ALTERATION OF THE CONCENTRATIONS OF DILUTE PALMITYL-CoA SOLUTIONS BY SURFACE ADSORPTION

R. E. Barden and W. W. Cleland

Department of Biochemistry, University of Wisconsin,
Madison, Wisconsin 53706

Received January 8, 1969

Surface active molecules containing both a hydrophilic group and a hydrophobic group of 8 or more carbons in the same molecule exhibit a strong tendency to adsorb at surfaces and the quantity adsorbed reaches a maximum at or near the critical micelle concentration (1). The critical micelle concentration of palmityl-CoA is 3-4 μM (2), and at such low concentrations, surface adsorption could conceivably cause a significant decrease in the concentration of the bulk phase. We wish to report that at 3 μM initial concentration, 0.23 mμmoles of palmityl-CoA per cm^2 are absorbed at the air-liquid interface. Unless the surface-volume ratio is adjusted so that the quantity of palmityl-CoA adsorbed is an insignificant portion of the total amount present, significant decreases in the concentration of the bulk phase are observed.

Methods and Materials. Approximately 0.25 μmoles of 1-[^{14}C]-palmityl-CoA (40 c/mole, Tracerlab) was dissolved in 5 ml of 8 mM K phosphate, pH 6.4, together with 2-3 mg of unlabeled palmityl-CoA (P. L. Biochemicals). A small amount of radioactive impurity was removed by adding 0.5 ml of 10% perchloric acid, centrifuging at low speed, and washing the precipitate with dry acetone in ether, as described by Seubert (3). The ether-insoluble thioester was dissolved in 3 ml of 8 mM K phosphate, pH 6.4, and an aliquot was chromatographed on Whatmann #1 paper. With the solvent n-butanol:acetic acid:water (5:2:3), the only radioactive peak observed had the R_f of palmityl-CoA, 0.56. In all experiments the palmityl-CoA was dissolved in 8 mM K phosphate, pH 6.4, and the concentrations of the stock solutions were determined spectrophotometrically, assuming a molar absorptivity of 15,400 at 260 mμ (4). Radioactivity was measured with a scintillation counter. Samples containing no more than 0.2 ml of water were mixed with 2.5 ml of ethanol and then diluted with 15 ml of toluene containing 4 gm of 2,5 diphenyloxazole and 0.25 gm of p-bis [2-(5-phenyloxazolyl)]-benzene per liter; samples containing 1 ml of water were counted in 15 ml of Bray's solution (5).

Results. When 2 ml of 3.2 μM 1-[^{14}C]-palmityl-CoA were placed in a glass vial for 30 min, and 1 ml of the bulk phase then transferred to a second vial, it was found that the bulk phase removed contained 5770 cpm, while the residual material contained 6890 cpm. A 1 ml aliquot of the original stock solution gave 6170 cpm. This phenomenon depends on the area of the air-liquid interface, as shown by the data in Table 1, and the average concentration of palmityl-CoA adsorbed at the surface is 1.4 x 10^{14} molecules (0.23 mμmoles) per cm^2.

An experiment demonstrating the same phenomenon in another fashion is shown in figure 1. In this case vials of constant diameter were used and the volumes of the palmityl-CoA solution placed in them were varied. The depletion of the bulk phase concentration was appreciable only when the volume was small; this result rules out adsorption on glass as the cause of the effect. The adsorption of palmityl-CoA on the surface is not instantaneous, but is a process which is complete only after 15-20 min when the solution was 4 mm deep, with a time for half approach to equilibrium of about 4 min. Deeper solutions may well require longer to reach equilibrium, although this was not tested.

The process of surface adsorption does not seem readily reversible.

