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MOLECULAR WEIGHT, SHAPE AND STRUCTURE OF MIXED MICELLES OF TRITON X-100 AND SPHINGOMYELIN

S. YEDGAR[a], Y. BARENHOLZ[a],* and V. G. COOPER[b]

[a]*Laboratory of Neurochemistry, Department of Biochemistry Hebrew University, Hadassah Medical School and* [b]*Microwave Division, Racah Institute of Physics, Hebrew University of Jerusalem, Jerusalem (Israel)*

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SUMMARY

The sedimentation coefficients of mixed micelles of Triton X-100 and sphingomyelin have been measured for Triton molar fractions between 0.32 and 0.79. These measurements, together with previously reported diffusion coefficients, are used to calculate the molecular weights of the mixed micelles, and their aggregation numbers. It is found that the molecular weight decreases steadily with increasing Triton molar fraction. However, the Triton aggregation number remains nearly constant at about 196, so that the decreasing molecular weight is reflected in the sphingomyelin aggregation number, which decreases from 442 to 50 as the Triton molar fraction increases from 0.32–0.79. Intrinsic viscosities of the mixed micelle solutions are found to be independent of Triton molar fractions, with a value of 5.5 ml/g. From these measurements a number of inferences are drawn regarding the micellar shape and structure.

INTRODUCTION

Enzymatic hydrolysis of water-insoluble lipids usually requires a detergent, since the enzymatic activity without detergent is too low to be studied with meaningful accuracy. With the addition of a suitable detergent, the activity is generally enhanced [1]. Such was the case in our recent studies with sphingomyelin as substrate (Yedgar et al. [2]), where it was found that the addition of a non-ionic detergent, Triton X-100, influenced the enzymatic reaction rate. The recent work of Dennis [3] shows a similar influence of Triton X-100, in this instance with the substrate lecithin.

The effect of the detergent can be either on the substrate or on the enzyme, or indeed it can act on both. The present work is an investigation of the first of these possible influences, that of the detergent Triton X-100 on the substrate sphingomyelin.

Since Triton X-100 is a surface active agent, which forms micelles in water, it is quite reasonable to conclude that the detergent solubilizes the sphingomyelin,

* Present address: School of Medicine, Department of Biochemistry, University of Virginia, Charlottesville, Va., U.S.A.

which is an insoluble swelling amphiphile, by forming mixed micelles of substrate and detergent. By this process the soluble lipid substrate is transformed into a large number of small particles. One might reasonably seek the explanation for the enhanced enzymatic activity in, say, the increased surface area of the micelles, which could increase the probability for contract between the enzyme and the substrate.

Quite frequently, in the absence of information on the physical properties of the substrate, resort is made to the enzymatic activity itself as an indicator of those properties. Ideally, however, independent information on the physical properties of the substrate–detergent system is required for a more fundamental understanding of the influence of the detergent on the enzymatic activity. Therefore in this work we have concerned ourselves with the size, molecular weight, shape and structure of the mixed micelles of sphingomyelin and Triton X-100, for a wide range of ratios of the two constituents. A study of the diffusion coefficients of these mixed micelles by light scattering, upon which much of the present work is based, is described in the preceding paper.

EXPERIMENTAL METHOD

(*I*) *Preparation and analysis of materials*

The enzymatic studies of Yedgar et al. [2], and the measurements of diffusion coefficients in the accompanying paper were carried out on partially hydrogenated bovine spinal cord sphingomyelin, whose preparation has been described by Gatt et al. [4]. A limited number of measurements in the preceding paper were also made with unhydrogenated sphingomyelin. In the present work too, most of the measurements were carried out on the same partially hydrogenated sphingomyelin, and a limited number of viscometric measurements were made with unhydrogenated bovine brain sphingomyelin. The preparation of the brain sphingomyelin was the same as for the spinal cord. Both species of sphingomyelin were chromatographically pure. All of the Triton X-100 was obtained from British Drug Houses. The micelles were prepared in 0.1 M sodium acetate buffer, pH 5.0. The preparation of the mixed micelles has been described in the preceding paper.

The partially hydrogenated sphingomyelin was analysed in order to determine the degree of hydrogenation. Chemical analysis was carried out by enzymatic degradation to ceramide and phosphorylcholine using phospholipase C of *Clostridium welchi*. 2 μmoles of sphingomyelin were dissolved in 1 ml diethyl ether and 0.5 ml of 0.1 Tris buffer, pH 7.4, containing 10 mM $CaCl_2$ and 0.5 mg enzyme. The mixture was incubated at room temperature until all the sphingomyelin was degraded (12 h). This was followed by hydrolysis of the ceramide to sphingosine bases and fatty acids (Gaver and Sweeley [5]).

