cannot distinguish between preassociative stepwise and concerted mechanisms.

Conclusions

Chiral [^{16}O,^{17}O,^{18}O]phosphate esters have provided a welcome clarification of a substantial number of mechanistic ambiguities in both chemical and enzyme-catalyzed phosphoryl-transfer reactions. All the phosphokinases that have been investigated with chiral [^{16}O,^{17}O,^{18}O]phosphate esters or anhydrides obey sequential kinetics and catalyze phosphoryl transfer with inversion of configuration. This has led to the confident assertion that single enzyme-catalyzed phosphoryl-transfer steps occur with inversion of configuration. By contrast the phosphomutases and phosphatases that have been studied all catalyze phosphoryl transfer with retention of configuration and all are known to have phosphoenzyme intermediates on their reaction pathway. The stereochemical course found for these three classes of phosphotransferases provided the necessary evidence for the more disparate stereochemical events observed with phosphodiesters to be interpreted with confidence.

Although the extensive use of chiral [^{18}O]-phosphorothioates for studying the stereochemical course of phosphoryl and nucleotidyl transferases have not been reviewed here, all the enzymes that have been studied with both chiral [^{18}O]phosphorothioate and chiral [^{16}O,^{17}O,^{18}O]phosphate esters (viz., glycerokinase, hexokinase, pyruvate kinase, polynucleotide kinase, snake venom phosphodiesterase, cAMP phosphodiesterase, adenylyl cyclase, methionyl-tRNA synthetase and tyrosyl-tRA synthetase)[54] have been found to follow the same stereochemical course. This gratifying result means that the stereochemical studies with chiral thiophosphate esters and anhydrides can now be confidently accepted as revealing the same stereochemical course as that followed by the natural substrate. This is particularly important for 5′-nucleotidase[55] and the myosin, mitochondrial, and sarcoplasmic reticulum ATPases[56–58] that cannot be investigated by other means.

It is a pleasure to acknowledge the collaboration of R. Bicknell, P. M. Cullis, R. L. Jarvest, B. V. L. Potter, and G. Tansley. Our work was supported by the Science and Engineering Research Council.

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Micelles of Nonionic Detergents and Mixed Micelles with Phospholipids

ROBERT J. ROBSON and EDWARD A. DENNIS*

Department of Chemistry, University of California at San Diego, La Jolla, California 92093

Received May 4, 1982 (Revised Manuscript Received December 6, 1982)

Nonionic surfactants are widely employed as detergents, solubilizers, and emulsifiers and are particularly effective in the solubilization of the protein and phospholipid components of biological membranes. Most of the commercial surfactants are polydisperse preparations with a distribution of molecular species. Only recently have synthetic, monodisperse compounds become readily available, such as dodecyl octaoxyethylene ether and octyl glucoside.

Robert J. Robson received a B.S. from Stanford University and a Ph.D. from the University of California at San Diego. Following a postdoctoral position at M.I.T. with H.G. Khorana, he joined in 1981 the Chevron Research Co. where he works as a Research Chemist in the Products Research Department.

Edward A. Dennis received a B.A. from Yale University and a Ph.D. from Harvard University where he worked with F. H. Westheimer on pseudorotation in phosphate ester hydrolysis. After postdoctoral research with E. P. Kennedy at Harvard Medical School, he joined the University of California at San Diego in 1970, where he is currently Professor of Chemistry. In addition to research on micelle and membrane structure, he also works on the mechanism of action of phospholipases.

A comprehensive treatise on nonionic surfactants edited by Schick[1a] in 1967 includes an abundance of information on the organic chemistry, physical chemistry, analytical chemistry, and biology of nonionic surfactants. This volume contains chapters on micelle formation,[1b] on thermodynamics of micelle formation,[1c] on solubilization,[1d] and on synthesis.[1e] Since 1967, another comprehensive volume on the preparation, chemistry, and industrial applications of poly(oxyethylene)-containing surfactants has appeared.[2] A volume on poly(oxyethylene)[3] has also been published, and sections of many monographs, review articles, and

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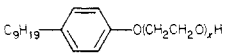
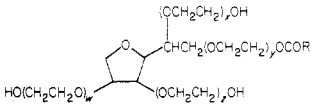
Table I
Structure of Poly(oxyethylene)-Containing Nonionic Surfactants[a]

