Short communication

Studies on the Interactions between Triton® X-100, Phosphatidylcholines and Cholesterol in Mixed Dispersions

B. Lundberg[a] and R. Klemets[b]

[a]Department of Biochemistry and Pharmacy, Åbo Akademi, SF-20500 Åbo, and [b]Wallac Oy, P.O.Box 10, SF-20101 Turku, Finland

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The nonionic detergent Triton® X-100 (TR, Rohm and Haas Co.) has been widely used for delipidation and activation of membrane-bound proteins.[1,2] In mixtures with phospholipids, vesicles and micelles are formed and their formation is responsible for the solubilization of intrinsic membrane proteins.[1] A more precise understanding of the formation and structure of these mixed micelles is important for the effective use of TR in enzymatic studies and protein purification. The interaction between TR and phospholipids has been quite well clarified.[3–5] However, the synergistic effect of cholesterol (CHL), another important membrane lipid, in TR/phospholipid systems has not been studied.

Experimental

The egg phosphatidylcholine (EPC),[6] dimyristoylphosphatidylcholine (DMPC) (Sigma Chemical Co.), and cholesterol (Merck), were judged to be pure by TLC on silica gel plates. Triton X-100, with an average number of 9.5 oxyethylene units, was obtained from BDH Chemicals. The radioactive lipids, phosphatidyl [N-methyl-^{14}C] choline (60 mCi/mmol), phosphatidylcholine, di-[1-^{14}C] palmitoyl (115 mCi/mmol), [7 (n)-^{3}H] cholesterol (9.5 Ci/mmol) and Triton X-100 [phenyl-^{3}H(N)] (1.58 mCi/mg) were purchased from The Radiochemical Centre, Amersham, UK.

Lipid mixtures were prepared by lyophilization from stock solutions and addition of the buffer (10 mM Tris-HCl, 100 mM NaCl, pH 7.4) to give the desired lipid concentration (about 1 mg/ml). The sonication was carried out in thin walled glass ampoules, which were filled with N_2 gas, with a bath type Branson sonifier for 30 min at 25 °C. Lipid systems forming micellar aggregates were prepared by vigorous shaking and then equilibrated for at least 1 day. The radioactivity of [^{14}C] PC, [^{14}C] CHL and [^{3}H] TR was measured by liquid scintillation counting. TR was determined fluorometrically (excitation 270 nm, emission 330 nm).

Gel filtration on a Sepharose CL-4B column (2.6×50 cm) was performed at room temperature with a flow rate of about 0.5 ml/min. The column was pre-equilibrated and run with the standard Tris buffer (above) to which was added 0.3 mM TR. Calibration curves were obtained with standard protein mixtures purchased from Pharmacia Fine Chemicals, Uppsala, Sweden (MW range 669.00–12.500). Calorimetric data were obtained with a Microcal MC-1 differential scanning calorimeter (Microcal, Amherst, MA). Lipid samples of 1–3 mg/ml in the Tris standard buffer were placed into the platinum sample cell with a calibrated microsyringe. The calorimetric scans were obtained under a constant pressure of 3 cm Hg at a heating rate of 20 °C/h.

Dedicated to Professor Per Ekwall on his 90th birthday.

Results

The solubilization of PC–CHL liposomes by TR in the form of mixed micelles was followed by turbidity measurements. The turbidity of the PC dispersions decreased with increasing TR concentrations to a plateau value. This was attained at a TR/PC mol ratio of about 2:1 for EPC and 1:1 for DMPC. When CHL was present in the dispersions at low mol fractions, clear solutions were formed at higher concentrations of TR than for pure PCs. At higher mol fractions of CHL (>0.25), the turbidity did not reach low and constant values even at a TR/PC mol ratio of 10:1. The solubilization was prevented at a lower CHL concentration with DMPC than with EPC. These results clearly show that the solubilization of PC–CHL membrane models is highly dependent on the amount of CHL in the bilayer. This finding is in accordance with the reduced extractability of PCs by TR in the presence of CHL obtained by Ray and Nemethy.[7] The difference in interaction between TR and EPC and DMPC, respectively, may be explained by tighter acyl chain packing of DMPC,[8] which could prevent the penetration of the lipid bilayer by the detergent.