The fatty acids were methylated with diazomethane. The methyl esters were then analysed using a Packard gas–liquid chromatograph on 15 % ethylene glycol succinate polyester columns.

(*II*) *Determination of molecular weights*

The molecular weights of the mixed micelles are determined according to the Svedberg equation (Schachman [6])

$$M_r = \frac{RTs_0}{D_0(1-\bar{v}\rho_0)} \tag{1}$$

where R is the gas constant and T is the absolute temperature, S is the sedimentation coefficient and D_0 the diffusion coefficient both in the limit of zero micelle concentration, $\bar{v}$ is the partial specific volume of the micelles, and where ρ_0 is the density of the solvent at 20 °C. The diffusion coefficient D_0 was measured in the preceding paper. The other experimental quantities were determined as follows:

(*I*) *Sedimentation coefficient.* Measurements of sedimentation velocity for the solutions of mixed micelles were carried out with a Spinco Model E analytical ultracentrifuge, equipped with Schlieren optics. Rotor speeds were 56 000 or 60 000 rev./min. Photographs were taken at 16 or 32 min intervals, and the duration of runs was between 80 and 300 min. The choice of interval and of run duration was determined in each case by the nature of the specimen and the speed with which sedimentation occurred.

Sedimentation coefficients were determined in the usual manner from the displacement of the Schlieren peak as a function of time [6] according to

$$S = \frac{1}{\omega^2}\frac{\mathrm{d}}{\mathrm{d}t}\ln r \tag{2}$$

where ω is the rotor speed in radian/s, and r is the radial displacement of the Schlieren peak from the rotor axis.

All measurements of sedimentation were made around 20 °C, and all observed values of S were corrected to standard conditions [6] according to

$$S_{20,w} = S\frac{\eta_{t,sol}}{\eta_{20,w}}\frac{(1-\bar{v}\rho)_{20,w}}{(1-\bar{v}\rho)_{t,sol}} \tag{3}$$

where η indicates a viscosity, and where $_{t,sol}$ and $_{20,w}$ indicate that the values of the subscripted parameters are for the solute at the measurement temperature, and for water at 20 °C, respectively. The temperature dependence of the solvent density was taken to be the same as that of water.

(2) *Measurement of density.* The quantities ρ and C are related according to the equation

$$\rho = \rho_0 + (1-\bar{v}\rho_0)C \tag{4}$$

where ρ is the density of the solution at solute concentration C (Becher and Arai [7]). Densities of the solvent and of micellar solutions at a range of densities were measured with a glass pycnometer of 2 ml capacity, using a water bath thermostatically controlled at 20 °C to within 0.1 °C. Specimens were brought to thermal equilibrium with the bath, and then each was weighed for three different volumes, on a Sartorius analytical balance. The relative accuracy of the density so determined was 0.04 %.

(*III*) *Measurement of viscosity*

Viscosity is an important parameter for indicating the shape and /or degree of hydration of particles (Tanford [8]). Viscosities of the solutions were determined by measuring their efflux times in a semi-micro Cannon Manning viscometer. The

viscometer and solutions were maintained at 20 °C, to within 0.1 °C, by a thermostatically controlled water bath. To check that the viscosity was not a function of velocity gradient, and that the behaviour of the solutions was therefore Newtonian, solutions with selected Triton molar fractions were examined in two, and in some cases three, viscometers, with a choice of water efflux times of 67, 107, and 141 s.

RESULTS

(*I*) *Analysis of the sphingomyelin*

Analysis of the sphingosine bases (Carter and Gaver [9]) yielded 53 % sphingosine and 44 % dihydrosphingosine. Hydrogenation did not significantly affect the fatty acid composition. As the results given below will show, the hydrogenation apparently has little effect on the micellar properties.

