  Bull. Chem. Soc. Jpn., 54, 2503—2506 (1981)

Formation of Micelles of Acylcarnitines in Glycerol

Lavinel G. Ionescu*,† and Daniel S. Fung

Department of Chemistry and Chemical Engineering, University of Detroit, Detroit, Michigan, U.S.A. 48221

†*Laboratório de Química de Superícies, Departmento de Química, Universidade Federal de Santa Catarina, Florianópolis, S. C., Brazil 88000*

(Received August 5, 1980)

Acylcarnitine chlorides form micelles in water and polyprotic solvents such as glycerol, ethylene glycol; 1,3-propanediol and 1,4-butanediol. The effect of the acyl chain on the formation of micelles was studied for compounds with chain lengths of 2 to 16 carbon atoms. The critical micellar concentration in glycerol was determined at 25 °C by means of difference spectroscopy using phenol as a probe. The acylcarnitine chlorides with chains containing less than 8 C atoms do not form micelles in glycerol. The critical micellar concentration varies from 0.060 mol dm^{-3} for octanoylcarnitine to 0.011 mol dm^{-3} for palmitoylcarnitine. The free energy of micellization, ΔG°_{mic}, varies linearly with chain length in the range of -6.95 kJ mol^{-1} to -11.2 kJ mol^{-1}. The "hydrophobic" or "solvophobic" effect was more pronounced in water than in glycerol and the free energy change per metylene group was -2.89 kJ mol^{-1} and -0.75 kJ mol^{-1}, respectively. The formation of micelles of acylcarnitine chlorides in glycerol is important in terms of the metabolism of fatty acids and their transport across biological membranes.

The principal pathway for the oxidation of fatty acids is generally considered to be β-oxidation. Several enzymes, known collectively as the fatty acid oxidase complex, are found in the mitochondrion closely associated with the enzymes of the respiratory chain. These catalyze the oxidation of fatty acids to acetyl-S-CoA, the system being coupled with the phosphorylation of ADP to ATP. Although the oxidation of fatty acids occurs inside the mitochondrion, the long chain fatty acids in the cytoplasm cannot pass through the mitochondrial membrane, unless they are enzymatically combined with carnitine to form a fattyacylcarnitine complex.

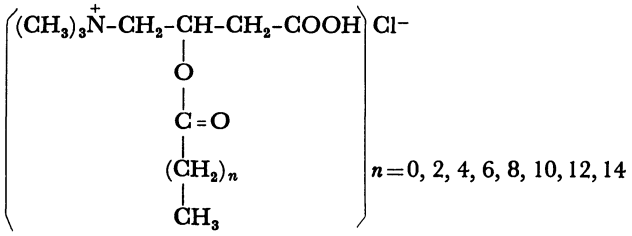
Long chain acylcarnitines are known to form micelles in water.[1,2] The critical micellar concentration (CMC) decreases by an order of magnitude as the alkyl chain length increases by units of two carbon atoms. The aggregation number of long chain acylcarnitines in water slightly increases concomitant with the increment in alkyl chain length.

In comparison with the amount of work done on the properties of surface active agents in water, the chemistry of surfactants in mixed aqueous solvents and nonaqueous solvents is a rather limited field. As part of a systematic study of the process of micellization[3-5] and the use of micelles as simple membrane models,[6] we have decided to search for micelle formation also in nonaqueous protic solvents such as glycerol, ethylene glycol; 1,2-propanediol and 1,4-butanediol. These solvents are very much like water and are known to form micelles with the nonionic detergent polyethylene glycol *p-t*-nonylphenyl ether, NPE_9 (Igepal CO-630) with an average of nine oxyethylene residues in the poly(oxyethylene) chain. The critical micellar concentration of Igepal CO-630 varies from 1.01×10^{-6} mol dm^{-3} in water to 1.55×10^{-2} mol dm^{-3} in 1,4-butanediol and the free energy of micellization, ΔG°_{mic}, ranges from -34.3 to -10.5 kJ mol^{-1} for the same solvents.[7] In general, the process of micellization appears to be thermodynamically favored and is similar to micelle formation in water. The main interaction responsible for micellization is of a hydrophobic type, not unique to water, but found in many other solvents. It is most pronounced in water and glycerol. This interaction is often given by the name "solvophobic" and its exact nature is hard to explain in terms of hydrogen bonding, OH groups, dielectric constant or other parameters.

