE-9 and critical micelle concentration determination

The critical micelle concentration of OPE-9 was determined by the method of Ray and Némethy [11]. The absorbance at 276.5 and 285 nm of solutions of known detergent concentration was measured at 25°C on a spectrophotometer. Detergent concentrations ranged from 0.01 to 1.6 mM in distilled water. The absorbances were plotted against the concentration of OPE-9, and the critical micellar concentration was determined by the intersection of the two straight lines joining the points far below and the points far above the critical concentration. From the slopes of the lines, ϵ_{max} values were obtained, and used for the calculation of the concentration of OPE-9.

Agarose chromatography

Agarose chromatography experiments were conducted with a 2.5 cm × 50 cm column of 6% agarose. The column was pre-equilibrated [6] and then run,

with buffer consisting of 10 mM Tris · HCl, pH 8.0/100 mM NaCl/0.02% sodium azide/0.4 mM OPE-9. The flow rate was approximately 0.5 ml · min^{-1}. Mixed micelles were prepared from solutions of OPE-9 at the appropriate concentration in column buffer which were added to dry phospholipid. The phospholipid was dispersed by vigorous shaking and stirring. The sample was allowed to sit a few minutes until the foaming subsided. Blue dextran 2000 and AMP were used in each sample as markers for the void volume and total volume, respectively. Phospholipid content of samples was determined by digestion in perchloric acid and a phosphate analysis according to Eaton and Dennis [12]. Palmitic acid content of samples was determined by radioactivity.

Results

Synthesis and characterization of OPE-9

OPE-9 was synthesized using a modification of the method of Mansfield and Locke [13] and Gingras and Bayley [14], and purified by high-pressure liquid chromatography using a modification of the procedure developed by Allen and Rice [10]. The synthesis was carried out in two steps shown in reactions 1 and 2.

$$\underset{\text{II}}{ClCH_2CH_2OCH_2CH_2OCH_2CH_2Cl} + \underset{\text{III}}{(CH_3)_3CCH_2(CH_3)_2C_6H_4OH} + NaOH \rightarrow$$

$$\underset{\text{IV}}{(CH_3)_3CCH_2C(CH_3)_2C_6H_4(OCH_2CH_2)_3Cl} + NaCl + H_2O \qquad \text{(Reaction 1)}$$

$$\underset{\text{IV}}{} + \underset{\text{V}}{NaO(CH_2CH_2O)_6H} \rightarrow \underset{\text{I}}{} + NaCl \qquad \text{(Reaction 2)}$$

To prepare the chloro derivative of OPE-3 (IV), a mixture of 1,2-bis(2-chloroethoxy)ethane (II), *p*-(1,1,3,3-tetramethylbutyl)phenol (III), and 50% sodium hydroxide in the mole ratio 4.3 : 1.0 : 1.3 was refluxed for 4 h and let stand overnight. Enough water was added to dilute the volume 7-fold; the mixture was thoroughly stirred, and the layers separated. The organic layer was diluted with toluene and washed with water four to five times. The organic layer was then distilled twice, b.p. 195—6°C (1 Torr), and purity checked on gas chromatography, which is capable of detecting impurities such as the starting materials and OPE-1, OPE-2, and OPE-3. The purity was greater than 99% and gave a yield of about 40% based on III.

The monosodium salt of hexaoxyethylene glycol (V) was prepared by covering the desired amount of this compound with a layer of *n*-heptane, and then adding with stirring an equal amount (mol/mol) of sodium in small portions. After the solution was stirred overnight, the temperature was raised by emersing the flask in an oil bath at 135°C until the salt formation was complete, and then stirring an additional 4 h. A 5% mole excess of the chloro derivative of OPE-3 (IV) was added dropwise over a 2 h period with stirring at the same temperature. The mixture was stirred an additional 2 h and let stand overnight at room temperature. 3 vols. of anhydrous ethyl ether were added,

