PHOSPHATIDYLCHOLINE EXCHANGE BETWEEN BRUSH BORDER MEMBRANE VESICLES AND SONICATED LIPOSOMES

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1. Introduction

The brush border membrane is that part of the plasma membrane of the enterocytes which is most highly specialized in both digestive and transport functions. For a better understanding of its physiological functions a knowledge of the membrane architecture is essential.

Phospholipid exchange activity is present in rat small intestine [1] and the isolation of phospholipid exchange proteins from rat small intestinal mucosa has been reported [2]. Phospholipid exchange has been assumed to play a role in the distribution of newly synthesized phospholipids between subcellular membranes. It may also be important for the process of fat absorption in the intestine [1].

Here we present evidence for the first time that in the presence of phosphatidylcholine exchange protein significant amounts of phosphatidylcholine can be incorporated into brush border membranes from rabbit small intestine when incubated with sonicated phosphatidylcholine liposomes. The extent of lipid transfer observed can be accounted for if it is assumed that practically all of the phosphatidylcholine in brush border membrane is available for exchange as a single pool. Measurements of D-glucose uptake show that the brush border membrane vesicles remained intact and sealed under the conditions of intermembrane phospholipid exchange. Possible interpretations of our results are either that all phosphatidylcholine is located on the outer surface of the membrane and readily accessible to the exchange protein or, that, if some phospholipid is present on the inner membrane surface, it must become available to the exchange protein by a rapid transverse movement of the phospholipid.

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2. Materials and methods

Egg yolk phosphatidylcholine was purchased from Lipid Products, South Nutfield, UK. Phosphatidyl[*N*-methyl-^{14}C]choline, glycerol tri[9,10(*n*)-^{3}H]oleate and D-[1-^{3}H]glucose were obtained from Amersham. All other chemicals were analytical reagent grade.

Brush border membrane vesicles were prepared as in [3]. The buffer used throughout this work was 10 mM Hepes/KOH (pH 7.5) containing 0.3 M D-mannitol and 1 mM EDTA. To test whether or not these membrane vesicles are closed and stable in the presence of sonicated egg phosphatidylcholine liposomes, D-glucose uptake was measured according to [4] in the presence of liposomes. Lipids were extracted from brush border membrane vesicles according to [5]. Phosphatidylcholine exchange protein was prepared from beef liver as in [6] except that the last purification step by gel filtration on Sephadex G-50 was omitted. The exchange protein was purified 710-fold. Single-bilayer liposomes of egg phosphatidylcholine were prepared by sonication [7]. Lipid phosphorus and protein were determined according to [8] and [9], respectively.

3. Results and discussion

When phosphatidylcholine liposomes (composed of [^{14}C]phosphatidylcholine with traces of the non-

exchangeable marker [^{3}H]triolein) were incubated with brush border membrane vesicles in the absence of exchange protein, 1–1.5% of [^{14}C]phosphatidylcholine originally present in the liposomes was transferred to the brush border membrane. Furthermore, the radioactivity ratio ^{14}C/^{3}H found in brush border membranes was identical with that of the original liposomes. Since [^{3}H]trioleate is a non-exchangeable marker [10], these results indicate that no specific transfer of phosphatidylcholine from single-bilayer liposomes to brush border membranes took place. In the presence of the phosphatidylcholine specific exchange protein from beef liver, the incubation of brush border vesicles with liposomes resulted in a marked decrease of the ^{14}C label in the supernatant with a concomitant increase of this label in the pellet, but the level of [^{3}H]trioleate in the supernatant did not change significantly.

From these results we conclude that in the presence of phosphatidylcholine exchange protein, membrane vesicles prepared from microvillus membranes as in [3] are susceptible to phospholipid exchange. Phosphatidylcholine is transferred from sonicated liposomes as the donor to brush border membranes as the acceptor.

