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PROTEIN-STIMULATED EXCHANGE OF PHOSPHATIDYLCHOLINE BETWEEN INTACT ERYTHROCYTES AND VARIOUS MEMBRANE SYSTEMS

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Summary

Phosphatidylcholine specific exchange protein from beef liver was found to catalyze the exchange of phosphatidylcholine between intact rat and human erythrocytes and various artificial membranes. Both multilamellar liposomes and single bilayer vesicles prepared from egg lecithin, cholesterol and phosphatidic acid (46:50:4, mol/mol) appeared to be effective phospholipid donor systems. Some merits and disadvantages of the various donor systems are discussed.

Specific exchange proteins from beef liver catalyzing the transfer of phosphatidylcholine between donor-acceptor pairs have recently been shown to function on intact erythrocytes using microsomes as donor [1], whereas earlier studies with this protein had been successful only with erythrocyte ghosts as phosphatidylcholine acceptor [2, 3]. Methodologically, greater flexibility could be attained with the intact erythrocyte as acceptor system if suitable donors could be employed the phospholipid composition of which was variable. In this regard we have examined the ability of a number of artificial membranes to function in this system. We report below the successful phosphatidylcholine exchange protein-mediated transfer of phosphatidylcholine between intact human and rat erythrocytes and multilamellar or unilamellar vesicles and vesicles prepared by ether vaporization.

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For all experiments the phosphatidylcholine specific exchange protein purified from beef liver according to the method of Kamp and Wirtz [4], using the modification of Wirtz et al. [5], was used. Buffer throughout refers to 10 mM Tris-HCl/150 mM NaCl/1 mM EDTA/25 mM glucose, pH 7.4

Microsomal membranes were prepared from Wistar rats injected intraperitoneally with 30 μCi of [*methyl*-^{14}C]choline [4]. Before incubation microsomes were sonicated for 1—2 min with a Branson sonifier (70 W) under N_2 in ice: 97% of the radioactivity present was found in the phosphatidylcholine In addition, all of the microsomal phosphatidylcholine is available in one pool for rapid exchange [6, 7]. Results on incubation of microsomal membranes (550 nmol phospholipid phosphorus) with intact human erythrocytes (605 nmol phospholipid phosphorus) and exchange protein (6.0 nmol) show a progressive exchange of phosphatidylcholine into erythrocytes, reaching approx. 65% in 4 h. In the absence of exchange protein, hardly any exchange occurs (Fig. 1).

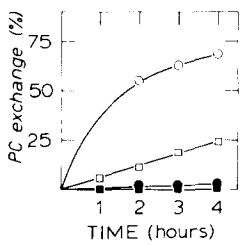

Fig. 1. Exchange of [^{14}C]phosphatidylcholine between rat liver microsomes or vesicles prepared by ether vaporization and human erythrocytes. For each point, ^{14}C-labelled microsomes (see text) containing 550 nmol of phospholipid phosphorus were incubated with 0.15 ml of packed human erythrocytes containing 605 nmol phospholipid phosphorus, in the presence (○) and absence (●) of 6.0 nmol phosphatidylcholine specific exchange protein in a total volume of 2 ml. After incubation, microsomes were separated from the erythrocytes by centrifugation at 2500 × *g* for 10 min, followed by three washes with 10 ml of buffer. The erythrocyte pellet was extracted as described by Rose and Oklander [12]. Phosphate was determined according to the method of Rouser et al. [13]. The radioactivity in cpm of ^{14}C in the extract was determined and the percentage of erythrocyte phosphatidylcholine (PC) which had equilibrated with the microsomal phosphatidylcholine was calculated as described previously [1]. In the case of vesicles prepared by ether vaporization, for each time point, 530 nmol of phosphatidylcholine of vesicles prepared by ether vaporization were incubated with 0.2 ml packed human erythrocytes containing 875 nmol of phospholipid phosphorus. The mixture was incubated in a total volume of 500 μl in the presence (□) or absence (■) of 1.6 nmol of exchange protein. After the incubation, the mixture was diluted with 5 ml of buffer and layered on top of 10 ml of 10% sucrose in buffer. After centrifugation at 2500 × g for 10 min, the erythrocytes were washed twice in the same way. The erythrocytes were then treated as described above.

Vesicles prepared by ether vaporization also serve as suitable donors to intact erythrocytes (Fig. 1). Vesicles prepared by ether vaporization were prepared according to the method of Deamer and Bangham [8] from egg lecithin, phosphatidic acid and cholesterol (46:4:50, mol/mol) using egg [^{14}C]lecithin as a tracer. Lipids dissolved in diethyl ether/methanol (9:1, v/v) at a concentration of 2.5 μmol/ml were injected into buffer as described above. The resultant vesicles prepared by ether vaporization were dialyzed against buffer overnight, centrifuged for 30 min at 35 000 × *g* (Sorvall, SS34 rotor) and resuspended in buffer, affording an overall yield of 65—75%. Incubation of vesicles prepared by ether vaporization (530 nmol) with intact human erythrocytes (875 nmol phospholipid) and exchange protein (1.6 nmol) results in exchange of erythrocyte phosphatidylcholine (Fig. 1) in

creasing linearly with time up to 25% exchange. Again, in the absence of exchange protein, virtually no exchange occurs.

