Solubilizing Effects Caused by the Nonionic Surfactant Octyl Glucoside in Phosphatidylcholine Liposomes

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ABSTRACT: The mechanisms governing the interaction of the nonionic surfactant octyl glucoside (OG) on phosphatidylcholine (PC) liposomes were investigated. Permeability alterations were detected as a change in 5(6)-carboxyfluorescein (CF) released from the interior of vesicles, and bilayer solubilization was determined as a decrease in the static light scattered by liposome suspensions. A direct relationship was established in the initial interaction steps (10–50% CF release) between the growth of vesicles, the leakage of entrapped CF, and the effective molar ratio of surfactant to phospholipid in bilayers (Re). This dependence was also detected during the solubilization range of Re values between 1.3 and 3.0, where the decrease in the surfactant-PC aggregate size and in the light scattering of the system depended on the Re parameter and, hence on the composition of these aggregates. The free OG concentration at subsolubilizing and solubilizing levels showed lower and similar, respectively, values than its critical micelle concentration (CMC). These findings indicated that the alterations in bilayer permeability were due to the action of surfactant monomers, whereas bilayer solubilization was determined by the formation of mixed micelles. This finding supports the generally accepted assumption that the concentration of free surfactant must reach the CMC for solubilization to occur. JAOCS 73, 877–883 (1996)

KEY WORDS: Carboxyfluorescein release, critical micelle concentration, mixed micelle formation, octyl glucoside, permeability alterations and bilayer solubilization, phosphatidylcholine liposomes, static light-scattering, surfactant/phospholipid molar ratios.

A number of studies have been devoted to improving our understanding of the principles that govern the interaction of surfactants with simplified membrane models, such as phospholipid bilayers (1–4). This interaction leads to the breakdown of lamellar structures and the formation of lipid–surfactant mixed micelles. A significant contribution has been made by Lichtenberg (5), who postulated that the critical effective surfactant/lipid ratio (Re) that produces saturation and solubilization depends on the surfactant critical micelle concentration (CMC) and on the bilayer/aqueous medium distribution coefficients (K), rather than on the nature of the surfactant. One of the most commonly used surfactants in membrane solubilization and reconstitution is octyl glucoside (OG) because of its reduced denaturing effect on proteins and its relatively high CMC value (6–11).

In earlier papers, we studied some parameters that are implicated in the interaction of different surfactants with liposomes at subsolubilizing and solubilizing concentrations (12–14). In this work, we seek to extend our investigations to correlate some physicochemical properties of surfactant–phosphatidylcholine (PC) aggregates formed during the interaction of OG with PC liposomes with the Re. This information may enhance our understanding of the complex phenomenon involved in the lamellar-to-micelle transitions during solubilization of PC liposome suspensions by this nonionic surfactant.

EXPERIMENTAL PROCEDURES

Materials and methods. PC was purified from egg lecithin (Merck, Darmstadt, Germany) according to the method of Singleton et al. (15), and was shown to be pure by thin-layer chromatography (TLC). The nonionic surfactant OG was purchased from Sigma Chemical Co. (St. Louis, MO). Triton X-100 was purchased from Rohm and Haas (Lyon, France). Piperazine-1,4 bis(2-ethanesulfonic acid) (PIPES) was obtained from Merck. PIPES buffer was prepared as 10 mM PIPES containing 110 mM Na2SO4 and adjusted to pH 7.20 with NaOH. Polycarbonate membranes and membrane holders were purchased from Nucleopore (Pleasanton, CA). The starting material 5(6)-carboxyfluorescein (CF) was obtained from Eastman Kodak (Rochester, NY) and purified by a column-chromatographic method (16).

Unilamellar liposomes of a defined size (about 200 nm) were prepared by extrusion of large unilamellar vesicles obtained previously by reverse-phase evaporation (12). To study the bilayer permeability changes, vesicles containing CF were freed of unencapsulated fluorescent dye by passing through Sephadex G-50 medium resin (Pharmacia, Uppsala, Sweden) by column chromatography (13). The concentration range of phospholipid in liposomes was 1.0–10.0 mM, which was determined by TLC coupled with an automated flame-ioniza-
tation detection system (Iatroscan MK-5; Iatron Lab. Inc., Tokyo, Japan) (17).

The size distribution and the polydispersity index (PI) of liposomes and surfactant-PC aggregates were determined with a photon-correlator spectrometer (Malvern Autosizer 4700c PS/MV; Malvern Co., Malvern, England). The measurements were made by distribution of particle number. Sample concentrations were adjusted to the appropriate range with PIPES buffer, and the measurements were taken at a lecture angle of 90° in water.