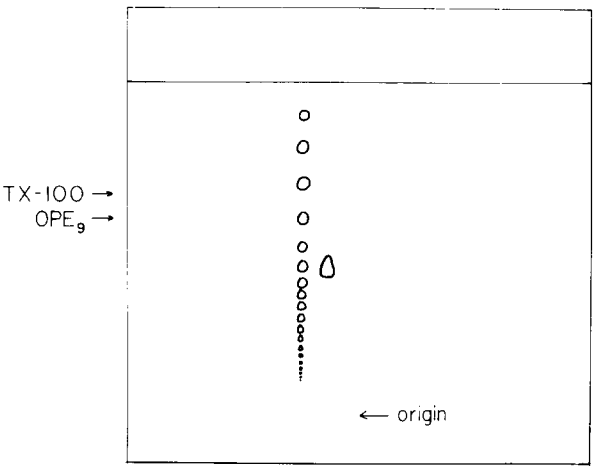


Fig. 1. Thin-layer chromatographic separation of the molecular components of Triton X-100 (TX-100) compared with pure OPE-9 developed with the solvent system ethyl acetate/acetic acid/water (30 : 5 : 4, v/v).

and then the solution was dried with sodium sulfate and filtered. The ether was evaporated off, and the OPE-9 product was analyzed by thin-layer chromatography. Yield was approximately 50% based on IV and overall yield based on III was 20%.

Purification of OPE-9 (the main contaminants other than unreacted starting materials were primarily shorter oxyethylene chain-length species although there were some longer chain-length species) was performed on high-pressure liquid chromatography as described in Experimental Procedure. For each run, the OPE-9 solution was neutralized with concentrated ammonium hydroxide (to eliminate the formation of acetate esters [10]), the layers separated, the solution dried with sodium sulfate, and the ethyl acetate was rotary evaporated at 30°C. Final drying was done under high vacuum (0.1 Torr) overnight.

The purity of OPE-9 was checked by thin-layer chromatography which can easily detect longer and shorter chain-length species. A typical thin-layer chromatogram of Triton X-100 which contains an extremely large range of OPE-*n*'s (differing by only one oxyethylene unit each) and OPE-9 is shown in Fig. 1. The OPE-9 is more than 99% pure; minor contaminants could be eliminated by preparative thin-layer chromatography, but was not necessary for the experiments reported here. OPE-9 is hygroscopic, and elemental analysis would not be able to distinguish OPE-9 from other OPE-*n* species of nearly the same chain length. The critical micellar concentration of OPE-9 was found to be 0.30 ± 0.02 mM (4 determinations). The cloud point [15] was determined for a 1% solution in water and found to be 67.5°C. The 220 MHz ^{1}H NMR spectrum and integration of areas was consistent with the structure, and was not significantly different from the spectrum of Triton X-100 [16]. Small quantities of OPE-9 have been synthesized previously [13], but the final product was purified using a crude step-wise elution on a silicic acid column. No experimental details were given for this purification, and contaminants as

