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Effective detergent/lipid ratios in the solubilization of phosphatidylcholine vesicles by Triton X-100

M. Aránzazu Partearroyo, M. Angeles Urbaneja and Félix M. Goñi

Department of Biochemistry, University of the Basque Country, PO Box 644, 48080 Bilbao, Spain

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Effective detergent:lipid ratios (i.e. molar ratios in the mixed aggregates, vesicles or micelles) have been estimated for the solubilization of phosphatidylcholine vesicles by Triton X-100. Effective molar ratios are given for both the onset and the completion of bilayer solubilization; small unilamellar, large unilamellar and multilamellar vesicles have been used. Effective detergent:lipid ratios are independent of phospholipid concentration, and their use allows a deeper understanding of membrane-surfactant interactions.

Surfactant; Detergent; Membrane solubilization; Liposome; Triton X-100

1. INTRODUCTION

Detergents are widely used tools in membrane biochemistry. Consequently, the interaction between lipid bilayers and surfactants has been the object of considerable attention [1–3]. Triton X-100 has found widespread application in biological research as a non-ionic detergent. Solubilization of phospholipid bilayers by Triton X-100 was first characterized by Dennis et al. (see [2] for a review), who found that a detergent/lipid ratio above 2 was required for solubilization to occur. Previous work from our laboratory has also been devoted to the sub-lytic and lytic effects of Triton X-100 on model membranes [4–7]. Lichtenberg and co-workers [8–10] have established the guidelines for improved quantitative studies on membrane-surfactant interaction by defining the so-called 'effective detergent/lipid ratios' and proposing convenient procedures for their estimation. The effective detergent/lipid ratio, R_e, is defined as the detergent/lipid molar ratio in the mixed aggregates, vesicles or micelles [10]. R_e can be easily calculated by performing solubilization experiments at different lipid concentrations, L, and noting the total detergent concentration producing solubilization, D_T. A relationship exists [10]:

$$D_T = R_e \{L + 1 / [(R_e + 1)]\} \quad (1)$$

where K is the distribution coefficient of Triton X-100 between the vesicles and the aqueous medium. The equation means that the total detergent concentration, D_T, required for obtaining any effective ratio R_e, has a linear dependence on L. R_e is given by the slope of the straight line defined by the equation, which should intercept with the x-axis at $-1/[K(R_e + 1)]$. In addition, the intercept with the y-axis corresponds to the concentration of free detergent in water, D_w, which in turn represents the apparent critical micellar concentration of the surfactant in the presence of lipid.

Following these ideas, we have calculated effective Triton X-100/PC ratios for the solubilization of three commonly used liposome preparations, namely multilamellar vesicles (MLV), small unilamellar vesicles (SUV) and large unilamellar vesicles (LUV). D_T values corresponding to both the onset and the completion of solubilization were recorded, from which saturation and solubilization effective ratios (respectively, R_e^{SAT} and R_e^{SOL}) could be computed.

2. MATERIALS AND METHODS

Triton X-100 (regular, batch No. 63F-0419) was purchased from Sigma (St. Louis, MO) and used without further purification. This detergent exhibits some heterogeneity in the length of the poly oxyethylene moiety, which is supposed to increase its solubilization properties [1]. Egg-yolk phosphatidylcholine was grade 1 from Lipid Products (South Nutfield, England). Liposomes were formed in a 50 mM Tris-HCl, pH 7.0 buffer. SUV were prepared by sonication [4] and LUV by extrusion through 0.1 μm Nucleopore filters [11]. Liposome suspensions were mixed with the same volumes of the appropriate detergent solutions (in the same buffer). The samples were left to equilibrate for 24 h at room temperature, and solubilization was assessed from the changes in light scattering [12]. Light scattering was

Abbreviations: SUV, small unilamellar vesicles; LUV, large unilamellar vesicles; MLV, multilamellar vesicles; R_e, effective ratio, molar ratio of detergent/lipid in the mixed aggregates (vesicles or micelles); R_e^{SAT}, R_e value at which the vesicles are saturated with the detergent; R_e^{SOL}, R_e value at which the solubilization of the lipid is completed; D_w, detergent concentration in the aqueous phase.

Correspondence address: F.M. Goñi, Department of Biochemistry, University of the Basque Country, PO Box 644, 48080 Bilbao, Spain. Fax: (34) (4) 464 8500.

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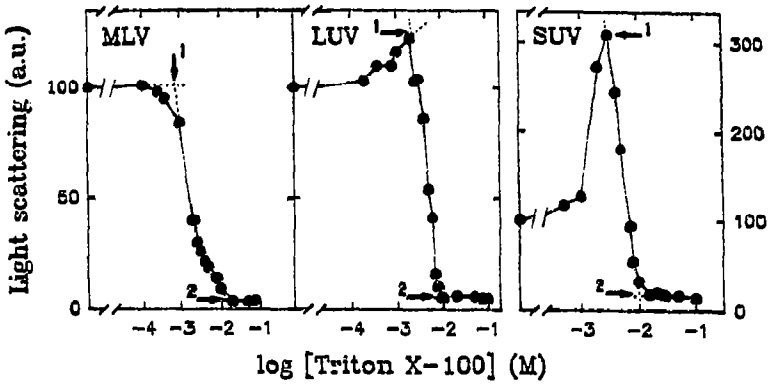

Fig. 1. Determination of D_{on} and D_{100}. The percent change in light scattering of three egg-yolk phosphatidylcholine liposome preparations (3.5 mM) in the presence of various concentrations of Triton X-100. Arrows 1 and 2 correspond, respectively, to D_{on} and D_{w}. Data points are the average of three independent measurements.

measured at 90°C in a Perkin Elmer LS-50 spectrofluorometer, with both monochromators adjusted at 500 nm. Total detergent concentrations producing the onset and the completion of solubilization (D_{on} and D_{100}, respectively) were determined graphically as shown in Fig. 1 for each kind of liposome preparation.

