Kinetic Studies of Liposome Solubilization by Sodium Dodecyl Sulfate Based on a Dynamic Light Scattering Technique

ARTICLE *in* LANGMUIR · AUGUST 1998

Impact Factor: 4.46 · DOI: 10.1021/la980219f

5 AUTHORS, INCLUDING:

Ornella Lopez
Pontificia Universidad Javeriana - Cali
25 PUBLICATIONS **194** CITATIONS
SEE PROFILE

Mercedes Cócera
Bicosome Co.
76 PUBLICATIONS **779** CITATIONS
SEE PROFILE

Ramon Pons
Institute of Advanced Chemistry of Catalonia…
101 PUBLICATIONS **1,364** CITATIONS
SEE PROFILE

Kinetic Studies of Liposome Solubilization by Sodium Dodecyl Sulfate Based on a Dynamic Light Scattering Technique

O. López,* M. Cócera, R. Pons, N. Azemar, and A. de la Maza

Departamento de Tensioactivos, Centro de Investigación y Desarrollo (C.I.D.), Consejo Superior de Investigaciones Científicas (C.S.I.C.), C/ Jordi Girona, 18-26, 08034 Barcelona, Spain

Received February 24, 1998. In Final Form: May 21, 1998

Introduction

The vesicle to micelle transformations induced by the action of surfactants in phospholipid vesicles are currently attracting much interest. These processes leads to the solubilization of lipid vesicles, and they represent good models for the study of solubilization of cell membranes.[1] A number of studies have been devoted to using techniques of light scattering[2−6] and cryo-TEM[7−9] to clarify the principles governing these transformations. In general, there is agreement that a growth of vesicles occurred in the initial interaction steps followed by the formation of a number of complex lipid−surfactant aggregates associated with the vesicle to micelle transformations. Thus, Edwards and Almgren,[7] Silvander et al.,[8] and Gustafsson et al.,[9] reported open bilayer fragments in coexistence with different micellar structures as intermediate aggregates in the interaction of varios anionic and cationic surfactants with phosphatidylcholine (PC) liposomes. However, recent studies using vesicles prepared from nonionic and oppositely charged surfactants proposed rapid and simpler mechanisms for vesicle to micelle transformations as single-step processes.[10−14]

In earlier papers we investigated the structural changes resulting in the interaction of alkyl sulfates with PC liposomes.[15−17] Although kinetic studies of formation of vesicles and micelles as independent processes have been reported,[18,19] kinetics of vesicle solubilization by surfactants remained elusive in spite its obvious importance. Hence, we present here a kinetic study of the vesicle to micelle structural transformations that take place in the solubilization of PC liposomes by sodium dodecyl sulfate (SDS). To this end, a dynamic light-scattering (DLS) technique (Ar laser source, useful in systems in which small and large particles coexisted) has been used. The use of this technique in solubilization kinetic studies opens up new avenues in the knowledge of the mechanisms that occur in this process. The anionic surfactant SDS has been selected given its frequent use in simplified membrane models such as PC liposomes[20−22] and those formed by stratum corneum lipids,[23,24] due to its irritating effect in biological membranes.[25−27]

Materials and Methods

PC was purified from egg lecithin (Merck, Darmstadt, Germany) using the method of Singleton[28] and was shown to be pure by TLC. Sodium dodecyl sulfate (SDS) was obtained from Merck and further purified by a column chromatographic method.[29] Tris(hydroxymethyl)aminomethane (TRIS buffer) obtained from Merck was prepared as 5.0 mM TRIS buffer adjusted to pH 7.4 with HCl and containing 100 mM of NaCl.

Vesicle Preparation and Solubilization. Unilamellar PC liposomes of a defined size (about 200 nm) were prepared by extrusion of large unilamellar vesicles (through 800−200 nm polycarbonate membranes) previously obtained by reverse phase evaporation.[16,30]

To study the kinetics of solubilization of PC liposomes by SDS, different surfactant concentrations in TRIS buffer (from 0.3 to 4.5 mM) were added to liposomes, the PC concentration remaining constant (0.5 mM). The study was carried out during 24 h using a dynamic light-scattering technique and paying special attention to the first 10 min to know in detail the initial steps of this process.