surfactant	examples of trade names
 poly(oxyethylene) *p-tert*-octylphenyl ethers	Triton X series Igepal CA series Nonidet P40
 poly(oxyethylene) *n*-alkylphenyl ethers	Triton N series Igepal CO series Surfonic N series Tergitol NP, TP, NPX series
$C_nH_{2n+1}O(CH_2CH_2O)_xH$ poly(oxyethylene) *n*-alkyl ethers	Brij series Gardinol WA series Lubrol W,AL series Emulphor ON series
$C_nH_{2n+1}O(CH_2CH_2O)_xH$ poly(oxyethylene) *sec*-alkyl ethers	Sterox AJ,AP series Emulphogen BC series Surfonic TD series
$RCOO(CH_2CH_2O)_xH$ poly(oxyethylene) esters of fatty acids	Sterox CD series Myrj series Emulphor EL,VN series Nopalcol series
 poly(oxyethylene) anhydrohexitol fatty esters	Tween series Emasol
$C_nH_{2n+1}S(CH_2CH_2O)_xH$ poly(oxyethylene) mercaptans	Nonic series Sterox SK,SE series
$RCONH(CH_2CH_2O)_xH$ poly(oxyethylene) alkaneamides	Ethomid series
$R(CH_2CH_2CH_2O)_x(CH_2CH_2O)_yH$ and $R(CH_2CH_2O)_x(CH_2CH_2CH_2O)_yH$ poly(alkylene oxide) block copolymers	Pluronic series

[a] Adapted from ref 1 and 6.

symposia proceedings have been devoted to this subject.[4]

The most common nonionic surfactants contain a poly(oxyethylene) chain as the hydrophilic portion and either an alkyl or an alkylphenyl group as the hydrophobic portion of the amphiphile. The term nonionic surfactant also includes anhydrohexitol derivatives, sugar esters, fatty alkanol amides, alkylmethyl sulfoxides, and fatty amine oxides.[1,5] The general chemical structures of and commercial names[6] for some of the more common polydisperse nonionic surfactants containing poly(oxyethylene) groups are shown in Table I. Among the most well-studied of these surfactants are those of the octylphenol poly(oxyethylene) variety such as Triton X-100 on which this Account will focus.

Phospholipids are amphiphatic molecules with a polar (hydrophilic) end that favors water interaction and an apolar (hydrophobic) end consisting of long alkyl chains. At interfaces (oil/water or air/water), these lipids form well-aligned monolayers with the polar group in contact with water and the nonpolar group(s) oriented such that they are shielded from water contact. In the solid state (devoid of water) the molecules align themselves in a lamellar arrangement such that the hydrocarbon chains form layers bordered by layers of polar groups. The phospholipids show different hydrated phases upon interaction with water. This phenomenon is called lyotropic mesomorphism. The lyotropic phases also show thermotropic mesomorphism.[7] When the temperature is above the mesomorphic transition temperature, water will readily penetrate the polar regions of the molecules, causing the crystal to swell. Multilamellar structures (liposomes) result with molecular weights in the billions.[8] Naturally occurring and synthetic phospholipids can form other types of aggregated structures in aqueous solution such as the single bilayer vesicles formed upon sonication.[9] The use of vesicles as membrane models has been recently reviewed.[10]

In the presence of detergents, lipid bilayers are solubilized to form mixed micelles if the mole ratio of detergent to lipid is above that needed for a lamellar–micellar transition, if the temperature is above a particular critical temperature, and if the detergent concentration is above its critical micelle concentration (cmc). The interactions of soluble amphiphiles (such

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as Triton X-100) with phospholipids in excess water is therefore quite complex. When the soluble amphiphile concentration is low, the surfactant interacts with the phospholipid bilayers without much loss in the general structure of the bilayer.[11] As the concentration of detergent is increased beyond a critical lamellar/micellar transition concentration, mixed micelles are formed. One goal of this review is to summarize studies on the formation and structure of these mixed micelles, as they have direct application to the solubilization of biological membranes by detergents, which has been reviewed elsewhere.[6,12]

Nonionic Detergent Micelles

The structure of the nonionic surfactant Triton X-100 is shown in Table I where x = 9–10. Triton X-100 is polydisperse (containing many different homologues) with a Poisson distribution of ethylene oxide chain lengths with a mean of 9.5 per octylphenyl moiety.[1e] Each oligomer of n oxyethylene units is termed OPE-n for *oct*ylphenoxy*e*thoxy*e*thanol. In the commercial synthesis, ethylene oxide is condensed with *p-tert*-octylphenol (formed by the reaction of isobutylene with phenol) to give Triton X-100. Some heterogeneity in the alkyl region of Triton X-100 has been suggested, since a small percentage of 2,4-dioctylphenol is formed upon preparation of octylphenol.[13] Thus the heterogeneity of the commercial product depends on the purity of the octylphenol employed (it can be easily purified by crystallization). The single species, molecularly homogeneous OPE-9 with exactly nine oxyethylene units, is not commercially available, but it has been synthesized.[14] The oxyethylene composition of these surfactants can be determined by NMR.[15]

Nonionic surfactants readily dissolve as monomers in aqueous solution up to the cmc. Generally, cmc's for nonionic surfactants are much lower than ionic surfactants of comparable alkyl chain length. As the oxyethylene chain is extended and the surfactant becomes more hydrophilic, the cmc increases. For the oxyethylene monoethers of octylphenol, the cmc is 0.1–0.8 mM for chains ranging from 1 to 40 oxyethylene units.[1b] The cmc is only slightly dependent upon salt concentration.[16] Even 0.5 M salt affects the cmc by less than a factor of 2 for most salts studied. Temperature has minimal effect on the cmc.[1b]