(*II*) *Sedimentation velocity*

1. *Homogeneity of the mixtures.* Measurements were carried out for Triton X-100 fractions between 0.16 and 1 (pure Triton). The Schlieren traces from a number of long runs (up to 5 h) were examined for indications of inhomogeneity. At Triton molar fractions below 0.32 at least three peaks could be resolved, characterized by widely differing sedimentation coefficients. For example, at the Triton molar fraction of 0.16, sedimentation coefficients of 4.9, 12.1 and 30.3 S were obtained for the three peaks, indicating severe inhomogeneity. As the results below will show, the lowest of these figures is roughly comparable to the sedimentation coefficient for mixed micelles at the Triton molar fraction of 0.32, the lowest molar fraction that yielded a homogeneous solution of mixed micelles. The larger sedimentation coefficients correspond to significantly larger particles, and indeed, electron micrographs indicated the presence of liposomes of various sizes. Apparently the excess of sphingomyelin above the maximum that can enter into mixed micelles separates into liposomes. These mixtures were excluded from further study, since they were heterogeneous mixtures of micellar and non-micellar particles.

At molar fractions between 0.32 and 0.79, a single, sharp, symmetrical peak was observed. Fig. 1a shows, as an example, the traces for the Triton molar fraction of 0.38, for centrifugation times of 16, 64, 96 and 144 min. Light scattering results in the preceding paper equally failed to indicate any polydispersity for these solutions. The specimens were taken to be effectively monodisperse, and eight Triton molar fractions in the range from 0.32–0.79 were examined.

At Triton molar fractions above 0.79, two peaks were observed, as shown by the Schlieren traces for the molar fraction 0.86 in Fig. 1b. The sedimentation coefficients are 1.3 S and 2.5 S for the left and right hand peaks, respectively. The overlapping of the two peaks allows no better than about 20 % accuracy in these figures. The left hand peak, whose sedimentation coefficient is equal to that of pure Triton, presumably indicates the presence of micelles of pure Triton X-100 which is in excess of the molar fraction that can enter into mixed micelles. The mixed micelles in this two phase system may be expected to retain a Triton molar fraction equal to the critical molar fraction of about 0.79, and hence to show a sedimentation coefficient of about 2.2 S. The higher value obtained here presumably results from the difficulty in locating the peaks in the two component traces. The exaggeration of the concentra-

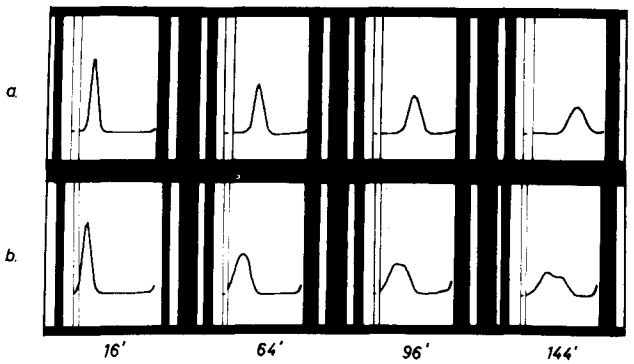

Fig. 1. Ultracentrifuge Schlieren traces for two Triton molar fractions: (a) a homogeneous system at 0.38, (b) a two-component system at 0.86.

tion of the pure Triton in Fig. 1b is due to the Johnston–Ogston effect (Schachman [6]). These inhomogeneous specimens were also excluded from the present study. The implications of this separation of phases are discussed below.

Pure Triton X-100 gave a single Schlieren peak. Though the micelles of pure Triton X-100 are not relevant to the measurements of enzymatic activity, measurements for pure Triton have been included to provide a limiting case for the influence of increasing Triton molar fraction, and also for comparison with data in the literature for pure Triton X-100 (Kushner and Hubbard [10]).

2. *Sedimentation coefficients.* Sedimentation coefficients were measured at several concentrations for a number of Triton molar fractions. For each concentration, $S_{20,w}$ was calculated. In Fig. 2 values of $S_{20,w}$ are shown plotted with respect to solute concentration for three Triton molar fractions: 0.32, 0.79 and 1.0. It is noteworthy that the concentration dependence of $S_{20,w}$ (i.e., the slope) increases monotonically with decreasing Triton molar fraction, suggesting that increasing presence of sphingomyelin promotes greater particle interaction. The slope was in

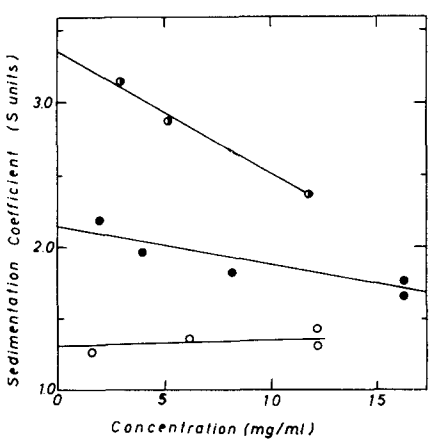

Fig. 2. The sedimentation coefficient $S_{20,w}$ as a function of mixed micelle concentration for three Triton molar fractions: ◑—◑, 0.39; ●—●, 0.79; ○—○, 1.0 (pure Triton).