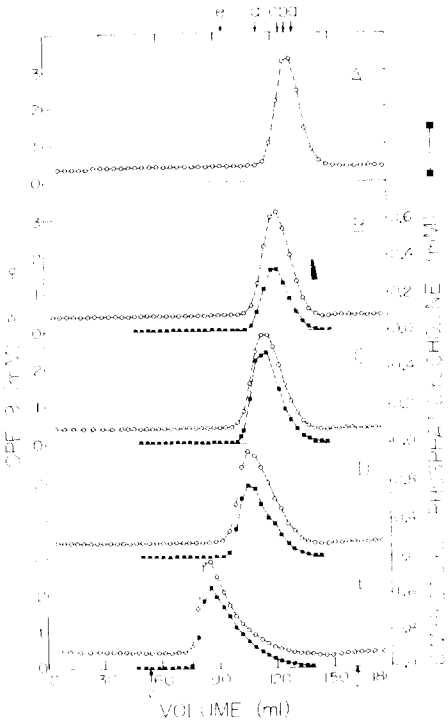


Fig. 2. Gel chromatography of dimyristoyl phosphatidylcholine and OPE-9 mixed micelles on 6% agarose at 28°C. The column was pre-equilibrated with and the elution carried out with buffer containing 0.4 mM OPE-9. The following samples (1.0 ml) were applied to the column: A, 50 mM OPE-9; B, 50 mM OPE-9 plus 6 mM dimyristoyl phosphatidylcholine; C, 50 mM OPE-9 plus 11 mM dimyristoyl phosphatidylcholine; D, 50 mM OPE-9 plus 17 mM dimyristoyl phosphatidylcholine; E, 50 mM OPE-9 plus 50 mM dimyristoyl phosphatidylcholine. The void volume (V) and total volume (T) of the column are indicated. The elution volumes of various proteins are indicated: a, ovalbumin; b, enolase; c, bovine serum albumin; d, alcohol dehydrogenase; e, β-galactosidase.

indicated by thin-layer chromatography may have been as high as 5% for each or any of the oligomers. Nonetheless, the reported critical micellar concentration [17] and cloud point [13] were not significantly different from those reported here.

Gel chromatography

The elution profile of OPE-9 micelles at 28°C on agarose is shown in Fig. 2 (column A). In order to avoid the net production of monomers as the micelles pass through the column, the columns were pre-equilibrated with, and run in buffer containing 0.4 mM OPE-9, which is slightly above its critical micellar concentration (0.3 mM measured at 25°C). When OPE-9 is not included in the buffer, tailing of the surfactant peak occurred at concentrations approximating the critical micellar concentration. The K_{av} is approx. 0.69; at 40°C, the micelle size increases somewhat and the K_{av} is approx. 0.58.

Column chromatography of OPE-9/dimyristoyl phosphatidylcholine mixed micelles at 28°C is shown in Fig. 2 (columns B–E). This temperature was chosen because it is well above the phase transition of dimyristoyl phos-

phatidylcholine (23°C). When 6 mM dimyristoyl phosphatidylcholine is included with 50 mM OPE-9 (column B), it co-elutes with the OPE-9 micelles. These mixed micelles are only slightly larger than pure OPE-9 micelles. The mole fraction of phospholipid eluted is fairly constant across the peak assuming a constant background of OPE-9, although this assumption may not be completely valid since the critical micellar concentration of OPE-9 is probably lowered by the presence of phospholipid, and any OPE-9 micelles in the buffer may equilibrate with the mixed micelles passing through the column and thereby dilute them. As the concentration of phospholipid is increased in the mixed micelle to 50 mM (a phospholipid mole fraction of 0.5) (columns C—E), the micelles become progressively larger. The mole fraction of phospholipid remains fairly constant across the peak corresponding closely to the mole fraction applied to the column. Fig. 3 shows that only small changes occur when the absolute concentration of the surfactant (and the phospholipid) is varied for mixed micelles at a constant mole fraction of 0.293.