At concentrations greater than the cmc, additional surfactant forms micelles, giving a clear isotropic solution. The process of micelle formation has an opposing force that limits the number of surfactant monomers per micelle (aggregation number). In the case of poly(oxyethylene)-containing nonionic surfactants this is most likely caused by the physical bulk of the oxyethylene chains.[4d] For nonionic surfactants the polar group is usually much larger than the hydrophobic group, in contrast to ionic surfactants where often the polar group can be treated as a point charge at the end of an alkyl chain. Micelle size has been studied extensively for nonionic surfactants, especially polydisperse and monodisperse alkylpoly(oxyethylene) ethers, as well as Triton X-100.

The effect of temperature on the apparent micelle size can be quite dramatic. Generally the micelle slightly increases in molecular weight with increasing temperature at lower temperatures, but considerable increases in apparent micellar weight occur as a function of temperature at temperatures approaching the cloud point. The cloud point[1d,17] (or lower consolute temperature[18]) is the sudden onset in turbidity of a nonionic surfactant solution upon increasing the temperature past a certain critical temperature. The hydration of the oxyethylene groups is the primary reason nonionic surfactants are soluble in water,[19] and increased temperature is believed to cause partial dehydration of the chains. This is equivalent to the oxyethylene chains becoming less hydrophilic. At the cloud-point temperature, or slightly higher, the solution separates into two isotropic phases, with one phase surfactant enriched and the other surfactant depleted. Fractionation of Triton X-100 occurs with the aqueous solution enriched in the more hydrophilic oligomers.[17b] Some reports suggest that hydration increases with a temperature increase,[20a,b] although this may be the result of assumptions on micellar shape. For Triton X-100, although little affected by surfactant concentration, the cloud point is dramatically affected by various additives (both electrolytes and solubilizates).[17a] Electrolytes lower the cloud point in direct proportion to their concentrations. Saturated hydrocarbons generally raise the cloud point, but more polar solubilizates (like aniline or benzene) are potent cloud-point depressants. Experiments have suggested that the cloud point can be viewed as a critical point phenomenon.[20c,d]

Gel Chromatography of Nonionic Surfactant Micelles

Micellar size has traditionally been determined by light scattering[21] and ultracentrifugation[22] techniques, although other techniques have been used, such as membrane osmometry,[23] nuclear magnetic resonance with a simple mass action model,[24] potentiometric titrations,[25] hydrodynamics,[26] and fluorescence.[27] Gel filtration is an equally useful method for micelle-size

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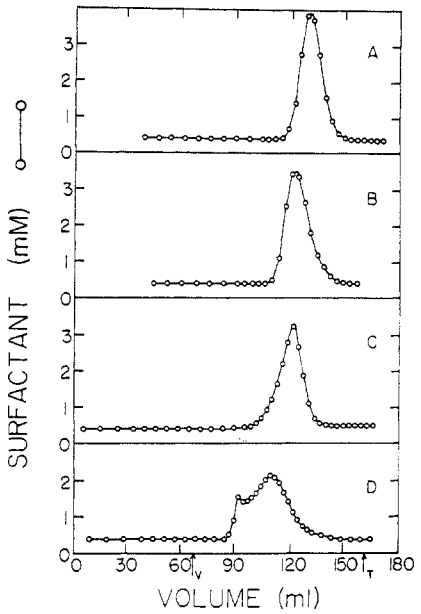

Figure 1. Gel chromatography of OPE-9 and Triton X-100 micelles on 6% agarose at 20 and 40 °C. The column was preequilibrated with and the elution carried out with buffer containing 0.4 mM surfactant. The following samples (1.0 mL) were applied to the column: (A, B) 50 mM OPE-9, (C, D) 50 mM Triton X-100. The column temperature was 20 °C for columns A and C and 40 °C for columns B and D. The void volume (V) and total volume (T) of the column are indicated. Data from ref 14.

determination,[28] but it has not been widely employed. Nonionic surfactant micelles can be chromatographed on an agarose gel.[11a,14] In order to avoid the net production of surfactant monomers as the micelles pass through the column, the columns are preequilibrated with and run in buffer containing surfactant slightly above the cmc. Figure 1 shows the elution profile of OPE-9 and Triton X-100 micelles at 28 and 40 °C. When no surfactant is included in the buffer, considerable tailing in the peaks occurs, with the concentration of surfactant in the tailing side approximately equal to the cmc. The elution profile of OPE-9 is much sharper than that of the polydisperse Triton X-100. If the width in elution volume is a measure of micelle-size distribution, then OPE-9 micelles have a narrower range of sizes than those of Triton-100 micelles. For Triton X-100 at 40 °C two peaks are observed, resulting from partial fractionation of the Triton X-100, with one peak having on the average six to eight oxyethylene units per phenyl ring and the other peak having on the average ten oxyethylene units per phenyl ring.