3. RESULTS AND DISCUSSION

When D_{on} and D_{100} are plotted as a function of lipid concentration, the straight lines predicted by equation 1 are found (Fig. 2). From those, the corresponding R_e values are readily calculated (Table I). R_e^{SOL}, i.e. R_e values at which the solubilization of the lipid is completed, are found of around 3–4 Triton X-100 molecules per phospholipid molecule, independently of the type of liposome preparation.

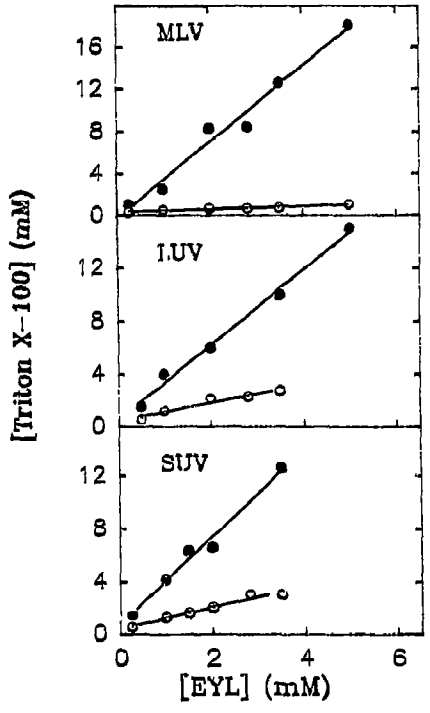

Fig. 2. Determination of R_e values. D_{on} (○) and D_{100} (●) are plotted as a function of lipid (EYL) concentration, for each type of liposome preparation; R_e values are estimated from the slope of the resulting straight lines.

R_e^{SAT} values, i.e. R_e at which the vesicles are saturated with Triton X-100, are higher for unilamellar than for multilamellar vesicles (0.7–0.8 vs. 0.15). R_e^{SAT} values are derived from measurements at the onset of solubilization (Figs. 1 and 2); the low R_e^{SAT} value for MLV may be explained by assuming that the outermost bilayers are solubilized before the system has reached equilibrium. It has been suggested [13] that, in the early stages of MLV solubilization, the outer layers are 'peeled off', giving rise to transient structures in the form of small unilamellar vesicles. Since in our calculations we consider the total amount of lipid, this leads to an apparently low R_e^{SAT} value for MLV. Independent measurements have shown that, in fact, Triton X-100 takes several hours to equilibrate across the various bilayers of MLV, while solubilization of the outer lamellae starts only seconds after surfactant addition [14]. For SUV, the R_e^{SAT} value is in good agreement with the slope of the 'maximum turbidity vs. lipid concentration' plot published by Alonso et al. [15], who found a value of about 0.67; this was expected since the point of maxi-

Table I

Effective detergent/lipid molar ratios and related parameters in the solubilization of phosphatidylcholine vesicles by Triton X-100

	Type of vesicles		
	SUV	LUV	MLV
R_e^{SAT}	0.78	0.71	0.15
D_w (mM)	0.46	0.37	0.29
K (mM^{-1})	1.7	1.9	0.52
R_e^{SOL}	3.7	3.0	3.6
D_w (mM)	0.53	0.45	0.23

Data are derived from plots as shown in Fig. 2.

mum turbidity of a detergent-treated SUV suspension is also the point at which solubilization starts. Also our R_e^{SAT} for SUV is reasonably close to the value found experimentally by Lasch et al. [16], i.e. 1.13. In general, our values for both R_e^{SAT} and R_e^{SOL} are in good agreement with the estimation of Dennis [17], who, after chromatographic and sedimentation studies of Triton X-100:phosphatidylcholine mixtures, found that egg phosphatidylcholine bilayers are able to incorporate the surfactant at molar ratios of Triton to phospholipid below about 1:1, whereas above a molar ratio of about 2:1 all of the phospholipid is converted into mixed micelles.

Values for D_w, free detergent concentrations in water (≈ apparent critical micellar concentrations of Triton X-100 in the presence of lipid) are also shown in Table I. Figures of the same order of magnitude as the critical micellar concentration in pure water (0.24 mM [18]) are found in all cases, in accordance with the theoretical predictions.

Schurtenberger et al. [8] proposed that, for ideal mixing of lipid and detergent, in dilute aqueous media, the distribution of detergent between lipid bilayers and aqueous media obeys a partition coefficient, K, that for low R_e values can be estimated as $K = R_e/D_w$. The corresponding data are also included in Table I, taking R_e as the value at the onset of solubilization.

Effective detergent/lipid ratios for surfactant-induced leakage of phospholipid vesicles have been calculated recently [16,19]. Values of R_e for leakage are lower than R_e^{SAT} (in this paper), as expected from previous studies [20].

The above results are of practical importance in view of the significant deviations that are often found between total and effective detergent:lipid ratios, particularly at low phospholipid concentrations. Previous results in the literature may require a re-appraisal in the light of the present data.

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