Dynamic Light-Scattering Experiments. The hydrodynamic diameters (HDs) of pure PC vesicles, pure SDS micelles, and particles formed during the interaction of SDS with liposomes were determined by means of a dynamic light-scattering (DLS) technique using a photon correlator spectrometer (Malvern Autosizer 4700c PS/MV) equipped with an Ar laser source (wavelength 488 nm). To acquire the full range of decay time necessary to determine the signal from both the large and the small particles, a low sample time value (2 μs) and a dilatation of 3 with parallel subcorrelators were used. Quartz cuvettes were filled with the samples, and all the experiments were thermostatically controlled. DLS determinations were made with a reading angle of 90° in all cases. Measurements of the overall

* To whom correspondence should be addressed. Telephone: (34-3) 400.61.61. Telefax: (34-3) 204.59.04.

(1) Lichtenberg, D. *Biochim. Biophys. Acta* **1985**, *821*, 470.
(2) Edwards, K. *Prog. Colloid Polym. Sci.* **1990,** *82,* 190.
(3) Paternostre, M.; Meyer, O.; Grabielle-Madelmont, C.; Lesieur, S.; Ghanam, M.; Ollivon, M. *Biophys. J.* **1995,** *69,* 2476.
(4) Partearroyo, M. A.; Alonso A.; Goñi, F. M.; Tribout, M.; Paredes, S. *J. Colloid Interface Sci.* **1996,** *178,* 156.
(5) Cladera, J.; Rigaud, J. L.; Villaverde, J.; Duñach, M. *Eur. J. Biochem.* **1997,** *243,* 798.
(6) Wenk, M. R.; Seelig, J. *Biophys. J.* **1997,** *73,* 2565.
(7) Edwards, K.; Almgren, M. *Langmuir* **1992,** *8,* 824.
(8) Silvander, M.; Karlsson, G.; Edwards, K. *J. Colloid Interface Sci.* **1996,** *179,* 104.
(9) Gustafsson, J.; Orädd, G.; Lindblom, G.; Olsson, U.; Almgren, M. *Langmuir* **1997,** *13,* 852.
(10) Söderman, O.; Herrington, K. L.; Kaler, E. W.; Miller, D. D. *Langmuir* **1997,** *13,* 5531.
(11) Seras, M.; Edwards, K.; Almgren, M.; Carlson, G.; Ollivon, M.; Lesieur, S. *Langmuir* **1996,** *12,* 330.
(12) Danino, D.; Talmon, Y.; Zana, R. *J. Colloid Interface Sci.* **1997,** *185,* 84.
(13) O'Connor, A. J.; Hatton, T. A.; Bose, A. *Langmuir* **1997,** *13,* 6931.
(14) Friberg, S. E.; Campbell, S.; Fei, L.; Yang, H.; Patel, R.; Aikens, P. A. *Colloid Surf. A* **1997,** *129−130,* 167.
(15) de la Maza, A.; Parra, J. L. *Langmuir* **1993,** *9,* 870.
(16) de la Maza, A.; Parra, J. L. *Langmuir* **1995,** *11,* 2435.
(17) de la Maza, A.; Parra, J. L. *Langmuir* **1996,** *12,* 3393.
(18) Campbell, S. E.; Yang, H.; Patel, R.; Friberg, S. E.; Aikens, P. A. *Colloid Polym. Sci.* **1997**, *275,* 303.
(19) Kahlweit, M.; *J. Colloid Interface Sci.* **1982,** *90,* 92.
(20) Inoue, T.; Yamahata, T.; Shimozawa, R. *J. Colloid Interface Sci.* **1992**, *149*, 345.
(21) Urbaneja, M. A.; Alonso, A.; González-Mañas, J. M.; Goñi, F. M.; Partearroyo, M. A.; Tribout, M.; Paredes, S. *Biochem. J.* **1990**, *270*, 305.
(22) Ruiz, J.; Goni, F. M.; Alonso, A. *Biochim. Biophys. Acta* **1989**, *937*, 127.
(23) de la Maza, A.; Parra, J. L. *Langmuir* **1996,** *12,* 6218.
(24) Downing, D. T.; Abraham, W.; Wegner, B. K.; Willman, K. W.; Marshall, J. M. *Arch. Dermatol. Res.* **1993**, *285*, 151.
(25) Schäfer-Korting, M. Clinical Trial Protocols for Anti-Inflammatory and other liposome Dermatics. In *Liposome Dermatics (Griesbach Conference)*; Braun-Falco, O., Korting, H. C., Maibach, H., Eds.; Springer-Verlag: Berlin, 1992; pp 299−307.
(26) Moon, K. C.; Maibach H. I. In *Exogenous Dermatoses: Environmental Dermatitits*; Menné, T., Maibach, H. I., Eds.; CRC Press: Boca Raton, FL, 1991; pg 217−226.
(27) Wilheim, K. P.; Surber, C.; Maibach, H. I. *J. Invest. Dermatol.* **1991**, *96*, 963.
(28) Singleton, W. S.; Gray, M. S.; Brown, M. L.; White, J. L. *J. Am. Oil Chem. Soc.* **1965,** *42,* 53.
(29) Rosen, M. J.; Hua, X. Y. *J. Colloid Interface Sci.* **1982,** *86,* 164.
(30) Paternostre, M. T.; Roux, M.; Rigaud, J. L. *Biochemistry* **1988,** *27,* 2668.