The agarose column was calibrated with proteins of known sizes and the Stokes' radii of the micelles were calculated. For Triton X-100, a value of 41–43 Å was obtained for temperatures 20–28 °C.[14] This is in close agreement with 48 Å at 20 °C found by Yedgar et al.;[29] 42 Å at 20 °C, 44 Å at 25 °C, and 46 Å at 30 °C determined by Corti and Degiorgio,[20c] based upon diffusion measurements; 42–43 Å at 4 °C found by Clarke[30] by gel filtration on Sepharose 4B; 42 Å at 20 °C calculated from the Stokes–Einstein relation from measured translational diffusion coefficients;[31] and 43 Å assumed

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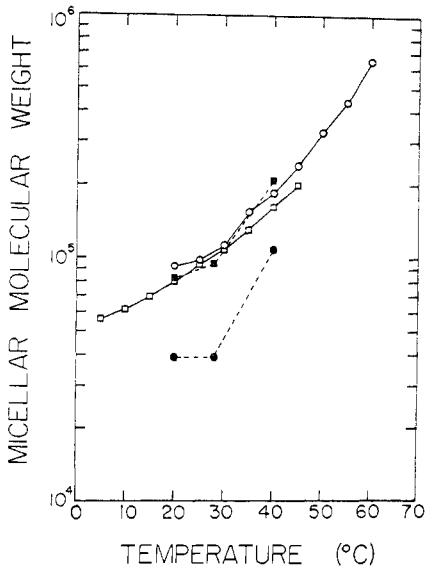

Figure 2. Temperature dependence of micelle molecular weight of Triton X-100 and OPE-9. Data for Triton X-100 obtained by light scattering (○),[20c] membrane osmometry (□),[23] gel chromatography (■). Data for OPE-9 obtained by gel chromatography (●).

by Kushner and Hubbard[21a] for a fully extended oxyethylene chain of Triton X-100, assuming a spherical micelle shape. For Triton X-100 at 40 °C, a value of 56 Å was obtained for the main peak in gel chromatography,[14] which is in general agreement with the effective hydrodynamic radius of 61 Å determined from diffusion measurements by Corti and Degiorgio[20c] at the same temperature.

Data from many laboratories on a variety of nonionic surfactants suggest that, as the temperature increases, the micelle size increases, and at temperatures just below the cloud point, the increase can be dramatic.[18,20b,23,32] Some of the published work describes a threshold temperature below which micelles exist with a minimum molecular weight. Above the threshold temperature a rapid rise in the micelle molecular weight is observed, approximating an exponential growth. Other evidence shows an exponential increase with temperature at all temperatures studied.[32f,g] The apparent micelle molecular weight for Triton X-100 as well as OPE-9 is shown in Figure 2 on a semilog plot as a function of temperature. The micelle molecular weight for OPE-9 follows the same trend as Triton X-100, with a 2–3-fold increase in apparent molecular weight from 28 to 40 °C.

Shape and Hydration of Nonionic Micelles

While a number of calculations have been made, the size, shape, and hydration of Triton X-100 micelles have not been definitely shown. For example, the correlation of micellar molecular weight measurements with hydrodynamic measurements such as intrinsic viscosity can provide an estimate of the limits of shape and hydration. Light scattering can theoretically give an es-

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timate of dissymmetry of micelle shape, but micelles are usually too small, and shape remains indeterminant. Deviation in the intrinsic viscosity of micelle solutions from the value for impenetrable spheres can be attributed to asymmetries in micelle shape or to hydration. The Triton X-100 micelle has until recently been considered to be spherical[20c,21a,29] with sufficient bound water to fit the intrinsic viscosity measurements of approximately 5.3 $cm^3\ g^{-1}$. However, the amount of bound water could be less and the shape could be nonspherical. Wright[33] has suggested, using transient electric birefringence techniques, that the Triton X-100 micelle may not be spherical and Paradies[31] has recently provided additional evidence.

It is important to know the size and shape of Triton micelles, and in order to understand these issues better, we have calculated the shape of the Triton X-100 micelle. Since the average micellar molecular weight of Triton X-100 is 90 000, it follows that the aggregation number is 140–150. In order to accommodate this volume of octylphenyl groups, yet maintain the restriction on the length of the hydrophobic group, a nonaqueous spherical micellar core of octylphenyl groups is not possible. Two models for the core were considered:[34] oblate and prolate ellipsoids of revolution. For a prolate ellipsoid the semiaxis dimensions of the core become 10 × 123 Å, and for an oblate ellipsoid the semiaxis dimensions are 10 × 35 Å.

