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TOPOGRAPHICAL DISSECTION OF SHEEP ERYTHROCYTE MEMBRANE PHOSPHOLIPIDS BY TAUROCHOLATE AND GLYCOCHOLATE

DAVID BILLINGTON, ROGER COLEMAN and YVONNE A. LUSAK

Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2 TT (U.K.)

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Summary

1. Glycocholate and taurocholate removed significant amounts of membrane phospholipid from intact sheep erythrocytes before lysis of the cells occurred. The pre-lytic extract was enriched in sphingomyelin and correspondingly depleted in phosphatidylserine, phosphatidylinositol and phosphatidylethanolamine when compared to the original membrane.

2. In contrast, the phospholipid profiles of glycocholate and taurocholate extracts of unsealed ghosts, made at the same bile salt concentrations, were similar to that of the whole membrane.

3. These observations are related to the topography of the phospholipids in the membrane and to some aspects of bile formation.

Recent studies have shown that the membrane damaging properties of bile salts are in the order deoxycholate > cholate > glycocholate and taurocholate [1,2]. Further, when intact human erythrocytes or pig lymphocytes are incubated with glycocholate, a proportion of the membrane protein and phospholipid is released into the medium prior to the onset of lysis [3,4]. In this study we report the time and concentration dependence of taurocholate and glycocholate to cause lysis of sheep erythrocytes and the phospholipid profile of extracts made at pre-lytic bile salt concentrations. Sheep erythrocytes were chosen because the profile and topography of the membrane phospholipids differs from that of the human erythrocyte in that phosphatidylcholine is almost entirely replaced by sphingomyelin, such that the outer leaflet of the lipid bilayer contains predominantly sphingomyelin [5,6].

Sheep blood was obtained fresh from the local slaughterhouse and collected in glucose-citrate solution (A.C.D). Blood samples were centrifuged at 2500 × *g* for 10 min and the plasma and buffy coat were removed. The packed

cells were washed 3 times in 5 volumes of 0.154 M NaCl 1.5 mM *N*-2-hydroxyethylpiperazine-*N*′2-ethanesulfonic acid (HEPES), pH 7.4 and resuspended in this medium to an approximate concentration of 2 μmol phospholipid per ml (assayed in lipid extracts by the method of King [7]). Sheep erythrocyte ghosts were prepared in 40 imosM HCO_3^-, pH 7.4 containing 1 mM EDTA [8]; such ghosts are essentially haemoglobin free and unsealed. They were finally resuspended in 0.154 M NaCl 1.5 mM HEPES, pH 7.4 to approximately 2 μmol phospholipid per ml. One volume of the erythrocyte or ghost suspension was incubated, at 37°C, with 3 volumes of 0.14 M NaCl 15 mM HEPES, pH 7.4 in the presence of different concentrations of bile salt. Supernatants were obtained by centrifuging at either 14 000 × *g* for 1 min in a microcentrifuge or at 150 000 × *g* for 60 min in a M.S.E. Model 50 ultracentrifuge (see appropriate legends).

The extent of lysis was assayed by comparing the absorbance at 525 nm of 5-fold dilutions (with water) of the supernatants with a 20-fold water dilution of an uncentrifuged control. Lipid extracts [9] of the supernatants were assayed for phospholipid phosphorus basically by the method of Bartlett [10], except that samples were digested with 72% $HClO_4$ [11]. The phospholipid profile of the lipid extracts was determined by quantitative thin-layer chromatography [12].

Glycocholate and taurocholate (Grade A, more than 98% pure) were obtained from Calbiochem Ltd., Hereford, U.K.

The time course of haemolysis of erythrocytes by 4 different concentrations of taurocholate is shown in Fig. 1a. Taurocholate at 1% (w/v) caused little haemolysis whilst with greater concentrations a lag of 5—15 min occurred before the onset of haemolysis. At 1.25% (23 mM) taurocholate, 10—12% of the membrane phospholipid was removed prior to the onset of haemolysis (Fig. 1b). Further studies as to the profile of this phospholipid released were therefore restricted to incubations under essentially non-lytic conditions (i.e. for 10 min in the presence of 1.25% taurocholate). In a similar series of experiments (not shown), erythrocytes incubated for 10 min with 1.1% (23 mM) glycocholate were not lysed and 8—10% of the membrane phospholipid was released into the incubation medium.

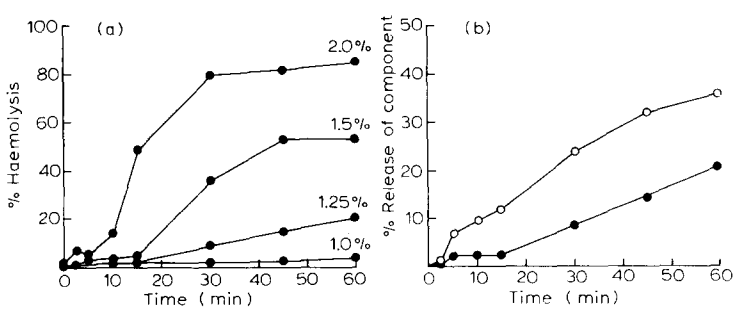

Fig. 1. The time course of haemolysis and phospholipid release from sheep erythrocytes by taurocholate. Intact erythrocytes were incubated in a final volume of 1.5 ml at 37°C in the presence of (a) different concentrations of taurocholate and, (b) 1.25% (w/v) (23 mM) taurocholate. Supernatants were obtained by centrifugation at 14 000 × *g* for 1 min. (●), haemoglobin; (○), phospholipid.

