Biophysical Chemistry 97 (2002) 1–5

Biophysical Chemistry

www.elsevier.com/locate/bpc

Letter

Quantitative assessment of human erythrocyte membrane solubilization by Triton X-100

P.S.C. Preté, S.V.P. Malheiros, N.C. Meirelles, E. de Paula*

Departamento de Bioquímica, Instituto de Biologia, Universidade Estadual de Campinas (Unicamp), C.P. 6109, CEP 13083-970, Campinas, SP, Brazil

Received 21 November 2001; received in revised form 11 February 2002; accepted 11 February 2002

Abstract

We report here a quantitative analysis of the interaction of the non-ionic surfactant Triton X-100 with human erythrocyte membranes. By applying a classical treatment for the interpretation of the action of surface active compounds to the hemolytic curves, we could calculate parameters such as R_e—the effective surfactant/lipid molar ratio for erythrocyte membrane saturation (R_e^{sat}) and total lysis (R_e^{sol})—and K_b, the binding constant of Triton X-100 to human erythrocyte membranes. The K_b (5900 M^{-1}) and R_e (1.58 and 2.14) values presented here are in good agreement with literature data for Triton X-100 solubilization of model phospholipid membranes.

Keywords: Erythrocyte; Triton X-100; Membrane; Surfactants; Hemolysis; Solubilization

Triton X-100 is the non-ionic surfactant most frequently used in biochemistry, with a wide range of applications to biological systems [1–3]. Solubilization of lipid membranes triggered by Triton X-100 is a well-described phenomenon [4–7] and its hemolytic action has also been studied [8–12], although without an appropriate quantitative approach. The high solubilizing capacity of Triton X-100 is related to its hydrophobic character, as can be evaluated from its *HLB* (13.5) [13] and *cmc* (2.5×10^{-4} M) [14] values. For instance, the lytic potency of Triton X-100 is higher [15] than that of non-ionic detergents belonging to the Renex [16], Tween [17] or C_nE_m series [18], with *HLB* values between 14 and 19.

It is interesting to note that although a large number of studies devoted to the interpretation of the hemolytic phenomena occurring with the use of Triton X-100 as the solubilizing agent have been published [8,9,19–22], none of them presented a straight-forward quantitative analysis of the phenomenon. Only Loizaga et al. [11] reported that a Triton X-100/protein (4.7:1 w/v) was necessary to induce 50% hemolysis of human erythrocytes.

The major objective of the present study was to quantitatively analyze the interaction of Triton X-

*Corresponding author. Tel.: +55-19-3788-6143; fax: +55-19-3788-6129.

E-mail address: depaula@unicamp.br (E. de Paula).

0301-4622/02/$ - see front matter
PII: S0301-4622(02)00043-1

100 with erythrocyte membranes using the approach described by Lichtenberg [23,24].

Fig. 1a presents the hemolytic curves obtained with increasing concentrations of Triton X-100 in erythrocyte suspensions of $Ht = 0.075\%$ under isotonic conditions in PBS buffer, pH 7.4. Arrows indicate the surfactant concentration for the onset of solubilization (C^{sat}) and for 100% (C^{sol}) solubilization. The erythrocyte suspension was prepared from fresh human blood and the hemolytic activity was measured by hemoglobin release into the supernatant, as described by Malheiros et al. [25].

The analysis of membrane solubilization proposed by Lichtenberg [23] was applied, considering C^{sat} and C^{sol} as the surfactant concentration required to induce saturation (the onset of hemolysis) and total membrane solubilization (total lysis), respectively [25].

C^{sat}- and C^{sol}-values for different erythrocyte concentrations (0.075, 0.15, 0.30 and 0.45%; Table 1) were plotted as a function of the lipid concentration in these hematocrits [26] in Fig. 1b. The straight line obtained is predicted by Eq. (1) [4,27]:

$$D_t = R_e\left[L + 1/K_b(R_e + 1)\right] \qquad (1)$$

where D_t is the total surfactant concentration (C^{sat}, C^{sol}) and L is the lipid concentration in the system. The slope of the resulting straight lines allows R_e calculation and the y-intercept corresponds to the concentration of free detergent in water, D_w [23,28]. Finally, K_b (M^{-1}), the molar binding constant of the surfactant to the erythrocyte membrane, could easily be derived, according to Eq. (2) [23,28]:

$$R_e^{sat} = K_b \cdot D_w / (1 - K_b \cdot D_w) \qquad (2)$$

The R_e values obtained seem quite reasonable, considering the physicochemical properties of Triton X-100 (Table 2); the values of 1.58 and 2.15 for the onset of solubilization and for complete solubilization, respectively, are comparable to those obtained by other authors for the solubilization of liposomes.

In 1974, Dennis [29] reported that egg phosphatidylcholine bilayers were able to incorporate Triton X-100 up to a detergent/lipid molar ratio of 1:1; the author also reported that at ratios above 2:1, all the lipids were solubilized in mixed micelles. In a further study, Partearroyo et al. [4] described the solubilization of the same phosphatidylcholine vesicles in terms of the effective detergent/lipid molar ratios in the membrane, and obtained R_e^{sat}- and R_e^{sol}-values of 0.7:1 and 3:1, respectively [4].

The hemolytic process induced by surface-active compounds can be described as a bilayer-to-micelle transition depending on the surfactant/lipid ratio; in intermediate ratios these two types of aggregates are detected. R_e^{sat} and R_e^{sol} determine the limits, in terms of detergent/lipid ratios, for the co-existence of mixed-membranes and mixed-micelles [24].