The molecular conformation of the oxyethylene chain in Triton X-100 or OPE-9 micelles is not known precisely, but three possible rigid conformations of polyoxyethylene have been considered: the fully extended zigzag conformation (3.5–3.6 Å/monomer),[35] the meander conformation (1.8–2.0 Å/monomer),[35] and the 7/2 helix.[36] In aqueous solution, the polyoxyethylenes are typical random-coiled polymers.[3,37] On the basis of a Raman spectral study of several surfactants both as neat liquids and as micelles,[38] the oxyethylene chain of Triton X-100 and OPE-9 most likely resembles a random coil. Comparison between calculated and observed Stokes' radii and intrinsic viscosities suggests that the poly(oxyethylene) chain must be randomly coiled in certain alkyl poly(oxyethylene) ether micelles.[39] Thus, a value of 17 Å for the average length of the oxyethylene chain in a random-coil configuration was assumed for calculations.

These calculations give half-axis dimensions for the prolate micelle of 27 Å × 140 Å and half-axis dimensions for the oblate micelle of 27 Å × 52 Å. This can be compared to recent small-angle X-ray scattering experiments on Triton X-100 at 20 °C, indicating an oblate ellipsoid with half-axis dimensions of 32 Å × 50 Å,[31] and conductance measurements coupled with intrinsic viscosity measurements at 15 °C, suggesting half-axis of 20 Å × 54 Å.[40] Both values are more consistent with an oblate than a prolate micelle; furthermore, calculations[34] of the amount of bound water expected for both types of micelles suggest that an oblate elliposid would be the most reasonable model for both the hydrophobic core and the total micelle. However, it should be noted that the polydispersity of the oxyethylene chains could result in a nonuniform distribution of oxyethylene groups around the micelle, allowing the overall shape to be closer to spherical than the average calculations suggest.

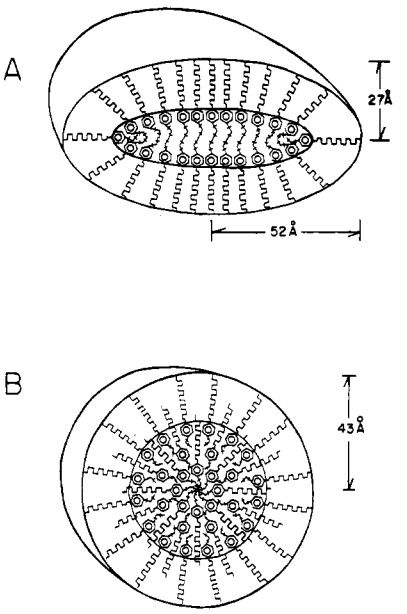

Figure 3. Schematic view of the oblate ellipsoid model (A) and spherical model (B) for an OPE-9 micelle. The micelle shapes were calculated[34] on the basis of a Stokes radius of 44 Å at 40 °C and a hydration (taken from the value for the Triton-X-100 micelles at 25 °C) of 1.2 g of water/g of OPE-9. With 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 nonclassical model, some oxyethylene units must be included in the hydrophobic core. It is assumed that the hydrophobic region extends one oxyethylene chain length (16 Å) beyond the hydrophobic core, making the radius of the whole micelle about 44 Å.

We have suggested that the possibility must also be considered that a sharp boundary does not exist between the hydrophobic interior and the oxyethylene chains, in contradiction to the classical pictures of micelles.[4d,41] If the first few oxyethylene groups at the octylphenyl end of some Triton X-100 molecules were contained in the hydrophobic core, it would be possible to accommodate a spherical model for the hydrophobic region. The total micelle could then be either spherical or oblate, depending on the arrangement of oxyethylene chains. It should be noted that the polydispersity of oxyethylene chain lengths allows for nonuniform arrangements as well and the surface (hydrophobic core and/or the whole micelle) need not be smooth.

Experimentally determined hydration of Triton X-100 suggests 1.2 g of water/g of surfactant, based on assumptions of spherical shape[20c,21a,29] and oblate ellipsoids of revolution.[31] Geometric calculations for an oblate ellipsoid or a nonclassical sphere suggest 1.1–1.2

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g water/g of surfactant.[34] Thus, these methods do not allow one to distinguish between the extremes of a classical oblate ellipsoid and a nonclassical spherical micelle as illustrated in Figure 3.