TABLE I

Triton molar fraction	Sedimentation coefficient, S	Diffusion coefficient, $D_0 \cdot 10^7$ (cm^2/s)	Molecular weight, M	Aggregation numbers			Stokes radius, r_s (Å)	Equivalent radius, r_e (Å)	Ratio r_s/r_e
				N_{Tr}	N_{SPM}	N_{total}			
1.0	1.32	4.46	86 000	134		134	48.0	31.5	1.52
0.79	2.20	3.93	163 000	192	50	242	54.5	39.0	1.40
0.76	2.46	3.84	187 000	210	66	276	55.8	40.8	1.37
0.66	2.69	3.57	220 000	209	110	319	60.0	43.0	1.40
0.61	2.83	3.46	238 000	209	133	342	61.9	44.2	1.40
0.49	2.93	3.14	272 000	186	196	382	68.2	46.2	1.48
0.44	3.04	3.02	293 000	180	228	408	70.9	47.4	1.50
0.39	3.42	2.89	334 000	179	282	461	74.1	49.4	1.50
0.32	4.47	2.72	479 000	210	442	652	78.8	55.8	1.41

fact found to depend linearly on Triton molar fraction. This knowledge allowed accurate extrapolation to zero concentration for a number of intermediate molar fractions, with fewer data points. The values of S_0, the sedimentation coefficient at zero solute concentration, are given in Table I, for all Triton molar fractions studied. As in the preceding paper, the critical micellar concentration for the mixed micelles should be low enough that this extrapolation to zero solute concentration, instead of zero micelle concentration, introduces negligible error.

(*III*) *Density and partial specific volume*

In Fig. 3 the densities of the solutions are shown as functions of the total solute concentration. It is clear that the density is essentially a function of the total solute concentration only, since it is not measurably dependent on the Triton molar fraction. The best fit for ρ(in g/ml) is given by

$$\rho = 1.002 + 0.0837\ C$$

with C, the solute concentration, in units of g/ml. The standard deviation of the linear coefficient is 2 %.

The value of $\bar{v}$ is found from Eqn 4. The term $(1-\bar{v}\rho_0)$ is of course to be identified with the linear coefficient determined here. Since this coefficient was found to be independent of Triton molar fraction, the quantity $\bar{v}$ is similarly taken to be a constant for all the mixed micelles studied. The value of $\bar{v}$ is 0.915 ml/gm.

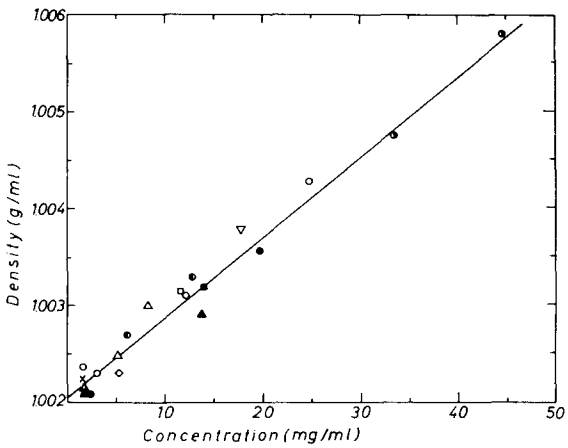

Fig. 3. Density of the mixed micelle solution as a function of mixed micelle concentration for 11 Triton molar fractions: ×, 0.32; ◇, 0.39; ◐, 0.44; ▲, 0.49; ▽, 0.61; ●, 0.66; ■, 0.76; △, 0.79; □, 0.86; ◑, 0.93; ○, 1.0 (pure Triton).

(*IV*) *Viscosity*

As was stated above, some of the viscosity measurements were carried out on the same spinal cord sphingomyelin as was used in the other measurements reported here (and in the preceding paper), but recourse was also made to bovine brain sphingomyelin. As for the diffusion measurements in the preceding paper, here again no systematic differences were found between the viscosities measured for the two species of sphingomyelin.