Column chromatography of OPE-9/dipalmitoyl phosphatidylcholine mixed

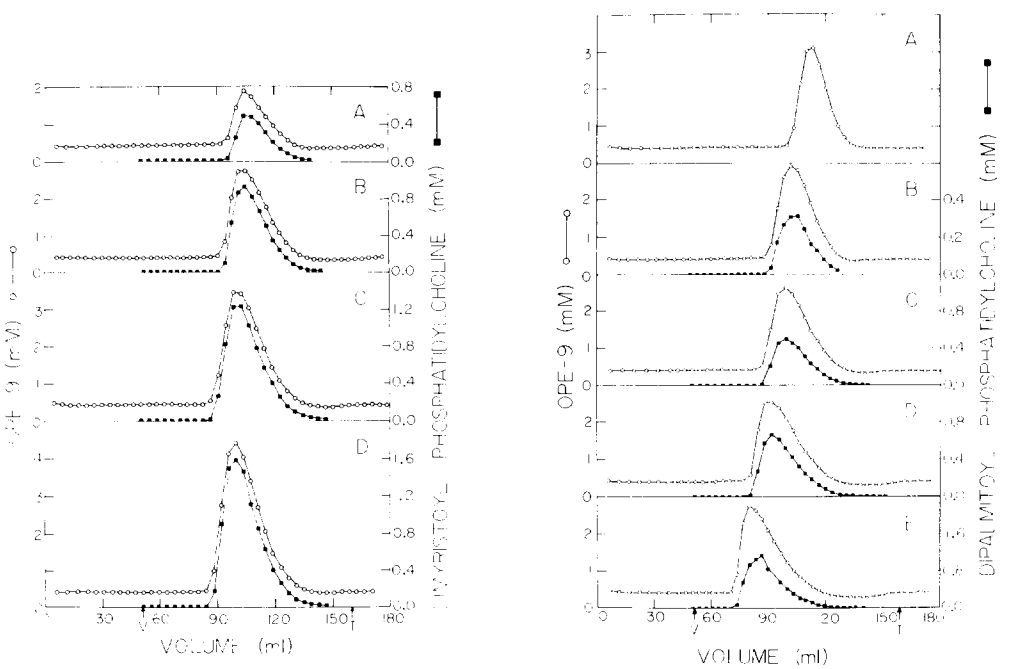


Fig. 3. Chromatography of dimyristoyl phosphatidylcholine and OPE-9 mixed micelles under the same conditions employed in Fig. 2, keeping the mole fraction of phospholipid constant at 0.293. The following samples were applied to the column: A, 25 mM OPE-9 plus 10.4 mM dimyristoyl phosphatidylcholine; B, 50 mM OPE-9 plus 20.8 mM dimyristoyl phosphatidylcholine; C, 75 mM OPE-9 plus 31.3 mM dimyristoyl phosphatidylcholine; D, 100 mM OPE-9 plus 41.7 mM dimyristoyl phosphatidylcholine.

Fig. 4. Chromatography of dipalmitoyl phosphatidylcholine and OPE-9 mixed micelles under the same conditions employed in Fig. 2, except that the column was maintained at 40°C. The following samples were applied to the column: A, 50 mM OPE-9; B, 50 mM OPE-9 plus 6 mM dipalmitoyl phosphatidylcholine; C, 50 mM OPE-9 plus 11 mM dipalmitoyl phosphatidylcholine; D, 50 mM OPE-9 plus 17 mM dipalmitoyl phosphatidylcholine; E, 50 mM OPE-9 plus 25 mM dipalmitoyl phosphatidylcholine.

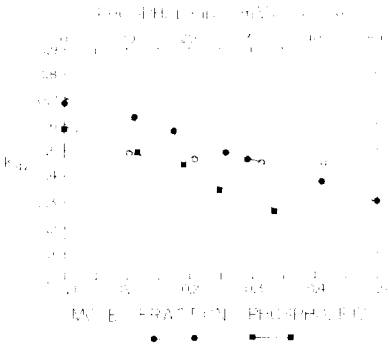


Fig. 5. A plot of the K_{av} of the eluted peaks from the agarose columns as a function of the mole fraction of phospholipid applied, phospholipid/(phospholipid + OPE-9), for dimyristoyl phosphatidylcholine at 28°C (●) and dipalmitoyl phosphatidylcholine at 40°C (■). The K_{av} of the eluted peak is also shown as a function of the bulk concentration of phospholipid applied for dimyristoyl phosphatidylcholine at 28°C (○) at a constant mole fraction of phospholipid of 0.293. The lines shown are least squares fits to the data.