The surface tensions of buffered solutions that contained increasing amounts of OG were measured by the ring method (18) with a Krüss processor tensiometer K 12 (Krüss GMBH, Hamburg, Germany). The CMC of the OG (18.0 mM) was determined from the abrupt change in the plot of surface tension vs. surfactant concentration.

To evaluate the alterations caused by OG on lipid bilayers, the effective Re in an aggregate (liposome or micelle) is calculated from the following equation (Ref. 5):

\[
\text{Re} = \frac{(S_T - S_W)}{(PL - PL_{mon})}
\]

where \(PL\) is the phospholipid concentration (mM), \(S_T\) is the total OG concentration (mM), and \(S_W\) is the OG concentration in the aqueous medium (mM). The monomeric \(PL\) concentration \(PL_{mon}\) is negligible due to the low solubility of \(PL\) in water.

The determination of Re and \(S_W\) was carried out on the basis of the linear dependence between the surfactant concentrations required to achieve these parameters and the PL in liposomes, which is described by the equation:

\[
S_T = S_W + \text{Re [PL]}
\]

where Re and \(S_W\) are the slope and the ordinate at the origin (zero PL) of each curve, respectively.

**Permeability alterations and solubilization of liposomes.**

The permeability alterations, caused by OG, were determined by monitoring the increase in fluorescence intensity of the liposome suspensions due to the CF released from the interior of vesicles to the bulk aqueous phase (16). Fluorescence measurements were made with a Shimadzu (Kyoto, Japan) RF-540 spectrofluorophotometer. On excitation at 495 nm, a fluorescence maximum emission of CF was obtained at 515.4 nm. The presence of OG did not cause direct quenching of the aforementioned spectrofluorophotometric CF signal. OG solutions were added to equal volumes of liposome suspensions (lipid concentration ranging from 2.0 to 20.0 mM), and the resulting mixtures were left to equilibrate for 40 min. This interval was chosen as the minimum period of time needed to achieve a constant level of CF release. The experimental determination of this time interval will be shown in the Results and Discussion section. The fluorescence intensity measurements were taken at 25°C. The percentage of CF released was calculated from the following equation (Ref. 13):

\[
\%\text{CF release} = \frac{(I_T - I_0)(I_{mon} - I_0)}{100}
\]

where \(I_T\) is the initial fluorescence intensity of CF-loaded liposome suspension in the absence of OG, \(I_T\) is the fluorescence intensity measured 40 min after adding OG to a liposome suspension, and \(I_{mon}\) corresponds to the fluorescence intensity remaining after the complete destruction of liposomes by the addition of Triton X-100 aqueous solution (Rohm and Haas) (16).

With regard to liposome solubilization, it has been previously demonstrated that static light-scattering constituted a convenient technique for the quantitative study of the bilayer solubilization by surfactants (3, 19, 20). Accordingly, the solubilizing perturbation produced by OG in PC liposomes was monitored by this technique. The overall solubilization can be characterized by two parameters termed \(R_{e SAT}\) and \(R_{e SOL}\), which correspond to the Re ratios at which light-scattering starts to decrease with respect to the original value and shows no further decrease (21). These parameters correspond to the OG/lipid molar ratios at which the surfactant saturates liposomes and leads to a complete solubilization of these structures.

OG solutions were added to equal volumes of liposome suspensions, and the resulting systems were left to equilibrate for 24 h. This time was chosen as the optimum period needed to achieve a complete equilibrium surfactant/liposome for the lipid concentration range used (3, 20). Light-scattering measurements were made in the spectrofluorophotometer at 25°C with both monochromators adjusted to 500 nm. The assays were carried out in triplicate, and the results given are average values.

**RESULTS AND DISCUSSION**

**Mean vesicle size and stability of liposome suspensions.** The vesicle size distribution of liposomes after preparation (\(PL\) ranging from 1.0 to 10.0 mM) varied little (around 200 nm). The PI, defined as a measure of the width of the particle size distribution obtained from the “cumulant analysis,” remained below 0.1, which indicates that all liposome suspensions showed a homogeneous size distribution. The size of vesicles, after addition of equal volumes of PIPES buffer and equilibration for 24 h, showed values similar to those obtained after preparation, with a slight increase in PI (between 0.12 and 0.14). Hence, the liposome preparations appeared to be reasonably stable in the absence of surfactant under the experimental conditions used in solubilization studies.

**Interaction of OG with liposomes.** It is known that complete equilibrium may take several hours in surfactant/lipid systems (3, 5). However, in subsolubilizing interactions, a substantial part of the surfactant effect takes place within approximately 30 min after its addition to the liposomes (22).

To determine the time needed to obtain a constant level of CF release from liposomes in the range of the PC investigated (1.0 and 10.0 mM), a kinetic study of the interaction of OG with liposomes was carried out. Liposome suspensions were...