Comparison of the results taken with viscometers of different efflux times indicated that the solutions were Newtonian at all Triton molar fractions examined, and at all concentrations, up to the highest used.

Viscosities were measured for mixed micelles with Triton molar fractions of 0.32, 0.49 and 0.76, and for pure Triton X-100. These are representative of the entire range of Triton molar fractions studied. For each molar fraction, viscosities were measured at about eight concentrations up to a maximum of about 0.02 g/ml, with five efflux times averaged at every concentration. In addition, reference measurements for water and for pure buffer were taken. At each concentration, viscosity was calculated according to the equation (Tanford [8])

$$\eta_s = \frac{t_s}{t_w} \frac{\rho_{20,sol}}{\rho_{20,w}} \eta_w \tag{5}$$

where t_s and t_w are the efflux times for the solution and for water respectively. Reduced viscosities were then calculated at each concentration [8], according to

$$\eta_{red} = \frac{1}{C}\left(\frac{\eta_s}{\eta_0} - 1\right) \tag{6}$$

where η_0 is the viscosity at zero solute concentration (pure buffer). The results are plotted in Fig. 4. The reduced viscosities show no concentration dependence and, moreover, no systematic dependence on Triton molar fraction. Differences among the lines of best fit probably result from variation in day to day standardization of temperature of about 0.1 °C.

The intrinsic viscosity, η, the intercept of η_{red} at zero solute concentration is taken to be a constant, independent of Triton molar fraction. The average for the four molar fractions measured is 5.5 ml/g, with an average deviation of 0.5 ml/g.

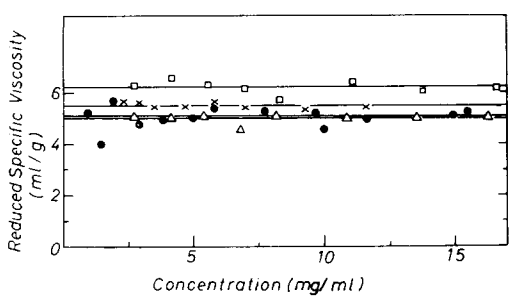

Fig. 4. Reduced viscosity as a function of mixed micelle concentration for four Triton molar fractions: ×—×, 0.39; □—□, 0.49; △—△, 0.76; ●—●, 1.0 (pure Triton).

(V) Additional parameters

For purposes of discussion, the sedimentation data in Table I has been supplemented by a number of additional parameters: (1) The value of D_0, the diffusion coefficient at zero solute concentration, has been calculated for each Triton molar fraction from the best fit given in the preceding paper. (2) Molecular weights have been calculated according to Eqn 1, using the measured values of S_0, the interpolated values of D_0, and the value of 0.0837 determined for the factor $(1-\bar{v}\rho_0)$. The result for pure

Triton X-100 is in good agreement with the value of 90 000 found by Kushner and Hubbard [10]. (3) The aggregation numbers N_{Tr} and N_{SPM}, for the Triton X-100 and the sphingomyelin, respectively, have been calculated according to the relationship

$$N_{Tr} M_{Tr} + N_{SPM} M_{SPM} = M_r \tag{7}$$

where the Triton molecular weight M_{Tr} has a mean value of 645 [11], the sphingomyelin molecular weight M_{SPM} has a calculated value of 775, and where

$$\frac{N_{Tr}}{N_{Tr} + N_{SPM}} = X_{Tr} \tag{8}$$

X_{Tr} being the Triton molar fraction. The total aggregation number, equal to $N_{Tr} + N_{SPM}$, is also shown. Here again the monomer concentration is assumed to be negligible. (4) The Stokes radius r_s of the micelles has been calculated from the interpolated values of D_0 according to the well known relationship

$$r_s = \frac{k_b T}{6\pi\eta D_0} \tag{9}$$

(5) The radius r_e of the "equivalent sphere" [8] which contains the unhydrated mass of the micelle has been calculated according to

$$\tfrac{4}{3}\pi r_e^3 = \frac{M_r \bar{v}}{N_0} \tag{10}$$

where N_0 is Avogadro's number. The ratio r_s/r_e, which is equivalent to the familiar ratio of frictional coefficients f/f_0 (Perrin [12], Oncley [13]) is also shown.

DISCUSSION

We now examine three aspects of the experimental results for their implications regarding the micellar structure: the existence of a critical Triton molar fraction, the aggregation numbers, and the degree of hydration or non-sphericity indicated by the ratio r_s/r_e and by the intrinsic viscosity. A number of structural models will then be evaluated in the light of these considerations.