micelles at 40°C is shown in Fig. 4. The phase transition of dipalmitoyl phosphatidylcholine is 41°C, but since agarose columns are not stable for long periods at high temperatures, cloud point effects begin to be significant above 40°C, and the phase transition temperature is probably lowered somewhat by the presence of the surfactant [18], these columns were run at 40°C. As with the dimyristoyl phosphatidylcholine, when dipalmitoyl phosphatidylcholine is added to the OPE-9 sample, the micelles become progressively larger, and the phospholipid mole fraction remains fairly constant across the peak corresponding to the mole fraction applied to the column. A graph of the K_{av} versus

TABLE I

STOKES' RADII (R_s) FOR OPE-9 AND TRITON X-100 MICELLES AND MIXED MICELLES WITH PHOSPHOLIPIDS

Sample	T (°C)	Mole fraction phospholipid	R_s (Å)
Triton X-100	28	0.0	41
OPE-9	28	0.0	32
OPE-9/dimyristoyl phosphatidylcholine	28	0.113	38
OPE-9/dimyristoyl phosphatidylcholine	28	0.176	44
OPE-9/dimyristoyl phosphatidylcholine	28	0.258	54
OPE-9/dimyristoyl phosphatidylcholine	28	0.293	58
OPE-9/dimyristoyl phosphatidylcholine	28	0.411	70
OPE-9/dimyristoyl phosphatidylcholine	28	0.500	86
OPE-9/phosphatidylserine	28	0.117	37
OPE-9/phosphatidylethanolamine	28	0.104	39
OPE-9/lysophosphatidylcholine	28	0.043	38
OPE-9/lysophosphatidylcholine	28	0.145	45
OPE-9/palmitic acid	28	0.111	37
OPE-9	40	0.0	44
OPE-9/dipalmitoyl phosphatidylcholine	40	0.117	54
OPE-9/dipalmitoyl phosphatidylcholine	40	0.191	61
OPE-9/dipalmitoyl phosphatidylcholine	40	0.247	76
OPE-9/dipalmitoyl phosphatidylcholine	40	0.336	90
OPE-9/sphingomyelin	40	0.09	47

mole fraction phospholipid is shown in Fig. 5 for both the dimyristoyl and the dipalmitoyl phosphatidylcholine mixed micelles; both show a significant increase in size with phospholipid mole fraction. A plot of K_{av} versus phospholipid concentration is also shown and suggests that the mixed micelle size is relatively independent of the absolute concentration of phospholipid and surfactant over a 4-fold range if the mole fraction is kept constant.

Based on the standardization of the agarose column with proteins, the Stokes' radii of OPE-9 micelles and mixed micelles were calculated utilizing the equation $(K_{av})^{1/3} = a - bR_s$ [19]. Here, a and b are constants and R_s is the Stokes' radius. R_s values are given in Table I for OPE-9 and Triton X-100 micelles as well as mixed micelles of OPE-9 with dimyristoyl and dipalmitoyl phosphatidylcholine and several other lipids. These results show that mixed micelles at similar mole fractions and temperatures have similar Stokes' radii, independent of the identity of the phospholipid component.

Discussion

OPE-9 micelles

Because the nonionic surfactant OPE-9 is homogeneous and not polydisperse, interpretation of results previously obtained with polydisperse Triton X-100 should be simplified [6,7]. In the first place, the elution profile of OPE-9 is much sharper than that of the polydisperse Triton X-100, giving a peak width at half height of 14 ml (OPE-9) vs. 20 ml (Triton X-100) measured at 28°C. Furthermore, the size of OPE-9 micelles as measured by gel chromatography is significantly smaller than the micelles of the polydisperse surfactant.