S0743-7463(98)00219-4 CCC: $15.00
Published on Web 07/15/1998

Table 1. Particle Size Distribution Data Corresponding to the Interaction of SDS at 1.5 and 4.5 mM with PC Liposomes (0.5 mM PC) during 24 h[a]

	1.5 mM SDS				4.5 mM SDS			
	1st peak		2nd peak		1st peak		2nd peak	
time (min)	nm	%	nm	%	nm	%	nm	%
10	3.6	4.2	186.7	95.8	4.7	15.2	185.6	84.8
30	3.9	3.9	188.2	96.1	5.2	27.3	187.6	72.7
60	4.1	4.3	185.3	95.7	5.0	33.6	174.5	66.4
90	4.6	6.5	188.0	96.7	5.8	43.1	172.0	56.9
120	6.4	6.4	181.3	93.6	5.7	51.1	170.4	48.9
140	6.1	6.2	184.8	93.8	5.6	63.9	167.6	36.1
180	6.2	10.6	180.3	92.4	5.8	71.5	166.3	28.5
220	5.8	13.4	177.3	86.6	6.0	86.7	165.8	13.3
260	5.6	13.9	183.0	86.1	6.0	90.9	164.4	9.1
360	5.9	15.7	184.1	84.3	5.4	93.8	162.3	6.2
480	5.9	14.2	177.0	85.8	5.3	100		
1440	6.0	15.2	174.6	84.8	5.2	100		

[a] Results are given as diameters, and the percentage corresponds to intensity values.

process (24 h) were performed in intervals of 30 min. To study in detail initial interaction steps, a series of measurements (SDS concentration 1.5, 2.5, 3.5, and 4.5 mM) were carried out every 30 s during the first 10 min of the process. This period of time was the minimum needed to obtain statistically reliable data based on "cumulant analysis". The analysis of the data was performed using CONTIN software provided by Malvern Instruments, England. The goodness of the CONTIN results was tested by fitting a single or a biexponential to the correlation function. If a biexponential had to be fitted, first a single exponential was fitted to a long time range and the second exponential was then fitted to the residual. Both methods agreed fairly well. The results are given as diameters, and the percentage corresponds to intensity values.

Results and Discussion

We first determined the size distribution curves both for the micellar SDS solutions before they were mixed with liposomes and for pure PC liposomes. The curves for micellar SDS solutions (SDS concentration ranging from 1.5 to 4.5 mM) always showed a monomodal distribution with a peak at 2 nm. The curve for pure PC liposomes also exhibited a monomodal distribution with a hydrodynamic diameter of 186 nm.