TABLE I

COMPARISON OF THE PHOSPHOLIPID PROFILE OF TAUROCHOLATE AND GLYCOCHOLATE EXTRACTS OF SHEEP ERYTHROCYTES AND GHOSTS WITH THAT OF CONTROL CELLS AND GHOSTS

Intact, washed sheep erythrocytes or ghosts were incubated in a final volume of 8 ml for 10 min at 37°C with either 1.25% (w/v) (23 mM) taurocholate or 1.1% (w/v) (23 mM) glycocholate. Supernatants were obtained by centrifugation at 150 000 × *g* at 4°C for 60 min. The results are mean percentage phospholipid composition ± S.D. with the number of observations in parentheses. The results were statistically assessed using an unpaired *t*-test and the significant differences are designated $^{*}(P < 0.05)$, $^{**}(P < 0.01)$, $^{***}(P < 0.001)$. The mean phospholipid composition of sheep erythrocyte membranes, as determined in 5 other laboratories [14—18], is also presented.

	Whole cells (9) plus ghosts (7)	Taurocholate extract of:		Glycocholate extract of:		Literature values for sheep erythrocyte membranes
		Whole cells (9)	Ghosts (4)	Whole cells (10)	Ghosts (6)	
Origin	0.6 ± 1.4	1.6 ± 3.7	0.6 ± 1.2	1.0 ± 2.2	1.3 ± 1.5	—
Sphingomyelin	48.7 ± 5.2	75.5 ± 11.9***	36.0 ± 3.7*	76.6 ± 6.2***	45.5 ± 9.1	51.4 ± 2.4
Phosphatidylcholine	1.0 ± 1.0	8.8 ± 6.0***	2.5 ± 2.9	3.2 ± 4.5	0	1.6 ± 3.2
Phosphatidylserine + phosphatidylinositol	11.9 ± 3.8	2.9 ± 2.4***	23.1 ± 2.3**	3.9 ± 5.4***	13.1 ± 11.4	12.2 ± 6.2
Phosphatidylethanolamine	34.9 ± 4.0	9.6 ± 7.4***	33.8 ± 1.5	11.4 ± 4.1***	37.5 ± 4.0	30.5 ± 6.4
Phosphatidic acid and solvent front	2.8 ± 1.8	1.5 ± 1.5	4.1 ± 2.0	2.1 ± 3.3	1.0 ± 2.0	2.8 ± 1.9
% Haemolysis	—	2.3 ± 1.4	—	<1.3	—	—
% Phospholipid release	—	12.6 ± 3.8	20.5 ± 3.9	10.3 ± 2.2	9.8 ± 2.4	—

The profile of the phospholipid released from intact sheep erythrocytes by both taurocholate and glycocholate was significantly different from that of the whole membrane; the relative proportions of sphingomyelin and phosphatidylcholine were increased whilst those of phosphatidylserine plus phosphatidylinositol and phosphatidylethanolamine were correspondingly decreased (Table I). Similar incubations with ghosts yielded phospholipid extracts that were similar in their profile to that of the whole membrane (Table I).

These observations can be explained if both the topography of bile salt attack and the asymmetric distribution of phospholipids in the erythrocyte membrane are considered. During incubations with intact erythrocytes the bile salt will only have access to the outer leaflet of the lipid bilayer. Since the outer leaflet of mammalian erythrocyte membranes is largely composed of choline-containing phospholipids [5,13] and sheep erythrocyte membranes contain little (or no) phosphatidylcholine (Table I and refs. 14—18), the outer leaflet of the sheep erythrocyte membrane would be expected to contain predominantly sphingomyelin, explaining both the enrichment of sphingomyelin and the depletion of phosphatidylserine plus phosphatidylinositol and phosphatidylethanolamine in the bile salt extracts. On the other hand, since the preparation of ghosts used was "unsealed", the bile salts would have access to both leaflets of the bilayer, explaining why the profile of phospholipids extracted was essentially similar to the whole membrane. In the case of taurocholate there was a slight enrichment of phosphatidylserine plus phosphatidylinositol and a corresponding depletion of sphingomyelin (Table I) and this may represent a preference of taurocholate for phosphatidylserine and phosphatidylinositol over sphingomyelin for micelle formation.

The phospholipid profile of prelytic glycocholate extracts of human erythrocytes has similarly been shown to resemble that of the outer leaflet of the bilayer of this cell type. However the concentration of glycocholate required to extract approximately 10% of the membrane phospholipid from sheep erythrocytes, without causing cell lysis, was 1.1% compared to 0.6% with human erythrocytes [3]; this may reflect species differences between the cells (e.g. phospholipid composition, surface pressures, size etc.).

It therefore seems that glycocholate and taurocholate are able to remove a proportion of outer leaflet membrane phospholipid without causing sufficient damage to bring about membrane disruption. Recently, Pearlstein and Seaver [19] have similarly demonstrated the release of outward-facing membrane components from intact fibroblasts and BHK cells by non-lytic concentrations of the non-ionic, synthetic detergent Nonidet P40.

Bile is known to contain appreciable quantities of phospholipid [20] and glycoprotein enzymes resembling those located on the outer face of the plasma membrane [21]. The ability of glycocholate and taurocholate to specifically remove phospholipid from the outer face of the sheep erythrocyte membrane supports the proposal [22] that biliary phospholipid is derived from the outer leaflet of the bile canaliculus membrane.

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