Confidence in the standardization near the size of Triton X-100 micelles is warranted since the K_{av} of Triton X-100 was 0.61 (at 28°C and applied as a 50 mM solution in the same buffer as the other column studies). This corresponds to a Stokes' radius of 41 Å for Triton X-100, which is in close agreement with calculations for Triton X-100 micelle sizes based on sedimentation velocity [20] and light scattering measurements [21,22]. For the OPE-9 micelles, the Stokes' radius is 32 Å at 28°C and 44 Å at 40°C. Light scattering measurements of the molecular weight of Triton X-100 micelles [21] at 25°C account for about 140 monomers (M_r = 90 000). Intrinsic viscosity measurements have indicated that there is approximately 1.2 g of water per g of surfactant for Triton X-100 micelles [20—22], assuming a spherical micelle model. No measurements have been made on the hydration of OPE-9 micelles, but if 1.2 g water per g surfactant is assumed, then one can calculate how many monomers of OPE-9 would comprise the average micelle. From the Stokes' equation, $4\pi R_s^3/3 = Mv/N_0$, where v is the partial specific volume and N_0 is Avogadro's number, one can calculate the molecular weight, M, of the hydrated micelle assuming that the micelle is spherical. Using the same partial specific volume as has been calculated for Triton X-100 (0.91 ml · g^{-1} [20]), then the anhydrous molecular weight of the OPE-9 micelle at 40°C is about 100 000, which corresponds to about 170 OPE-9 monomers per micelle.

We [23] have shown in previous calculations on the structure of Triton micelles that at 25°C the Triton X-100 micelle composed of 143 monomers should be spherical only if one assumes a non-classical micelle model with a

nondistinct hydrophobic/hydrophilic interface. If one assumes a classical micelle model, then ellipsoids of revolution are reasonable models for the shape, the oblate micelle being preferred over the prolate micelle model. Similar preferences for other surfactants are discussed by Tanford [24]. The above is based on volume/density calculations for the hydrophobic core and an assumption about the contribution of the oxyethylene chains, which agrees with intrinsic viscosity and volume calculations. Similar conclusions probably apply to the OPE-9 micelle, with an oblate ellipsoid model (axial ratio 2 : 1) favored over a prolate model (axial ratio 6 : 1) for the OPE-9 micelle at 40°C, which is a similar size to that of Triton X-100 at 28°C. We do not have good evidence at this time to distinguish between a classical micelle model with a distinct hydrophobic/hydrophilic boundary represented by the oblate micelle and a non-classical micelle model with a nondistinct hydrophobic/hydrophilic interface which could result in a spherical micelle.

Mixed micelles with phospholipids

Previous agarose chromatography of mixed micelles of the polydisperse surfactant Triton X-100 [6] with dimyristoyl phosphatidylcholine showed that even at a Triton/phospholipid mole fraction of 0.08, two peaks were observed: a small peak of larger mixed micelles (termed "quasi-micelles") and a peak of mixed micelles near the same size as pure Triton X-100 micelles. In contrast, mixed micelles of OPE-9 and dimyristoyl phosphatidylcholine show a narrow size distribution and symmetrical peaks over a wide range of phospholipid concentrations. Unlike the situation with Triton X-100, no shoulder at the high molecular weight side of the peaks is observed, and no peak at the void volume is observed even at a 0.5 mole fraction. As can be seen from Fig. 5, a continuous variation in size of the mixed micelles with added phospholipid is observed as measured by K_{av} versus the mole fraction phospholipid: the Stokes' radii for several phospholipid concentrations (mole fraction) are shown in Table I.

Mixed micelles of dipalmitoyl phosphatidylcholine and OPE-9 display similar characteristics to dimyristoyl phosphatidylcholine mixed micelles on agarose columns although the column was run at a higher temperature because of the higher phase transition temperature for dipalmitoyl phosphatidylcholine as compared to dimyristoyl phosphatidylcholine. At 40°C, all the micelles, even those of pure detergent, are somewhat larger. The fact that in Fig. 5 the lines of K_{av} versus mole fraction phospholipid for both dimyristoyl phosphatidylcholine and dipalmitoyl phosphatidylcholine are parallel is indicative that the two types of mixed micelles are both changing in a similar and regular manner with changing mole fraction phospholipid. Presumably the displacement of the two lines is due mainly to a temperature effect on the size of the micelles.