There is some evidence in the literature that would allow a "nonclassical" micelle to be a reasonable model. This includes solubilities of oxyethylene compounds in aromatic and alkane solvents, a specific charge-transfer complex between benzene and ether oxygens of poly(oxyethylene),[42] proposals for desolvation of the first six ethoxy groups of the oxyethylene chain upon micelle formation,[16] partition experiments of OPE's in water/isooctane mixtures,[43] and size and hydration changes with structural changes.[44] It is generally considered that the binding, association, and/or entrappment of water in the oxyethylene chains is quite extensive,[1b,d] but the precise extent to which water interacts with these chains is still a matter of controversy. The penetration of water into all types of micelles has been suggested and its presence would be important for considerations of micelle size, shape, structure, free energy changes associated with micelle formation, catalysis in micelles, solubilization processes, etc.[45]

A varying degree of solvation along the poly(oxyethylene) chains in Triton X-100 micelles has been suggested by NMR experiments.[15,46] The fact that in micellar solutions there are several chemically shifted peaks for the oxyethylene protons as compared to the surfactant dissolved in chloroform was interpreted as a gradual change in hydration of the oxyethylene groups along the chains. The extent of water penetration into the hydrocarbon core of alkyl and alkyphenyl poly(oxyethylene) ethers is minimal as determined by NMR chemical shifts and spin–lattice relaxation-time measurements. Additionally, the low solubility of water in hydrocarbon argues strongly against the presence of water.[45] For Triton X-100 it has been suggested that there are approximately 4–5 moles of water/mol of ethylene oxide from intrinsic viscosity and molecular weight measurements[20c,21a,29] at 25 °C. Generally, with constant alkyl chain length, increasing the oxyethylene chain increases the measured value of hydration. It should be noted that this can be misleading, since the amount of measured water includes water bound (hydrogen bonded to the oxyethylene groups) and associated water located within a shell or region enclosing the micelle. Future advancement of our understanding of nonionic micelle structure requires a better understanding of the degree of both water and poly(oxyethylene) penetration in both the hydrophobic core and extended polar region of the micelle.

Nonionic Detergent Phospholipid Mixed Micelles

Natural phospholipids in an excess of water form smectic mesophases best thought of as bilayer structures. Synthetic phospholipids with fatty acyl chains shorter than those found in nature exhibit properties different than the long-chain homologues. The diacetyl-, dipropyl-, and dibutyroylphosphatidylcholines are water soluble as monomers.[47] The dihexyl-, diheptanoyl-, and dioctanoylphosphatidylcholines form micelles when dissolved in water at concentrations above their cmc.[48] Lysophospholipids are also water soluble and form micelles like other single-chain amphiphiles. The formation of globular micelles by long-chain phospholipids is forbidden by geometric arguments.[4d] The surface area per headgroup is about the same as single-chain amphiphiles, but there are twice the number of alkyl chains so the required wedge shape cannot be achieved. Long-chain phospholipids exist as monomers in aqueous solution only at extremely low concentrations, probably in the nanomolar range. Smith and Tanford[49] have measured the upper limit for monomer dipalmitoylphosphatidylcholine in water to be 4.7×10^{-10} M at 25 °C. Of course, some of the fatty acids in natural phospholipids contain double bonds and this may increase their solubility somewhat.

Elworthy et al.[4c] have defined solubilization as "the preparation of a thermodynamically stable isotropic solution of a substance normally insoluble or very slightly soluble in a given solvent by the introduction of an additional amphiphilic component or components". The solubilization of membranes and pure lipid bilayers by detergents has been reviewed by Helenius and Simons[6] and recently by us.[12b] When a detergent is added to phospholipid multibilayers in an aqueous milieu, a fraction of the detergent interacts with the bilayers and the remainder exists free in solution.

Subsequent to detergent saturation of the bilayer, mixed micelles begin to form. A mixture of detergent-saturated bilayers and phospholipid-saturated mixed micelles exist until enough detergent is added to convert all the bilayers to mixed micelles.[6,12b] Thus, the structures of the phospholipid and detergent mixtures depend on the ratio of the components (as well as temperature, ionic strength, etc.). Since nonionic surfactants are excellent gentle membrane solubilizers, it is important to understand the formation and structure of nonionic surfactant mixed micelles. In addition, these mixed micelles are often used as membrane models for mechanistic studies of lipolytic enzymes[50] and for studies of membrane protein interactions with particular classes of phospholipids.[51]

Gel Chromatography of Surfactant/Phospholipid Mixed Micelles

The formation, structure, and composition of mixed micelles of the polydisperse surfactant Triton X-100 and monodisperse OPE-9 and various phospholipids were characterized by elution on agarose gel columns

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as described in an earlier section. When mixed micelles containing egg phosphatidylcholine and Triton X-100 at low mole fractions of phospholipid were applied to the column maintained at 28 °C, the phosphatidylcholine and Triton X-100 components eluted together. As the lipid content of the mixed micelles was increased further, the micelles became larger and a shoulder separated completely into a distinct peak, with the lipid mole fraction variable across both peaks.[14]