Glycerol and ethylene glycol form intra- and intermolecular hydrogen bonds,[8,9] although they are much less strong than those in water. Both have been used widely in protein conformation studies and as simple membrane simulators. These dense liquids approximate portions of membranes in terms of the anhydrous environment that they provide. Glycerol has been applied as a viscous agent in the construction of a medium having a viscosity closer to the intracellular environment in the study of the allosteric enzyme glycogen phosphorylase b.[10] Reactivation effects by glycerol and ethylene glycol of inactivated δ-aminolevulic acid synthetase were observed. It was suggested that the protein conformation around the pyridoxal 5′-phosphate binding site of synthetase was stabilized by the polyprotic alcohols.[11]

Materials and Methods

Acylcarnitine chlorides have the generalized structure given below. The compounds studied ranged from acetylcarnitine chloride (n=0) to palmitoylcarnitine chloride (n=14) and differed in the chain



length of the fatty acid by units of two carbon atoms. They were prepared in our laboratory according to the method of Ziegler, Bruckner and Binon.[12] The synthesis essentially involved the preparation of the acyl chlorides from the corresponding acids by means of thionyl chloride, followed by reaction of the acyl

chloride with carnitine employing trichloroacetic acid as the solvent. The acylcarnitines were subsequently recrystallized from isopropyl alcohol and dried over P_2O_5 under high vacuum. Acetyl chloride and trichloroacetic acid (reagent grade) were obtained from Allied Chemical Company. Butanoic, hexanoic, octanoic, decanoic, lauric, myristic, palmitic acid, and *dl*-carnitine hydrochloride were of the highest quality available and were purchased from Aldrich Chemical Co. Thionyl chloride and phenol were of reagent grade and were supplied by Fisher Scientific Co. Glycerol (reagent grade) was purchased from Eastman Organic Chemicals and used without any further purification.

In the original experiments, the CMC was determined by surface tensiometry and only lauroylcarnitine chloride was used. The surface tension measurements were performed at 25 °C with a Du Nouy Tensiometer Model No. 70547 or a Fisher Surface Tensiomat Model 21. Both instruments were calibrated before use. Solutions of lauroylcarnitine chloride in glycerol ranging 1×10^{-9} to 0.1 mol dm^{-3} were prepared. Ten milliliter aliquots of solution in a glass dish with a diameter of 6 cm were used for the measurements. The first determination in a given series was the measurement of the surface tension of deionized distilled water, followed by pure glycerol at 25 °C. Subsequent measurements were of the various surfactant solutions in order of increasing concentration. By plotting the surface tension versus the concentration or the logarithm of the concentration of the surfactant solution, a curve with a sharp initial drop and subsequent levelling off was obtained. The first concentration at which the levelling off took place was taken as the critical micellar concentration.[3,4] Typical results obtained are illustrated in Fig. 1.

Because of the experimental difficulties encountered in the measurement of the surface tension of the viscous glycerol solutions, an alternate method using phenol as a probe for micelle formation was employed for the determination of the CMC. This is essentially a difference spectrophotometric technique developed by Ray and Némethy[13,14] The absolute absorption spectrum of 5.0×10^{-4} mol dm^{-3} phenol in glycerol was recorded with a Beckman Model 24 Spectrophotometer. Phenol has a well-defined λ_{max} at 272 nm. The difference spectra were determined with the same intrument using an expanded absorbance scale (0—0.1, 0—0.25, or 0—0.5). A solution of 5.0×10^{-4} mol dm^{-3} phenol in glycerol was placed in the reference cuvette. The sample cuvette contained a solution of the same concentration of phenol in glycerol and varying amounts of the corresponding acylcarnitine chloride. The spectra of the solutions were measured from 240 to 350 nm. The difference absorption spectra were determined for all the acylcarnitine chlorides mentioned above, namely, acetyl-, butyryl-, hexanoyl-, octanoyl-, decanoyl-, lauroyl-, myristoyl-, and palmitoylcarnitine chloride.