Applying the Stokes' equation to the mixed micelles may not be completely valid since v is unknown and the mixed micelles are probably not spherical. However, one can get an upper limit on the micellar molecular weight if one does assume sphericity and a similar v and hydration to pure Triton micelles. With these assumptions, the dimyristoyl phosphatidylcholine/OPE-9 mixed micelles at 28°C range from approximately 70 000 for a mole fraction of 0.11 to 10^6 for a mole fraction of 0.50. Similarly, the dipalmitoyl phosphatidyl-

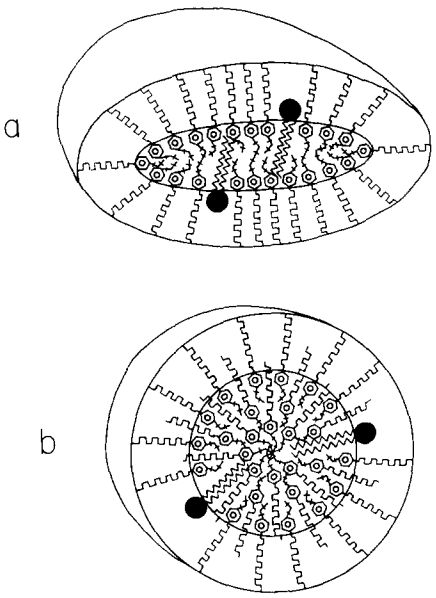


Fig. 6. Schematic view of the oblate ellipsoid model (a) and spherical model (b) for a mixed micelle containing a low mole fraction of phospholipid (<0.1). The micelle model shapes were calculated based on a Stokes' radius of 44 Å at 40°C and a hydration (taken from the value for the Triton X-100 micelle at 25°C) of 1.3 g water/g OPE-9. Using volume/density calculations for the hydrophobic core, (a) is a classical micelle with the shape of an oblate ellipsoid with an approximately 2 : 1 axial ratio. For the spherical micelle model (b), the octylphenyl groups cannot pack in a spherical core to form a classical micelle. Therefore, in this model some oxyethylene units must be included in the hydrophobic core. It is not possible to precisely calculate the arrangement of groups in this model because one does not have the limits imposed by a distinct hydrophobic/hydrophilic boundary, but all of the octylphenyl groups are shown in the core plus the relevant portion of the oxyethylene chains that are attached to the octylphenyl groups. It is assumed that the hydrophilic region extends one oxyethylene chain length (16 Å) beyond the hydrophobic core making the radius of the whole micelle about 44 Å.

choline/OPE-9 mixed micelles at 40°C range from about 180 000 for a mole fraction of 0.12 to 10^6 for a mole fraction of 0.34. Obviously more hydration and nonspherical shapes will lower the values of the calculated molecular weights. A schematic representation of a mixed micelle of OPE-9 and phospholipid at a very low mole fraction phospholipid is shown in Fig. 6 based on our considerations [23] of the possible structure of a Triton X-100 micelle. Both the classical oblate micelle model and the spherical micelle model with a nonclassical hydrophobic/hydrophilic interface are shown.

These results suggest that the polydispersity of Triton X-100 is responsible for the formation of multiple mixed micelle species as monitored by gel chromatography. OPE-9 solubilizes phospholipid to form only one narrow size distribution of mixed micelles at each mole fraction phospholipid, and the mole fraction may be increased to at least 0.5 (for dimyristoyl phosphatidylcholine) without the formation of large bilayer-like structures. Furthermore, mixed micelles of OPE-9 and phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingomyelin, lysophosphatidylcholine and palmitic acid at a mole fraction of 0.1—0.15 are all similarly sized, suggesting that they have similar overall structures. OPE-9 may therefore be of considerable value in

the kinetic analysis of phospholipase A_2 as well as other lipolytic enzymes [1,12] and in membrane solubilization studies [2].

Acknowledgements

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