This partitioning into two peaks was caused by the polydispersity of Triton X-100. The average oxyethylene chain length for the Triton in the larger micelles is shorter than nine oxyethylene units, and the oxyethylene chain distribution for the smaller mixed micelles is approximately nine or ten or slightly longer. The exact reason for the fractionation is unclear although it may be related to the effect seen with Triton X-100 micelles eluted at 40 °C. It may also be related to the fact that the short-chain poly(oxyethylene) Triton species are much more hydrophobic than the long-chain oligomers and may preferentially interact with the lipid molecules and the fact that phospholipids lower the cloud point of Triton X-100.[11b,17b] At 40 °C, micelles even in the absence of any phospholipid show surfactant fractionation as was shown in Figure 1. It is therefore not surprising that the addition of lipid accentuates this phenomenon. It is clear that the polydispersity of the surfactant Triton X-100 and resultant fractionation of the molecular species complicates our understanding of the precise formation of mixed micelles. To surmount this difficulty, the homogeneous surfactant OPE-9, containing exactly nine oxyethylene units, was used to characterize mixed micelle formation with a variety of lipids.

For column chromatography of OPE-9/dipalmitoylphosphatidylcholine mixed micelles at 40 °C, the micelles became progressively larger with increasing mole fractions of phospholipids, and the phospholipid mole fraction remained fairly constant across the peak, corresponding to the mole fraction applied to the column.[14] Mixed micelles of OPE-9 and dimyristoylphosphatidylcholine at 28 °C show a narrow size distribution and symmetrical peaks over a wide range of phospholipid concentrations. Unlike the results with Triton X-100, no shoulder at the high molecular weight side of the peaks was observed, and no peak at the void volume is observed even at 0.5 mole fraction of dimyristoylphosphatidylcholine.

A graph of K_{av} vs. mole fraction of phospholipid for both dimyristoylphosphatidylcholine and dipalmitoylphosphatidylcholine are parallel, as shown in Figure 4, indicating that the two populations of mixed micelles are changing in a similar and regular manner with changing mole fraction of phospholipid. Presumably the displacement of the two lines is due mainly to a temperature effect of micellar size. The K_{av} value does not depend on the total amount of mixed micelles as also shown in Figure 4.

Thermotropic phase transitions of phospholipids also affect mixed-micelle formation.[52] Mixed micelles of Triton X-100 and dipalmitoylphosphatidylcholine do not form at room temperature except at very low mole fractions of lipid, and mixed micelles of Triton X-100

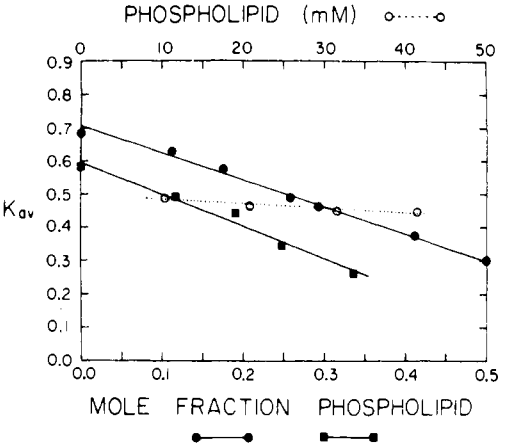

Figure 4. A plot of K_{av} of the eluted peaks from the agarose columns as a function of the mole fraction of phospholipid applied, phospholipid/(phospholipid + OPE-9), for dimyristoylphosphatidylcholine at 28 °C (●) and dipamitoylphosphatidylcholine at 40 °C (■). The K_{av} value of the eluted peak is also shown as a function of the bulk concentration of phospholipid applied for dimyristoylphosphatidylchloine at 28 °C (○) at a constant mole fraction of phospholipid of 0.293. The lines shown are least-squares fits to the data. From ref 14.

and sphingomyelin at 20 °C show anomalous two-peak elution profiles. It was important to know if this was the result of the polydispersity of the oxyethylene chains, or if it was an inherent property of the lipid affecting micelle formation. There is no evidence that pure surfactant micelles and mixed micelles can coexist as has often been stated[53] on the basis of the conclusions of Yedgar et al.[29] In the mole fraction range of 0.11–0.50 with OPE-9 and dipalmitoylphosphatidylcholine mixtures, there are two types of mixed micelles (containing both surfactant and phospholipid) that elute from the column at 20 °C, irrespective of the applied lipid mole fraction. An increase in lipid increases the relative concentration of the mixed micelles containing the larger mole fraction of phospholipid. The smaller mixed-micelle species become saturated, and the new mixed-micelle species form that are in slow equilibrium. When the temperature of the mixed-micellar solution is below a temperature that approximates the phase transition of the lipid (at least for sphingomyelin and dipalmitoylphosphatidylcholine), column chromatography shows more than one population of thermodynamically stable mixed micelles may exist in solution simultaneously, depending on the lipid mole fraction.[52]