The DLS kinetic study of the SDS−PC liposome interaction (24 h, PC concentration 0.5 mM), using a 0.3 mM SDS concentration showed always a monomodal distribution with a peak of about 186 nm (mixed vesicles). Hence, no changes in the aggregation state of the system was detected throughout the process. The SDS concentration 0.3 mM was lower than its critical micelle concentration (cmc), which was 0.75 mM according to the surface tension measurements under the experimental solution conditions. This low cmc value was due to the presence of 100 mM NaCl in the buffered medium.

When the experiments were carried out at SDS concentrations higher than its cmc (1.5 and 4.5 mM, see Table 1), two peaks in the size distribution curves were observed (small and large particles). Thus, the system containing 1.5 mM SDS showed 10 min after the surfactant addition a peak for a HD of 186 nm (attributed to the formation of surfactant−PC mixed vesicles) with a very slight change in both the size and proportion of these particles throughout the process. The small particles detected (initial size 4.2 nm) increased in size with time to become stable with a size of about 6 nm. Comparison of the size of these particles with that of pure SDS micelles (2 nm) indicated that the small particles (after 10 min of interaction) did not correspond to pure SDS micelles and, consequently, may be attributed to the formation of surfactant−PC mixed micelles. It is noteworthy that although the proportion of these small particles progressively rose during the first 6 h (up to 15%), their size became stable (of about 6 nm) 2 h after the surfactant addition. Hence, in the interval from 6 to 24 h the system remained stable with a coexistence of mixed vesicles and mixed micelles. The lack of particles with intermediate sizes raises a question about the formation of intermediate aggregates during the solubilization of PC liposomes by SDS previously reported.[8,16]

In the experiment carried out at the SDS concentration 4.5 mM (Table 1), the aforementioned two peaks were detected up to 360 min and only one peak (small particles of 5−6 nm) was detected up to the end of the experiment. Thus, the liposome suspension was completely solubilized 360 min after the addition of SDS. The mean size of the large particles slightly decreased with time (from 186 to 163 nm after 360 min), whereas its proportion showed an abrupt fall during the initial 250 min of treatment. The size of the small particle showed a slight growth to reach a HD of about 6 nm (90 min after SDS addition), and its proportion markedly rose to reach 100% after 480 min. This experiment also shows the absence of intermediate aggregates during the process.

From this study we may assume that the interaction of SDS with PC liposomes led to a dynamic structural equilibrium regardless of the surfactant proportion in the system. Thus, when the surfactant concentration was lower than its cmc (sublytic concentration, in agreement with our previous work[16]), this equilibrium did not affect the size of the vesicles and, consequently, the aggregation state of the system. When the SDS concentration was higher than its cmc but insufficient to solubilize completely PC liposomes, an equilibrium was achieved between the mixed vesicles and the mixed micelles formed also without vesicle growth. When the SDS concentration was sufficient to completely solubilize liposomes, the equilibrium involved the complete formation of mixed micelles. It is noteworthy that the time needed to achieve these three equilibrium states was directly dependent on the surfactant concentration and, consequently, on the surfactant−PC molar ratio.

From a structural viewpoint, two findings sharply contrast with those reported by our research group using DLS (He−Ne laser source) and negative-stained TEM techniques[16] and with those reported by Silvander et al.[7] and Edwards et al.,[8] also using DLS (He−Ne laser source) and cryo-TEM techniques: on one hand, the formation of mixed vesicles (surfactant incorporation into liposomes) without growth of these bilayers structures and, on the other hand, the absence of complex intermediate aggregates during solubilization (only equilibrium of mixed vesicles and mixed micelles). This last finding seems to be in line with the very recent solubilization studies of Seras et al.[11] and Danilo et al.,[12] using mixtures of vesicle-forming surfactants, in which the absence of some complex intermediates previously described has been reported.

