PHOSPHOLIPID DISTRIBUTION IN HUMAN En(a−) RED CELL MEMBRANES WHICH LACK THE MAJOR SIALOGLYCOPROTEIN, GLYCOPHORIN A

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1. Introduction

The human erythrocyte membrane has been extensively studied as a general membrane model [1,2]. It provides the best example of phospholipid assymmetry in biological membranes [3]. Phosphatidylcholine (PC) and sphingomyelin (SPH) are mainly present in the outer lipid layer whereas the major part of the amino phospholipids, phosphatidylethanolamine (PE) and phosphatidylserine (PS) reside inside [4]. The mechanism(s) by which the asymmetric distribution is generated and maintained is obscure although evidence is accumulating which suggests an important role for proteins in the stabilization of phospholipid distribution [5,6].

If and how intrinsic membrane proteins do effect phospholipid distribution could be studied using membrane mutants. We have used red cells of the rare blood group variant En(a−) which lack the main red cell sialoglycoprotein A [7–9] to study phospholipid distribution. These data show that the phospholipid distribution is, with respect to the polar head groups, completely normal which implies that glycophorin A does not play a dominating role in maintenance of phospholipid distribution in the human red cell membrane.

2. Materials and methods

En(a−) red cells and control erythrocytes, simultaneously drawn, were obtained through the Red Cross Blood Transfusion Service, Helsinki and analyzed 24 h later in Utrecht. The cells were washed 3 times in a buffer containing 150 mM NaCl, 3 mM sodium azide, 25 mM glucose and 10 mM Tris–HCl (pH 7.4) (STEG) buffer. After the final wash the packed cells were suspended in 3 vol. STEG buffer. Part of the cells (a 0.5 ml suspension) was spun down immediately, lysed in 10 mM EDTA made in water and frozen under N_2. This material was used for phospholipid analysis of whole membranes. Another 0.5 ml of the suspension was incubated in a final volume of 2 ml STEG buffer containing 10 mM $CaCl_2$ and 20 IU phospholipase A_2 from *Naja naja* (Sigma, St Louis) at 37°C on a clinical blood rotator for 2 h. The cells were then washed with STEG buffer containing 10 mM EDTA, lysed in 10 mM EDTA and frozen. A third identical sample was incubated with phospholipase A_2 as above but for 1 h only. Twenty IU of sphingomyelinase (*Staphylococcus aureus*), purified according to [10], were then added and the incubation was continued for another 1 h. Subsequent washings were carried out as above. The lipids were extracted, according to [11], and separated by two-dimensional thin-layer chromatography [12]. Phosphorus was determined as in [13].

3. Results

As reported in [14], the En(a−) membranes have a phospholipid composition which is virtually identical to the composition of control erythrocytes. The result of 3 independent analyses showed that of the total phospholipid content 29.5% was PC, 25.3% was SPH, 25.9% was PE and 12.2% was PS. The residual being lyso-PC 1.8 and minor phospholipids.

Incubation of En(a−) and control erythrocytes with phospholipase A_2 resulted in identical hydrolysis

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Table 1
Degradation of phospholipids in intact erythrocytes by phospholipase A_2 and sphingomyelinase

	Control		En (a−)		Data from [4]	
	PL-ase A_2	SPH-ase	PL-ase A_2	SPH-ase	PL-ase A_2	SPH-ase
Δ PC	67 ± 0.9	78 ± 1.5	64 ± 0.5	78 ± 1.6	68	76
Δ SPH		79 ± 0.9		80 ± 2.0		82
Δ PS		8 ± 0.3		2 ± 0.3		
Δ PE	4 ± 0.7	21 ± 0.7	5 ± 0.8	17 ± 1.4	8	20

Cells, control and En (a−), were incubated with phospholipase A_2 (PL-ase A_2) alone or in combination with sphingomyelinase (SPH-ase) as in section 2. The amount of residual phospholipid was analyzed. The table shows the amount of each lipid class that can be hydrolyzed in the intact cells given in % of the total amount of the respective phospholipid present. The last 2 columns are taken from [4] for comparison. The results are means of 3 determinations ± SD values

patterns. Table 1 summarizes these data and the results in [4]. From the total PC, 67% and 64% was hydrolyzed by phospholipase A_2 in control and En(a−) cells, respectively, which is in good agreement with data published for normal cells. Also similar amounts of PE were hydrolyzed. Subsequent treatment with sphingomyelinase resulted in the hydrolysis of 78% of the PC, 17–21% of the PE, 78–80% of the sphingomyelin and a minimal amount of PS. Again these data confer to those published (see table 1).

We can conclude that, as in normal erythrocytes, the En(a−) erythrocytes have an asymmetric phospholipid distribution: 78% of the PC, 80% of the sphingomyelin and ~20% of the PE is present in the outer layer of the membrane, the residual being inside. In various other aspects En(a−) cells behaved during lipolytic treatment like control cells: no lysis was observed, echinocytes were formed by phospholipase A_2 treatment alone and the combined action of both phospholipases resulted in the formation of stomatocytes.

4. Discussion

Normal human erythrocyte membranes have an asymmetric phospholipid distribution [3,4]. The reason for the generation and maintenance of this asymmetry has, however, remained unclear. One attractive possibility would be that the asymmetry is due to specific intrinsic membrane proteins. The major red cell sialoglycoprotein, glycophorin A, is one of the best characterized intrinsic membrane glycoproteins [1,2]. It contains an intramembraneous hydrophobic amino acid sequence which evidently interacts with membrane lipids. Normal cells contain ~10^6 copies of glycophorin A/cell [15], whereas En(a−) red cells completely lack the protein [9,16]. The availability of this naturally occurring red cell variant gives an excellent possibility to study the influence of glycophorin A on the structure and function of red cell membranes.

Earlier studies have shown alterations in the En(a−) red cell membrane evidently secondary to the absence of glycophorin A. The band 3 protein is overglycosylated [7,17], but its rotational diffusion in the membrane was not changed although it normally seems to be associated with glycophorin A [18]. However, studies using spin-labelled fatty acids showed that the core region of En(a−) membranes was more fluid than that of normal membranes [19]. Furthermore, external labelling with trinitrobenzenesulfonate more efficiently labelled PE of En(a−) cells that that of normal cells [14]. These results indicated a changed phospholipid bilayer.

The phospholipid localization of En(a−) membranes has now been studied in more detail using non-penetrating phospholipases. The results clearly show that the distribution of the major phospholipids is not changed from that of normal cells. This fact clearly rules out an involvement of glycophorin A in this phenomenon.

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