The quantitative aspect of the solubilization process was further examined by measurements of the maximum uptake of CHL in thermodynamically stable EPC/TR systems. The results are given in Fig. 1. Liposomes of EPC can incorporate 1 mol CHL per mol phospholipid in a stable bilayer structure. When the uptake of CHL was studied with EPC/TR systems, a decreased solubilization capacity was noted at increasing mol fractions of TR. The capacity of pure TR micelles to solubilize CHL was about 1 mol CHL per 10 mol of TR. The low CHL solubilizing capacity of TR is in agreement with other detergent systems like bile salts,[9] which also need phospholipids to solubilize CHL.

Column chromatographic results are presented in Fig. 2. Samples of pure EPC and this lipid with low concentrations of TR run in the void volume (V_0) and TR micelles eluted as a symmetric peak at a K_{av} value of 0.56. When the TR/EPC molar ratio in the samples exceeded 1:2, a second peak with a K_{av} value near that of pure TR micelles appeared (Fig. 2a). Peak I near V_0 was caused by large aggregates (liposomes) and peak II by smaller structures (mixed micelles). If the molar ratio of TR/EPC in the bilayers and micelles is calculated, the results (inserted in Fig. 2a) show that the TR/EPC ratios are similar for both bilayers and micelles. When the molar ratio between TR and EPC was increased above 1:1, the bilayer peak decreased and the mixed micelle peak dominated. At a TR/EPC ratio of 4:1, all of the phospholipid was included in the mixed micellar peak (Fig. 2b) and the micellar size was slightly larger than that of pure TR micelles (Stokes' radii 5.5 and 5.1 nm, respectively).

When CHL was included in the samples, it coeluted with the two other components. When the TR/EPC molar ratio was 1:1, the CHL-containing system gave 2 peaks (Fig. 2c), while mixed

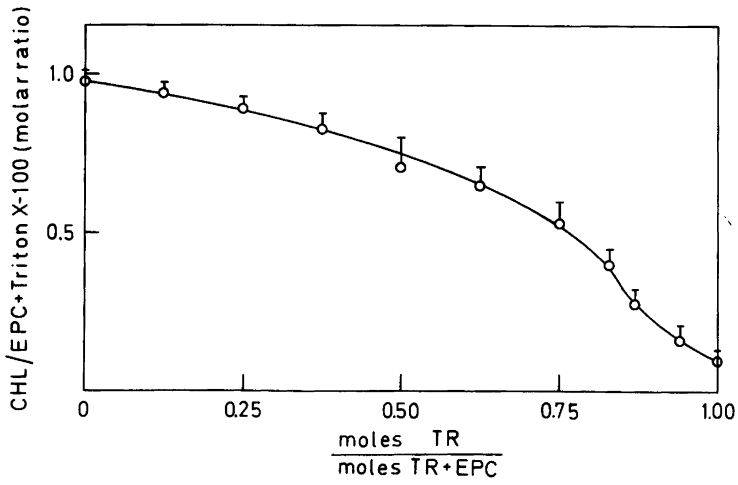

Fig. 1. CHL solubilization limits in the EPC/TR/CHL system, expressed as mol ratio between CHL, and EPC plus TR and plotted as a function of the molar fraction of TR calculated on TR plus EPC.
The concentration of EPC plus TR was 1 mg/ml in all samples. Each point represents the mean ± S.E.M. of 3 measurements.