Figure 1 shows the variation in the proportion of mixed vesicles (Figure 1A) and mixed micelles (Figure 1B) as a function of time for different SDS concentrations (PC concentration 0.5 mM). It may be seen that for a 0.3 mM SDS concentration (Figure 1A) the percentage of mixed vesicles was 100% during all the process, indicating that at this surfactant concentration neither solubilization nor other effects took place in the liposome structure. The curves for 1.5 and 4.5 mM SDS confirm that the time needed to achieve equilibration (no change in both the proportion and the size of particles) was directly dependent

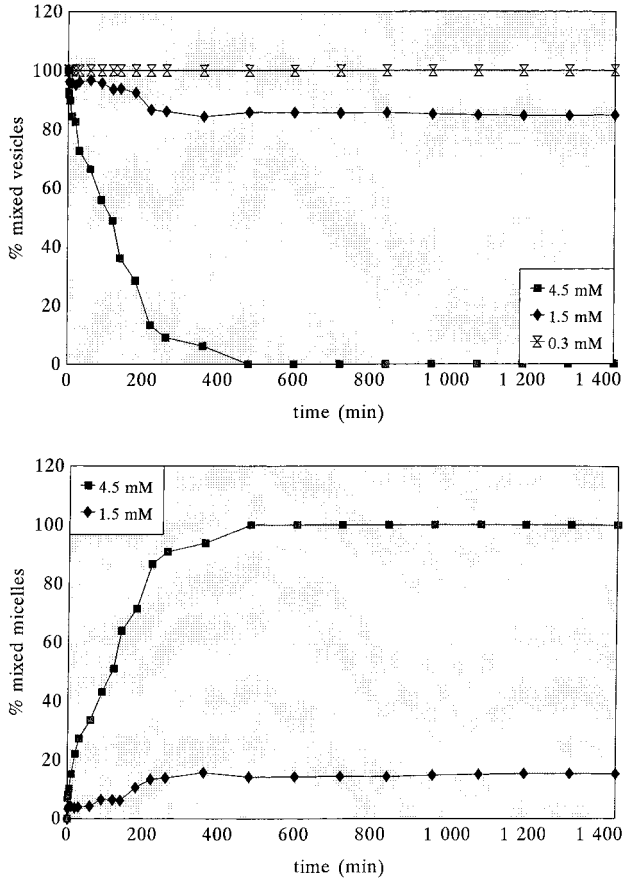

Figure 1. Variation in the percentage of mixed vesicles (A, top) and mixed micelles (B, bottom) versus time (24 h) in the interaction of PC liposomes (0.5 mM PC) with SDS at different concentrations. Surfactant concentrations: (⧖) 0.3 mM; (◆) 1.5 mM; (■) 4.5 mM.

on the SDS concentration and that the equilibration was achieved even if the solubilization of liposomes was not completed (equilibrium mixed vesicles−mixed micelles).

To study in detail the first steps of this interaction, additional DLS experiments were performed at short periods of time (each 30 s) and various SDS concentrations (1.5, 2.5, 3.5, and 4.5 mM, PC concentration 0.5 mM). The data obtained are shown in Table 2. After 30 s only the peak for the large particles (of about 185 nm) was detected for all the SDS concentrations tested. The absence of pure SDS micelles at this short period of time indicated that the adsorption of surfactant into liposomes was almost instantaneous in all cases. This finding raises a question about whether the micellar SDS solutions added to liposomes were adsorbed into these bilayer structures as micelles or as individual monomers, thus breaking the added surfactant micellar structure. Although the present information does not explain this aspect, a surfactant monomeric adsorption could be expected either through the hydrophilic holes created by these monomers on the PC polar heads or via formation of short-lived complexes of surfactants−PC polar heads, in agreement with the studies of Lasic on the phase behavior of membranes.[31] This assumption may be extended to the system for a SDS concentration lower than its cmc (0.3 mM). In this case a monomeric surfactant adsorption into PC bilayers could be expected without growth of the mixed vesicles formed.

(31) Lasic, D. D. *Liposomes: from Physics to Applications*; Elsevier Science Publishers B. V.: Amsterdam, 1993; Chapter 2.