TABLE I

Surface Adsorption of Palmityl-CoA

Vial radius, cm	0.5	0.65	0.9	1.1
Air-liquid surface, cm^2	0.79	1.3	2.5	3.8
Bulk phase concentration, µM	2.60	2.35	2.18	2.03
Δ concentration, µM	0.30	0.55	0.72	0.87
Molecules removed from bulk phase x 10^{-14}	1.2	2.3	3.0	3.7
Molecules per cm^2 of air-liquid surface x 10^{-14}	1.5	1.8	1.2	1.0

Each vial contained 0.7 ml of 2.9 µM [^{14}C]-palmityl-CoA. After 20 minutes, 0.1 ml aliquots of the bulk phase were removed and counted along with an 0.1 ml aliguot of the original solution. The average number of molecules absorbed per cm^2 is 1.4 ± 0.3 x 10^{14}.

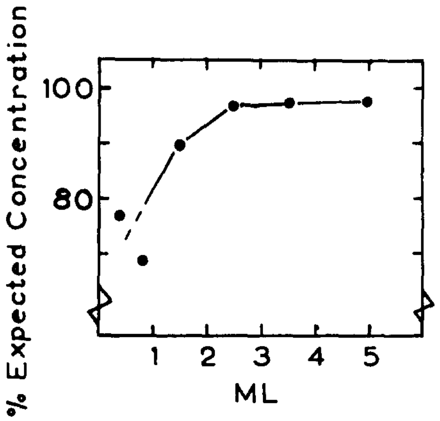

Figure 1. Percent of expected concentration as a function of sample volume in vials of constant radius. The indicated volumes of a 3.1 µM 1-[^{14}C]-palmityl CoA were placed in glass vials (radius equals 0.65 cm) for 30 minutes, and 0.1 ml aliquots of the bulk phase were then counted and compared with an 0.1 ml aliquot of the original solution.

To show this 1.3 ml aliquots of 3.1 µM 1-[^{14}C]-palmityl-CoA were placed in each of two 15 x 50 ml glass vials lying on their side, in which position the air-liquid surface area is greatly increased compared to that in a vertical position. After 1 hour a 0.1 ml aliquot was removed from the bulk phase of one of these vials and assayed; the other vial was carefully placed in an upright position. At the same time 1.3 ml of the original palmityl-CoA solution was placed in another vial standing upright. After 1 hour a 0.1 ml aliquot was removed from the bulk phase of each upright vial and assayed. The vial that had been only in a vertical position gave 528 cpm, the vial that had only been horizontal gave 437 cpm, while the one that was horizontal and then returned to a vertical position gave only 426 cpm, indicating that material had not returned to the bulk phase when the surface area was decreased.

Discussion. These experiments show that palmityl-CoA is highly surface active at dilute concentrations in the range of its critical micelle concentration (3 µM) and that at these low concentrations surface adsorption can result in a significant decrease in the concentration of the bulk phase if the surface-volume ratio exceeds 0.5 cm^2/ml. Obviously, adequate precautions to prevent concentration errors are needed when working with dilute solutions of such compounds. The critical micelle concentration of a surfactant is frequently determined by plotting the surface tension versus the surfactant concentration (6). With this method the assumption is made that the amount of surfactant adsorbed at the surface is not sufficient to alter the concentration of the bulk phase. Such an assumption would clearly be invalid for palmityl-CoA, unless deep vessels are used.

Acknowledgement. This work was supported in part by grant No. HE-05095 from NIH. The authors are grateful to Professor P. Mukerjee for helpful discussion.

References

1. Tamamushi, B.-I. In Colloidal Surfactants (E. Hutchinson and P. Van Rysselberghe, Eds.) New York: Academic Press, 1963.
2. Zahler, W. L., Barden, R. E., and Cleland, W. W. Biochim. Biophys. Acta, 164, 1 (1968).
3. Seubert, W. Biochem. Prep., 9, 55 (1962).
4. Bock, R., Ling, N., Morell, S., and Lipton, S. Arch. Biochem. Biophys., 62, 253 (1956).
5. Bray, G. A. Anal. Biochem., 1, 279 (1960).
6. Shinoda, K. In Colloidal Surfactants (E. Hutchinson and P. Van Rysselberghe, Eds.) New York: Academic Press, 1963.