(*1*) *Critical Triton molar fraction*

The sedimentation velocity results show that mixed micelles do not exist with Triton molar fractions from 1 down to 0.79; that is, at sphingomyelin molar ratios from 0.0–0.21. Thus, if sphingomyelin be "added" to a solution of Triton micelles in a molar fraction below about 0.21, it will nonetheless be solubilized by the Triton X-100 at the molar fraction of 0.21, corresponding to a minimum sphingomyelin aggregation number of about 50. The resultant mixed micelles co-exist with the remaining micelles of pure Triton. The conclusion to be drawn is that at the critical Triton molar fraction around 0.79 the micellar structure changes to one which can accept sphingomyelin. The Triton aggregation number N_{Tr} (which is discussed more fully below) also indicates such a transition, changing from 134 for pure Triton to a value close to 200 for all the mixed micelles. The structural changes that might be associated with this critical molar fraction are considered later.

(II) Aggregation numbers and aggregation properties

The most striking aspect of the aggregation numbers of the mixed micelles is that N_{Tr} remains roughly constant, with an average value of 196 and average deviation of 12. Therefore, as the sphingomyelin molar ratio increases, there is a steady increase in micellar size and weight associated with the ever increasing N_{SPM}. This phenomenon can be plausibly explained in terms of the aggregation properties of the sphingomyelin molecule. When sphingomyelin molecules aggregate, they tend to be bound together nearly parallel, to form a surface with very little curvature. This is well illustrated by the sonicated liposomes of pure sphingomyelin examined in the preceding paper. There, even with intense sonication to induce the formation of small particles, the smallest liposome radius obtained was about 250 Å. With hydrogenation of the sphingomyelin, which promotes stronger bonding [14], the smallest radius was about 700 Å. This phenomenon may be associated with the fact that most of the length of the sphingomyelin molecule, about 26 Å, is hydrophobic [14, 15]. Curvature would tend to allow the aqueous environment to penetrate between neighbouring molecules.

By contrast, most of the length of the Triton X-100 molecule, about 35 Å, is hydrophilic [16]. This promotes an open structure in which a large angle can exist between neighbouring molecules, corresponding to high curvature. (See the discussion below on the structure of the pure Triton micelle.) In these terms, one can see the role of the Triton X-100 in solubilizing the sphingomyelin as overcoming their low surface curvature, so that particles can be formed with sufficient curvature to close upon themselves within a small radius. In good approximation, the curvature that the sphingomyelin contributes in the formation of micelles is insignificant; otherwise the hydrogenation of the sphingomyelin would have a strong influence on the micellar size (as it does for the sonicated liposomes), and this was not found to be the case. In this approximation, it is the Triton alone which must accommodate the 4π steradians that the total micellar surface subtends (regardless of what its shape may be). The nearly constant Triton aggregation number therefore suggests that each Triton molecule contributes a fixed measure of "curvature", regardless of how many sphingomyelin molecules are present in the micelle.

The description of the aggregation properties in terms of surface curvature should be understood as a useful phenomenological description of the complicated interactions of the molecules with each other and with their aqueous environment. We shall apply it below to the evaluation of different structural models for the micelle.

(III) Hydration and non-sphericity of the micelles

Information on the degree of hydration and/or non-sphericity of the mixed micelles is provided in this work by the intrinsic viscosity and by the ratio r_s/r_e [8]. The intrinsic viscosity has an average value of 5.5 ml/g for several disparate Triton molar fractions, including pure Triton. This result is identical to the value for pure Triton X-100 found by Kushner and Hubbard [10]. The departure of this value from 2.5 ml/g, the value for impenetrable spheres, can be ascribed either to hydration or to departure from sphericity. Using the shape factors of Simha [17], one finds as limiting cases, (a) with perfect sphericity a hydration of 1.4 ± 0.2 g solvent/g solute, and (b) with zero hydration an ellipsoidal axial ratio (prolate or oblate) of 6 ± 1.

The ratio r_s/r_e gives similar information. Like the intrinsic viscosity, it shows no apparent dependence on the Triton molar fraction. Its average value is 1.45 ± 0.05.

If the micelles were unhydrated spheres this ratio should be 1. Using the shape factors of Perrin [12] for f/f_0, in a treatment similar to that for the viscosities, one finds as limiting cases a hydration of 1.8 g solvent/g solute or an ellipsoidal axial ratio of about 9. These values are higher than those indicated by the intrinsic viscosity. However, these two techniques seldom give better agreement than that obtained here (Oncley [13]). In any event, both indicate the same thing: either a high degree of hydration, or a large departure from sphericity, or some combination of both.