Table 2. Particle Size Distribution Data Corresponding to the Interaction of SDS at 1.5, 2.5, 3.5, and 4.5 mM with PC Liposomes (0.5 mM PC) during the Initial 10 min of the Interaction[a]

		1st peak		2nd peak	
[SDS] (mM)	time (min)	nm	%	nm	%
1.5	0.5			186.4	100
	1.0			185.3	100
	1.5			186.2	100
	2.0	3.4	3.8	184.4	96.2
	4.0	3.2	4.0	185.2	96.0
	8.0	4.1	4.5	185.9	95.5
2.5	0.5			183.7	100
	1.0			184.9	100
	1.5	2.9	5.4	185.2	93.2
	2.0	3.5	6.5	186.1	93.5
	4.0	3.8	7.2	185.3	92.8
	8.0	4.4	7.4	184.8	92.6
3.5	0.5			184.1	100
	1.0	2.5	6.2	183.8	93.8
	1.5	3.2	6.7	184.6	93.3
	2.0	4.2	7.3	184.5	92.7
	4.0	3.5	7.0	183.6	92.0
	8.0	3.8	8.4	180.3	91.6
4.5	0.5			185.6	100
	1.0	2.4	7.1	186.6	92.9
	1.5	3.8	7.7	185.8	92.3
	2.0	4.6	8.1	185.5	91.9
	4.0	4.8	9.7	185.9	90.3
	8.0	5.5	10.2	186.4	89.8

[a] Results are given as diameters, and the percentage corresponds to intensity values.

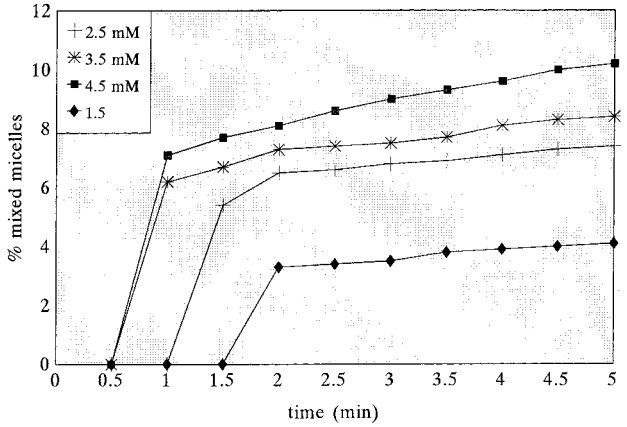

Figure 2. Variation in the percentage of mixed micelles versus time (initial 5 min) in the interaction of PC liposomes (0.5 mM PC) with SDS at different concentrations. Surfactant concentrations: (◆) 1.5 mM; (+) 2.5 mM; (*) 3.5 mM; (■) 4.5 mM.

Furthermore, the time needed by the surfactant adsorbed to form mixed micelles with PC molecules (time of latency) was longer than that needed for surfactant adsorption and inversely dependent on the surfactant concentration (Table 2). Thus, the higher the SDS concentration in the system, the lower the time needed to form mixed micelles. In addition, no growth of mixed vesicle was detected in these initial interaction steps.

Figure 2 shows the percentage of mixed micelles formed versus time for the initial interaction steps (SDS concentration ranging from 1.5 to 4.5 mM, PC concentration 0.5 mM). The formation of mixed micelles was in all cases a biphasic process, in which a rapid micellization process occurred after the time of latency (without mixed micelles) followed by a more slow and almost linear formation of mixed micelles.

In summary, kinetic studies on PC liposome solubilization by means of SDS (at sublytic and lytic concentra-

tions) and based on a DLS technique (Ar laser source) demonstrated that a rapid adsorption of surfactant molecules into the vesicles took place without growth of these bilayer structures. Afterward, the time needed to form mixed micelles was inversely dependent on the surfactant concentration. Although all the surfactant−PC systems investigated reached a dynamic equilibrium (mixed micelles−vesicles or only mixed micelles), the time needed to reach this equilibrium was directly dependent on the SDS concentration in the system. As for the transition from liposomes to mixed micelles, no intermediate aggregates were detected with only a slight variation in the size of both the small and large particles formed.

Acknowledgment. We are grateful to Mr. G. von Knorring for expert technical assistance. This work was supported by funds from DGICYT (Dirección General de Investigación Científica y Técnica) (Prog No. PB94-0043), Spain.

LA980219F