In general fifteen to twenty solutions of the surfactants were measured. These solutions were prepared by diluting stock solutions of acylcarnitines containing 5.0×10^{-4} mol dm^{-3} phenol in glycerol with glycerol containing the same amount of phenol. All the spectra were determined at 25 °C. A plot of the change in absorption at 272 nm, ΔA, *versus* the concentration or the logarithm of the concentration of surfactant gave a curve with a definite break at the CMC. Linear plots were taken as an indication that micelle formation did not take place. Figure 2 gives some typical experimental results obtained for lauroylcarnitine chloride.

Results and Discussion

A summary of the critical micellar concentrations and the free energies of micellization of acylcarnitine chlorides is given in Table 1. As can be seen, octanoylcarnitine chloride is the shortest acylcarnitine that forms micelles in glycerol. The value obtained for the CMC of lauroylcarnitine chloride by surface tension measurements was also 0.030 mol dm^{-3} and thus the agreement between the two different techniques was within experimental error. The CMC decreases with increasing carbon chain length, but the effect is much less pronounced in glycerol than in water.

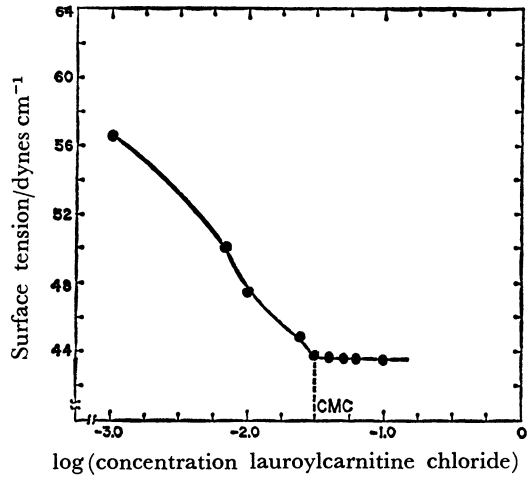


Fig. 1. Plot of surface tension *versus* the logarithm of the concentration of lauroylcarnitine chloride in glycerol at 25 °C.

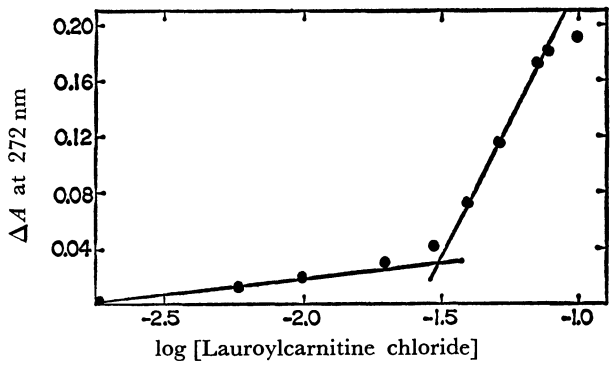


Fig. 2. Dependence of absorbance difference (ΔA) of phenol on the logarithm of the concentration of lauroylcarnitine chloride in glycerol at 25 °C.

Table 1. Critical micellar concentration and free energy of micellization for acylcarnitine chlorides in glycerol at 25 °C

Acylcarnitine chloride	Critical micellar concentration / mol dm^{-3}	ΔG_m° / kJ mol^{-1}
Acetyl-	None	—
Butyryl-	None	—
Hexanoyl-	None	—
Octanoyl-	0.060	−6.95
Decanoyl-	0.050	−7.45
Lauroyl-	0.030	−8.66
Myristoyl-	0.020	−9.67
Palmitoyl-	0.011	−11.2

The formation of normal micelles in nonaqueous polar solvents is of particular interest because in addition to providing novel insights into the process of micellization, it may lead to a better understanding of the interactions that take place between surfactant molecules and the solvent. For micelles in water, the process of micellization is generally explained by means of the hydrophobic interactions between the surfactant and water. "Hydrophobic interactions" is in many ways a convenient term that is used to describe an entire array of inter- and intramolecular interactions involved in micellization and in a certain way the term disguises our ignorance about the actual molecular dynamic processes that take place.