Distinguishing between hydration and non-sphericity is a familiar and difficult question, which can only be dealt with the in context of some model for the micellar structure. The case of pure Triton X-100 has been considered by Kushner and Hubbard [10]. They concluded that the micelles were spherical and highly hydrated. The authors based their argument on simple "disk" and "rod" models. They showed that no axial ratio for these models could be found that was consistent with the observed intrinsic viscosity and with their estimated Triton monomer length of 43 Å. Their picture of the monomers in a radial array is certainly consistent with the Stokes radius of 48 Å reported here for pure Triton, which is only slightly longer than their estimated monomer length. The interpretation of the data is somewhat dependent upon the estimated monomer length, since a shorter length allows a model with greater non-sphericity to be chosen. Rosch [16] has described two possible configurations of the oxyethylene chain. In the "meander" configuration the total length of the Triton X-100 molecule would be about 30 Å, whereas the "zig-zag" configuration would produce a total molecular length of about 45 Å. According to Rosch, the zig–zag model is the more appropriate for the relatively short oxyethylene chain in Triton X-100 .

Fig. 5a shows a cut-away representation of a spherical Triton micelle. The organization of the Triton molecules is indicated in the sectional plane, and the effective hydrodynamic envelope is shown without structural detail. The angular spacing between Triton molecules in the sectional plane (about 20 °) is appropriate to the calculated Triton aggregation number of 134, as is the centre-to-centre separation of the hydrophilic heads at the surface (about 16 Å). Since the oxyethylene chain has a thickness of about 2.5 Å [16], the structure is indeed open. The high degree of hydration

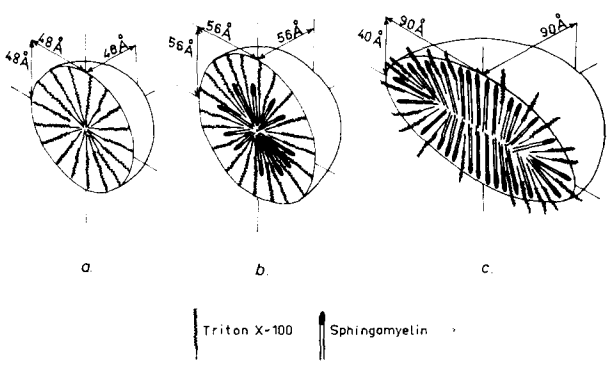

Fig. 5. Cut-away representations of micelles at three Triton molar fractions: (a) 1.0, (b) 0.79, (c) 0.32. The organization of the molecules is shown in the sectional plane; the hydrodynamic envelope is shown without structural detail.

which is implied by the spherical model (more than half of the micellar volume is apparently water) can be explained by this considerable free volume between the radially extended oxyethylene chains. Kushner and Hubbard [10] suggest that within this volume about half of the bound water is genuinely hydrated at oxygen atoms in the oxyethylene chain, while the other half is kinetically entrapped solvent.

(IV) Models for the mixed micelle structure

A similar approach is possible for the mixed micelles. Here, however, choosing a model is understandably more complicated, and must be done in the light of the micellar properties established above: there is a critical Triton molar fraction, surface curvature in the micelles is provided mainly by a fixed number of Triton molecules, and the micelles are highly hydrated and/or non-spherical. One additional consideration serves to restrict the choice of structural models. Because sphingomyelin is mainly hydrophobic, any arrangement approximating the "log boom" model of Becher and Arai [7], in which sphingomyelin molecules lie on the micellar surface exposed to water, is here rejected. Instead, the sphingomyelin must lie roughly normal to the micellar surface, presenting only its hydrophilic head to the aqueous environment.

We first consider what structural changes are likely to occur at the critical Triton molar fraction (around 0.79). The Triton configuration in Fig. 5a is open to the aqueous environment, and so is not suitable for the inclusion of sphingomyelin molecules. Only when a certain minimum number of sphingomyelin molecules are present are they able to impose their preference for an environment which is screened from the solvent. This screening is provided by the Triton. A likely arrangement is shown in a cut-away diagram in Fig. 5b. Here again the organization of the molecules is shown in the sectional plane. The arrangement shown, in which the Triton molecules are "staggered" with respect to the sphingomyelin molecules, provides a well defined and well screened hydrophobic core, with little overlap of the hydrophobic and hydrophilic portions of neighbouring molecules. The resulting radius of the hydrodynamic envelope is, as it should be, longer than the length of either the Triton or sphingomyelin momomers, approximating the Stokes radius of 55 Å at the Triton molar fraction of 0.79. With its predominance of Triton, this micelle retains the spherical and very open character of the pure Triton micelle.