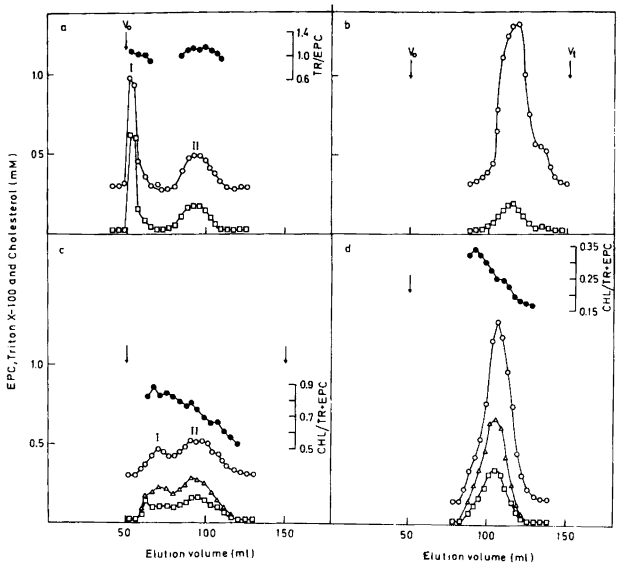

Fig. 2. Gel chromatography of EPC–TR–CHL dispersions. The following samples (1 ml) were applied to the column: (a) 13 mM EPC plus 13 mM TR, (b) 13 mM EPC plus 52 mM TR, (c) 13 mM EPC plus 13 mM TR and 24 mM CHL, (d) 13 mM EPC plus 52 mM TR and 27 mM CHL. V_o-void volume, V_t-total volume of the column. The symbols used are: EPC (□), TR (○), and CHL (△). Inserted in a, c, and d are ratios between components in the peaks.

micelles of TR/EPC (4:1) with incorporated CHL eluted as a single peak (Fig. 2d). The ratio between CHL and the 2 other components decreased with decreasing particle size (see inserts in Fig. 2c and 2d), while the ratios between EPC and TR were fairly constant throughout the micellar peaks. The mixed micelles which contained CHL showed a somewhat larger apparent molar mass than those without CHL. Fig. 3 presents the main chain melting transition of DMPC and this phospholipid containing varying mol ratios of TR and CHL. The chain melting transition temperature for DMPC was 23.9 °C and it remained quite unchanged on addition of both TR and CHL. It is

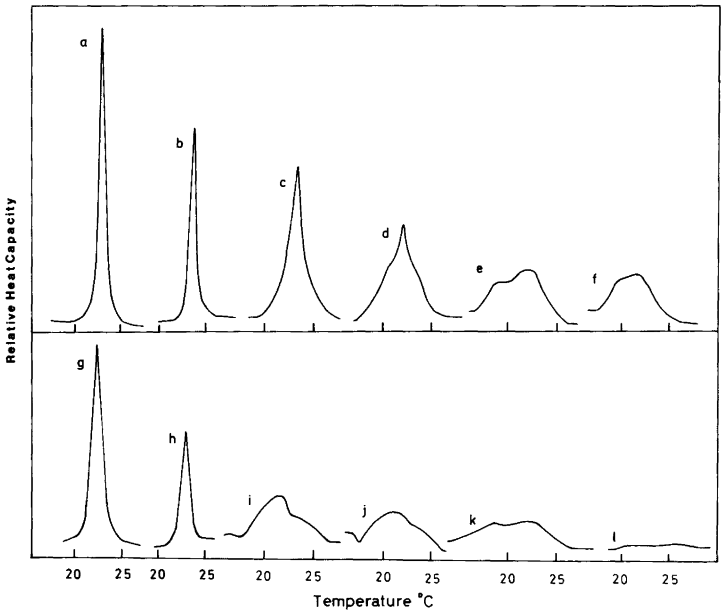

Fig. 3. DSC traces of mixtures of DMPC, TR and CHL in different molar ratios. (a) DMPC, (b) DMPC/TR 20:1, (c) DMPC/TR 10:1, (d) DMPC/TR 6:1, (e) DMPC/TR 4:1, (f) DMPC/TR 2:1, (g) DMPC/CHL 20:1, (h) DMPC/CHL 10:1, (i) DMPC/TR/CHL 20:1:1. (j) DMPC/TR/CHL 20:1:2, (k) DMPC/TR/CHL 20:2:1, (l) DMPC/TR/CHL 20:2:3.

well known that increasing concentrations of CHL broadens the gel to a liquid crystalline phase transition,[11] but the effect of TR has not been described. Addition of TR to the DMPC system gradually broadened the transition (Figs. 3a–f). However, although the transition became very broad, there was no marked reduction in the enthalpy. The broadening effect of TR and CHL on the chain melting transition was additive and CHL decreased the enthalpy of the transition; also in the presence of TR (Figs. 3g–l). The DSC results confirm that TR is in fact accomodated in the phospholipid bilayer. The effect of TR on the gel to liquid crystal transition is similar to that of CHL,[11] which has been found to increase the chain mobility and change the sharp, cooperative gel-liquid crystal transition to a diffuse, uncooperative event.[12]

From this study, it can be deduced that CHL has a profound effect on the interaction between TR and PCs. This fact has to be considered when TR is used for delipidation of biological membranes. Some types of membranes have a high CHL to phospholipid molar ratio (~0.8),[13] and the solubilization by TR might be incomplete or need extremely high concentrations of detergent.

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