In general, the formation of micelles in water is assumed to take place by the association of the hydrophobic parts of the surfactant molecules and the repulsion of water of solvation from their immediate environment. The thermodynamics of micelle formation has been discussed and treated extensively in the literature.[15,16] One approach assumes that the process of micellization involves the formation of a distinct micellar phase at the CMC and that the concentration of monomers in solution is constant, once micelles are formed. Then, the standard free energy of micellization, ΔG_{mic}°, is given by Eq. 1 to a good

$$\Delta G_{mic}^\circ = RT \ln \mathrm{CMC} \quad (1)$$

approximation. If one postulates that the aggregation number and the degree of ionization of the surfactant are temperature independent, the standard enthalpy (ΔH_{mic}°) and entropy (ΔS_{mic}°) can be evaluated by the temperature dependence of the CMC and the relationship given by Eq. 2.

$$\Delta G_{mic}^\circ = \Delta H_{mic}^\circ - T\Delta S_{mic}^\circ \quad (2)$$

The over-all process of micellization involves a decrease in the free energy of the system. According to Eq. 2, ΔG_{mic}° is the result of enthalpy and entropy contributions. For aqueous solutions, micellization is generally regarded as an entropy directed process and the preponderant contribution of the entropy term is explained by the disordering of the water structure and the breakup of the "Frank-Evans microcrystals" by the surfactant molecules.

The type of interactions involved in the formation of micelles in polar solvents other than water are called "solvophobic." The understanding of "solvophobic interactions" and micellization in nonaqueous media is considerably more nebulous. The driving force for micellization is less for such systems and the more positive ΔG_m° is usually believed to be primarily due to a decrease of the entropic contribution.[7,18] Few solvent systems are as highly ordered and as strongly hydrogen-bonded as water.

As has already been mentioned, the study of nonaqueous micellar systems is of importance also in terms of the understanding of membranes. In fact, the first dynamic membrane model, precursor of the current fluid mosaic or liquid crystal model, was a micelle.[17] In the over-all analysis of solvent-surfactant interactions it is desirable to sort out hydrogen bonding, solvation and the "solvophobic effect." However, the understanding and clear differentiation of these processes in certain systems may prove to be quite a formidable task.

We have attempted to quantify the solvophobic effect in water and glycerol by varying the chain length of the acylcarnitine surfactants. The free energies of micellization of these surfactants at 25 °C have been calculated for water and glycerol and are shown in Fig. 3. The values for water have been calculated from the experimental results of Yalkowsky and Zografi.[1] A simple analysis of the results indicates that the formation of micelles of acylcarnitine chlorides is thermodynamically more favored in water than in glycerol.

The change in ΔG_{mic}° for a given solvent as a function of chain length provides information about the nature of the interaction of the $-CH_2-$ groups with the given solvent and can be interpreted as the solvophobic effect. One can quantify such an interaction by determining the slope of the plots of ΔG_{mic}° *versus* chain length. Large breaks or deviations from linearity in such plots should be indicative of unusual interactions. The data in Fig. 3 illustrates a linear behavior for both solvents, but the slope is considerably steeper for water than for glycerol. The free energy

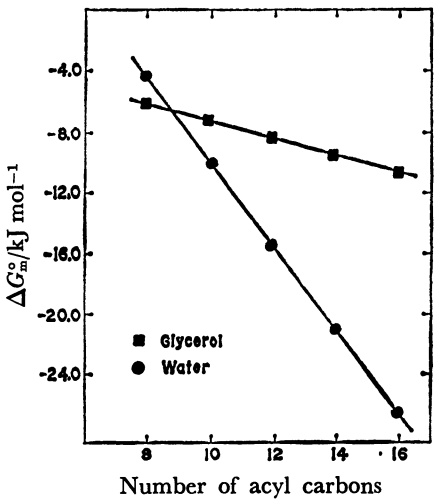


Fig. 3. Dependence of the free energy of micellization (ΔG_m°) on the acyl chain length of acylcarnitine chlorides in water and glycerol at 25 °C.

change per methylene group in water is -2.89 kJ mol^{-1} and in glycerol is -0.75 kJ/mol. The value obtained for water is in agreement with that commonly reported in the literature,[16] while the result for glycerol agrees well with -0.75 kJ mol^{-1} determined by Ray and Némethy for the polyether NPE_9 in ethylene glycol.[18] The solvophobic effect is thus considerably more pronounced in water and shows about the same change in going from water to glycerol and from water to ethylene glycol.