Multilamellar liposomes were prepared from egg lecithin, phosphatidic acid and cholesterol (53:5:42, mol/mol) essentially as described by Bangham et al. [9]; egg [^{14}C]lecithin was added as a tracer. After 1 h at room temperature, they were centrifuged at 20 000 × *g* for 30 min (Sorvall, SS34 rotor). The sedimented liposomes were gently resuspended in buffer; recovery based on determination of ^{14}C radioactivity varied between 90 and 95%. Results using these donors (6 μmol phosphatidylcholine) with intact human erythrocytes (840 nmol total phospholipid) showed that after 4 h there was exchange of 18% of erythrocyte phosphatidylcholine; in the absence of exchange protein, contamination by 0.6% liposomes resulted in radioactivity detection at about one-half of that exchanged.

Unilamellar vesicles were prepared by evaporating with a stream of N_2 a solution containing egg lecithin, phosphatidic acid and cholesterol (47:6:47, mol/mol) and traces of egg [^{14}C]lecithin, vortexing, sonicating for 30 min under N_2 in ice (power setting 70 W) and centrifugation at 100 000 × *g* for 45 min [10]. Vesicle recovery varied from 60 to 75%, as determined by supernatant radioactivity. Incubation of these vesicles (620 or 370 nmol) with exchange protein (1.6 nmol) and with human or rat intact erythrocytes (containing 840 and 790 nmol phospholipids) results in the exchange of phosphatidylcholine in both human and rat erythrocytes (Fig. 2). Exchange is linear in both cases up to 4 h, reaching 25 and 37% exchange for human and rat erythrocytes, respectively. In the absence of exchange protein, no exchange occurs.

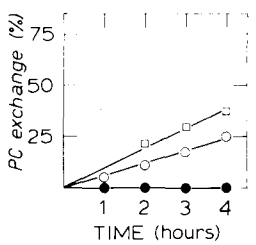

Fig. 2. Exchange of [^{14}C]phosphatidylcholine between sonicated vesicles and erythrocytes. Sonicated vesicles (620 or 370 nmol) were incubated with 0.2 ml packed human (○) or rat (□) erythrocytes, respectively, containing 840 nmol of phospholipid phosphorus. The incubation was performed in the presence (open symbols) or absence (closed symbol (●)) of 1.6 nmol of exchange protein in a total volume of 500 μl. After the incubation the mixture was extracted and analyzed as described in the legend to Fig. 1.

The importance of including cholesterol in the donor system is demonstrated by the determination of net cholesterol extraction from rat erythrocytes incubated with unilamellar vesicles composed of phosphatidylcholine, phosphatidic acid and varying mole fractions of cholesterol (Table I). Incubation with vesicles having 0.47 mol% cholesterol leads to no net loss of cholesterol. In contrast, after 3 h of incubation, those having 0.16 or 0 mol% cholesterol lead to 15 and 23% net cholesterol loss, respectively.

These results indicate that, in addition to microsomes, vesicles prepared by ether vaporization, multilamellar and unilamellar vesicles are all suitable

TABLE I

EFFECT OF DONOR CHOLESTEROL ON ERYTHROCYTE CHOLESTEROL DEPLETION

Incubation of rat erythrocytes (200 μl) in a total volume of 1.0 ml buffer containing phosphatidylcholine exchange protein (4.5 nmol) and unilamellar vesicles composed of 370 nmoles phosphatidylcholine and the indicated mol fractions of cholesterol. Phosphatidic acid (6 mol%) was also included in the vesicles; after 3 h the cells were collected by centrifugation and the lipids extracted and quantitated as described in the legend to Fig. 1. The cholesterol content was determined according to the method of Huang et al. [14].

Vesicles cholesterol (mol fraction)	Erythrocyte cholesterol depleted (%)
0	23
0.16	15
0.47	0

donors for intact erythrocytes in the phosphatidylcholine exchange protein-catalyzed transport of phosphatidylcholine. Each has its own advantages and disadvantages. Thus, vesicles prepared by ether vaporization allow selectable phospholipid composition and exhibit rates of exchange comparable to vesicles. Multilamellar vesicles also can be prepared from a variety of phosphatidylcholines but have ill-defined pools of phosphatidylcholine. Unilamellar vesicles possess all of the above advantages without corresponding disadvantages: though they have two pools of phosphatidylcholine, both are well defined [11].

Finally, it has to be mentioned that some problems may arise from the fact that after exchange and subsequent separation of donor and acceptor membranes, the erythrocytes still contain a small amount of donor material. The extent of this contamination is limited in all cases and does not exceed 3% of the added donor phospholipid. In the case of vesicles and vesicles prepared by ether vaporization the actual contribution of contaminating phosphatidylcholine to the recovery of [^{14}C]phosphatidylcholine from the erythrocytes is small. With multilamellar liposomes this amount is increased approx. 10-fold which is due to the fact that much more lipid has to be added to the incubation system in order to offer the same amount of exchangeable phosphatidylcholine.

Erythrocytes produced by exchange with unilamellar vesicles have native amounts of cholesterol as long as the donor contains phosphatidylcholine and cholesterol in nearly 1:1 amounts (Table I). In addition, other phospholipids, according to preliminary data, are also native in amounts (data not shown). Moreover, experiments carried out for times greater than 4 h show that phosphatidylcholine exchange can proceed to at least 75% of total phosphatidylcholine, making unilamellar vesicles at least as suitable as rat liver microsomes as donors to intact erythrocytes.

The principal advantage of vesicles over microsomes is that the composition of the former can be varied, not only with respect to phosphatidic acid to minimize non-specific ionic interaction, but also with respect to the alkyl side-chains in the 1- and 2-positions of phosphatidylcholine. Further studies employing vesicles of various types of phosphatidylcholine are underway and could provide information on the structure-function correlations of

membrane phosphatidylcholine and on phospholipid asymmetry in intact erythrocyte membranes.

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