We now consider the lowest Triton molar fraction for mixed micelles, 0.32. The results have indicated that the hydration/non-sphericity remains high, even at this low Triton molar fraction, where about two thirds of the micelle is sphingomyelin. It is questionable whether such a micelle could maintain the same hydration per unit weight as the pure Triton, so that some measure of non-sphericity is indicated. Indeed, some non-sphericity is indicated by purely geometrical considerations. If the shape were spherical, then the micellar radius would equal the Stokes radius of 79 Å (assuming that the hydration involves only entrapped solvent, and not external hydration layers). However, the staggered structure alluded to above has a maximum extent (hydrophobic length of the sphingomyelin plus hydrophilic length of the Triton) of about 60 Å, significantly short of the Stokes radius. This too indicates non-sphericity.

We consider a non-spherical shape for the micelle in terms of prolate and oblate ellipsoidal approximations. Various considerations may be brought to bear in choosing between these. However, the usefulness of treating the forces governing the shape of the micelle in terms of surface curvature has been shown above. This treatment

now suggests, firstly, that the Triton and sphingomyelin are not homogeneously distributed in the micelle; an ellipsoid has local variation in curvature, and this local variation must be accommodated by local variation in the Triton-sphingomyelin ratio. Now in every region of a prolate ellipsoid the local curvature in some direction is greater than the curvature of an equivalent sphere. Such a shape is therefore not favoured by a high molar ratio of sphingomyelin. In an oblate ellipsoid, on the other hand, regions of low curvature exist. Fig. 5c shows an oblate ellipsoidal micelle, in which a section parallel to the minor axis is exposed. The top and bottom surfaces have low curvature, which is accommodated by a clustering of sphingomyelin. The region of higher curvature around the "rim" of the ellipsoid contains a higher concentration of Triton. The clustering of the sphingomyelin at the centre on opposite surfaces satisfies the tendency of sphingomyelin to aggregate in a bilayer with hydrophilic heads directed outwards (Bangham [18]).

The sectional plane shows fairly accurately the correct number of Triton and sphingomyelin molecules that should lie close to such a cross section. (The molar ratio of the molecules in the micellar surface is assumed equal to their molar ratio in the solution. The apparent molar ratio along the perimeter of a cross section will equal the square root of that molar ratio.) The role of the Triton in introducing curvature while at the same time screening the sphingomyelin is shown clearly here. Because of the staggered structure, Triton molecules are "wedged" between neighbouring sphingomyelin molecules. The greater the local concentration of Triton, the greater the curvature.

The minor radius is close to the length of a sphingomyelin monomer; that is, about 40 Å (Vandenheuvel [15]), or perhaps slightly more because of the occasional protruding Triton molecule. To calculate the major axis, the total micellar volume must be determined. The volume of the unhydrated mass is by definition contained in the equivalent sphere. The volume of hydration must be estimated. We assume that a Triton X-100 molecule carries with it the same 1.8 g solvent/g solute as in the pure Triton micelle, and that the sphingomyelin molecule, whose hydrophilic length is 40 % that of the Triton X-100 molecule, carries with it 0.72 g solvent/g solute. An average hydration of 0.9 g solvent/g solute is obtained. The major radius is straightforwardly found to be 85–90 Å. Examination of Fig. 5c will show this to be a reasonable result if each sphingomyelin molecule occupies the same surface area in the micelle as it occupies in a monolayer, about 40 Å (Shah and Schulman [14]).

The axial ratio of this micelle is thus about 2. Micelles with this axial ratio and a hydration of 0.9 g solvent/g solute should have an intrinsic viscosity quite close to the observed 5.5 ml/g, and a ratio r_s/r_e of from 1.3–1.4, which is slightly short of the observed (average) value of 1.45.

The models in Figs 5b and 5c are limiting cases. For the Triton molar fractions from 0.79 down to 0.32, that is, with increasing presence of sphingomyelin, the micelles should tend from the spherical form of Fig. 5b to the oblate form of Fig. 5c.

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