Concluding Remarks

In summary, mixed-micelle formation depends both on the polydispersity (or monodispersity) of the nonionic surfactant and upon a temperature that correlates with the thermotropic phase transition of the pure phospholipid. The polydispersity of Triton X-100 makes the analysis of mixed-micelle formation more complicated because of the resultant fractionation of the different oxyethylene chain length species. Qualitatively, one can say that mixed micelles of Triton X-100 and phospholipid above their thermotropic phase transition form readily, and the sizes are not very dependent on the type of lipids at comparable lipid mole fractions. With dipalmitoylphosphatidylcholine at

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temperatures below its phase transition, only dilute mixed micelles form.[11a,52]

With OPE-9, the monodispersity of the surfactant allows one to differentiate between surfactant polydispersity and temperature as the cause of poor micelle-forming properties with some phospholipids. Mixed micelles with dipalmitoylphosphatidylcholine form readily even at 20 °C (unlike Triton X-100, where mixed micelles form only at a very low mole fraction of phospholipid), but two populations of micelles result. The larger sized species are probably best described as very small bilayers with interspersed OPE-9 or very large mixed micelles although they are only about twice the diameter of pure OPE-9 micelles at comparable temperatures. The smallest known bilayer for pure phospholipid is the single bilayer spherical vesicle formed upon sonication of multibilayers,[9] but with some detergent present, there would not be the need to vesiculate. For mixed-micelle formation with a lipid at low temperatures, lipid packing may be tighter laterally, leading to phase separation as is the case with pure lipid bilayers at temperatures below the thermotropic phase transition.[54]

The overall structure of mixed micelles of phospholipid and nonionic detergents is probably similar to that of the pure detergent micelles as illustrated in Figure 3 but with a few phospholipid molecules intercalated in the structure.[14] Much is known about the precise conformation of the phospholipid in the mixed micelle as derived from NMR studies on a large variety of phospholipids in Triton X-100 as well as in a number of other detergents.[55] In all cases, the phospholipid adopts a conformation in which the two fatty acyl chains are nonequivalent and are positioned at the interface with the carbonyl of the *sn*-2 chain more exposed and the carbonyl of the *sn*-1 chain more buried in the hydrophobic region.[55c] The exposure of these carbonyl groups in the phospholipid to the aqueous solution is greater in mixed micelles than in sonicated vesicles but less than for monomers as demonstrated by the susceptibility of the phospholipid to alkaline hydrolysis.[56] Thus, the packing of phospholipid in mixed micelles with Triton X-100 is such that hydroxide can get to the phospholipid and it does so with an equal rate of hydrolysis at the carbonyl of the *sn*-1 and *sn*-2 fatty acyl chains. If it could be definitively shown whether the classical or nonclassical model is better for pure nonionic detergent micelles, it is likely that the mixed micelles would be found to form the same type of structure.

We wish to thank Anthony Ribeiro who, as a graduate student, contributed much to our understanding of Triton micelles. Mary Roberts and Jacqueline deBony as postdoctoral associates and Professors Askel A. Bothner-By and Karol Mysels provided us with many germane suggestions and much help. Critical reading of this manuscript by Dr. Dov Lichtenberg and Dr. Andreas Pluckthun is appreciated. This work was supported by the National Science Foundation through Grant PCM 82-16963.

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Variational Unimolecular Rate Theory

WILLIAM L. HASE

Department of Chemistry, Wayne State University, Detroit, Michigan 48202

Received June 14, 1982 (Revised Manuscript Received December 3, 1982)

The Rice–Ramsperger–Kassel–Marcus (RRKM) theory plays a very important role in the interpretation of unimolecular reaction kinetics.[1-4] Since it is a statistical theory, a unimolecular rate constant for a dissociating molecule can be calculated without acquiring information concerning the molecule's intramolecular dynamics. The assumptions of the theory are fundamental. During dissociation of the molecule, energy is assumed to be randomized amongst all internal degrees of freedom. The classical mechanical equivalent of this statement is that a microcanonical ensemble is postulated for the phase space of the energized molecule. If the molecule is initially prepared with a nonrandom energy distribution, the theory further assumes that rapid intramolecular processes will render the distribution a random one on a time scale much shorter than the molecule's unimolecular lifetime.[4]

Another assumption of RRKM theory is a critical configuration[5] that separates internal states of the energized molecule (reactant) from those of the products. In the language of classical mechanics the critical con-

William L. Hase was born in Washington, MO, in 1945. Upon completing his undergraduate degree at the University of Missouri, Columbia, he went to New Mexico State University, where he received his Ph.D. degree in 1970. After spending 3 years as a postdoctoral research associate, 1 year at New Mexico State and 2 at the University of Columbia, Irvine, he joined the faculty at Wayne State University, where he is a Professor of Chemistry. Dr. Hase's research interests include the theory of gas-phase reaction kinetics (particularly unimolecular kinetics), derivation of potential energy surfaces for reactions involving polyatomic molecules, and computer simulation of both chemical reactions and intramolecular dynamics.

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(5) The critical configuration is often called an activated complex or transition state.

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