The concentration of free detergent in water, D_w^{sat} and D_w^{sol}, obtained by the y-axis intercepts of the straight lines in Fig. 1b were smaller (104 and 188 μM) than that pointed by the *cmc*-value, determined by surface-tension measurements (240 μM) [8,19,30,31]. These findings agree with the observations of Lichtenberg et al. [24] that consider D_w values as the *cmc* values in the presence of membranes, since the bilayer would facilitate the micelle formation.

The binding constant (5900 M^{-1}) determined here is higher than that estimated by Partearroyo et al. (1900 M^{-1}) for the binding of Triton X-100 to egg phosphatidylcholine unilamellar vesicles [4], or by Pantaler et al. (1570 M^{-1}) for Triton X-100/human erythrocyte binding [32]. In fact, Triton X-100 binding to erythrocyte membranes is

Table 1
Hemolytic effect of Triton X-100 on human erythrocyte membranes

Ht (%), *L* (μM)	C^{sat} (μM)	C^{sol} (μM)
0.075, 6.5	117	192
0.15, 13	131	222
0.30, 26	169	213
0.45, 39	184	255

L = lipid concentration in erythrocyte membranes corresponding to each hematocrit, calculated according to [26].

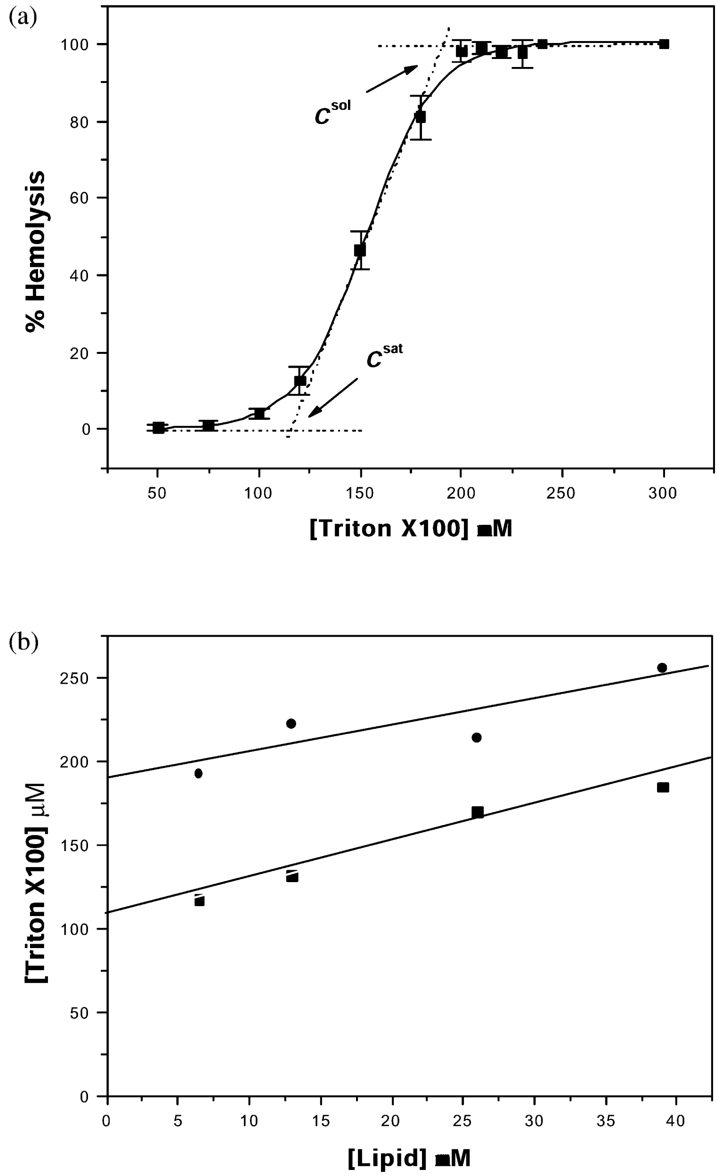

Fig. 1. Hemolytic effect of Triton X-100 on human erythrocytes. (a) $Ht=0.075\%$. (b) C^{sat} (■) and C^{sol} (●) were plotted as a function of erythrocyte lipid concentration—data from Table 1 for $Ht=0.075$, 0.15, 0.30 and 0.45%—and R_e values were calculated from the slope of the straight lines. 5 mM PBS buffer, pH 7.4, after 15 min incubation at 37 °C.

stronger than that observed for many non-ionic and ionic surfactants [33], explaining its hemolytic capacity.

The quantitative description of the lytic effect is of practical importance in the comparison of the solubilizing capacity of a large number of amphiphiles that interact with membranes [15]. The relationship between the aggregative and solubilizing properties of amphiphiles has been well characterized [3,8,19,23] and in recent studies, we have demonstrated that Lichtenberg's treatment could be useful to describe the hemolytic effect of

Table 2
Effective surfactant/lipid molar ratios and related parameters in the hemolysis of erythrocytes by Triton X-100; experimental condition as in Fig. 1

	Triton X-100
R_e^{sat}	1.58
R_e^{sol}	2.15
D_w^{sat} (μM)	104.1
D_w^{sol} (μM)	188.0
K_b ($\times 10^3$ M^{-1})[a]	5.9

[a] Taken from the saturation curves in Fig. 1.

both non-classical [25] and classical surfactants [18].

Acknowledgments

Grants from Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) and Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP, Proc. 01/03632-0) are gratefully acknowledged.

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