Preliminary experiments performed in our laboratory indicate that lauroylcarnitine chloride forms micelles also in ethylene glycol; 1,3-propanediol and 1,4-butanediol. All these solvents are similar to water in the sense that they form hydrogen bonds and are polar in nature. Glycerol and ethylene glycol form intra and inter hydrogen bonds, although they are much less strong than those in water.[8,9]

The experimental results clearly indicate that the long chain acylcarnitine chlorides, *i.e.*, octanoyl-, decanoyl-, lauroyl-, myristoyl-, and palmitoylcarnitine chloride form micelles in glycerol in addition to water. The shorter chain acylcarnitines do not form micellar aggregates in either of the two solvents. The results may be of biochemical interest for several reasons. It is well known that short chain fatty acids move freely in non-complexed form across membranes. Longer fatty acids, on the other hand, are known to cross the mitochondrial membrane in the form of acylcarnitine complexes. Glycerol has been widely used in protein conformation studies and as a simple membrane simulator. Its viscous nature and anhydrous environment may approximate portions of membranes.[19,20] In addition, the relationship between micelles and the liquid crystal membrane model has already been pointed out. The present work has shown that long chain alkanoylcarnitines form charged colloidal aggregates or micelles in an anhydrous environment akin to that of biological membranes. It would appear reasonable to suggest that micelles may play a role in the transport of long chain acylcarnitines across membranes.

The author (LGI) gratefully acknowledges financial support received from CNPq-National Research Council of Brazil (Grants Nos. 1111.5713/78 and 40.2548/79).

References

1) S. H. Yalkowsky and G. Zografi, *J. Pharm. Sci.*, **59**, 798 (1970).
2) S. H. Yalkowsky and G. Zografi, *J. Colloid Interface Sci.*, **34**, 525 (1970).
3) L. G. Ionescu, T. Tokuhiro, B. J. Czerniawski and E. S. Smith, "Solution Chemistry of Surfactants," ed by K. L. Mittal, Plenum Press, New York (1979), p. 487.
4) L. G. Ionescu, T. Tokuhiro, and B. J. Czerniawski, *Bull. Chem. Soc. Jpn.*, **52**, 922 (1979).
5) T. Tokuhiro, L. G. Ionescu, and D. S. Fung, *J. Chem. Soc., Faraday Trans. 2*, **75**, 975 (1979).
6) L. G. Ionescu and J. K. Tsang, *Rev. Roum. Biochim.*, **15**, 211 (1978).
7) A. Ray, *Nature*, **231**, 313 (1971).
8) M. Ueda, T. Urahata, A. Katyam, and N. Kuroki, *Seni Gakkaishi*, **32**, T 301 (1976).
9) J. L. Beaudoin, *J. Chim. Phys.*, **74**, 268 (1977).
10) S. Damjanovich, B. Somogyi, and J. Bot, *Stud. Biophys.*, **59**, 229 (1976).
11) T. Yubisui, M. Takeshita, and Y. Yoneyama, *Experientia*, **32**, 3989 (1976).
12) H. J. Ziegler, P. Bruckner, and F. Binon, *J. Org. Chem.* **32**, 3989 (1967).
13) A. Ray and G. Némethy, *J. Phys. Chem.*, **75**, 804 (1971).
14) G. Némethy and A. Ray, *J. Phys. Chem.*, **77**, 64 (1973).
15) D. G. Hall, *Trans. Faraday Soc.*, **66**, 1351 (1970).
16) N. Muller, "Reaction Kinetics in Micelles," ed by E. H. Cordes, Plenum Press, New York (1973), p. 1.
17) A. A. Benson, *J. Am. Oil Chem. Soc.*, **43**, 265 (1966).
18) A. Ray and G. Némethy, *J. Phys. Chem.*, **75**, 809 (1971).
19) A. Ray, *J. Am. Chem. Soc.*, **91**, 6511 (1969).
20) H. J. Sage and S. J. Singer, *Biochemistry*, **1**, 305 (1962).