Fig.1 illustrates the effect of phosphatidylcholine exchange protein on the transfer of [^{14}C]phosphatidylcholine between liposomes and brush border membranes:

(1) With increasing protein concentration progressively more [^{14}C]phosphatidylcholine was exchanged. At the maximum protein concentration used 24–33% of the [^{14}C]phosphatidylcholine originally present in liposomes was exchanged;
(2) With increasing concentration of acceptor membrane the exchange of phosphatidylcholine also increased.

Assuming that only the molecules on the outer monolayer of sonicated single-bilayer liposomes participate in the exchange process and that no net transfer of phosphatidylcholine from liposomes to brush border membranes occurs, the maximal transfer of [^{14}C]phosphatidylcholine from liposomes to brush border membrane can be calculated. If, for example, half of the total phosphatidylcholine in brush border membrane is assumed to be available for exchange, the maximum transfer is calculated as 15%. This is significantly lower than the experimental value of 23.4% (see lower curve in fig.1 for 1 mg brush border membrane protein/ml at the highest concentration of exchange protein). If all phosphatidylcholine in brush border membrane is available as a single pool, then a value of 24.8% is obtained consistent with the experimental value. This indicates that probably >90% of the total brush border membrane phosphatidylcholine acts in the exchange process as a single pool. We arrive at the same conclusion from calculations carried out for another ratio of donor to accep-

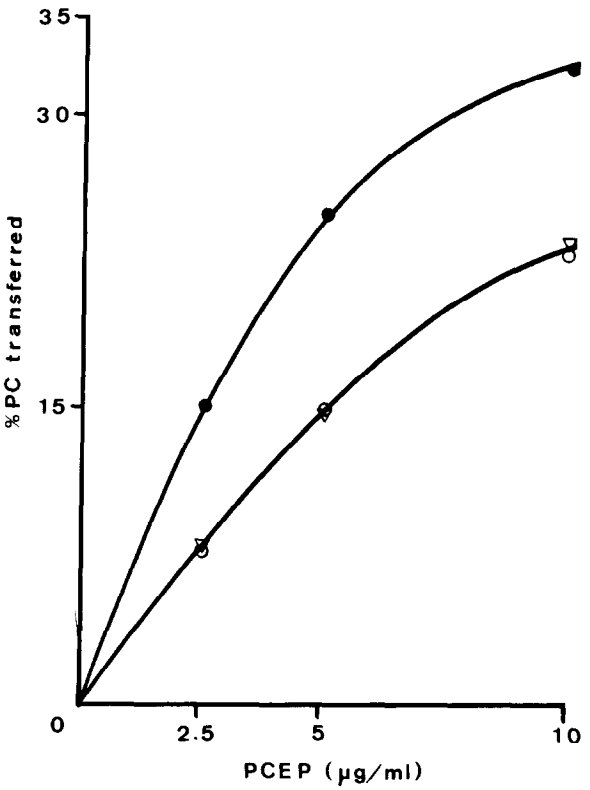

Fig.1. Effect of increasing concentrations of the phosphatidylcholine exchange protein (PCEP) on the transfer of [^{14}C]phosphatidylcholine from single bilayer liposomes to brush border membrane vesicles. The data are expressed as % of [^{14}C]phosphatidylcholine (PC) transferred from liposomes as estimated from either counting the radioactivity in the supernatant (–●–, –○–) or in the pellet (–△–). It should be noticed that both ways of determining the quantity of [^{14}C]phosphatidylcholine exchanged gave consistent results. Brush border membrane vesicles containing 80 nmol phosphatidylcholine/mg protein were dispersed in 1 ml buffer (cf. section 2) at 1 mg protein/ml (–○–; –△–) and 2 mg protein/ml (–●–) and incubated at 20° C for 40 min with liposomes (200 nmol) containing [^{14}C]phosphatidylcholine and a trace of [^{3}H]trioleate as a non-exchangeable marker [10]. The brush border vesicles were separated from liposomes by centrifugation at 27 000 × *g* for 30 min at 5°C.

tor membrane (2 mg membrane protein/ml, see upper curve, fig.1).

These results raise the question of whether under the conditions of the exchange reaction the membrane integrity was impaired. It is now well documented that lipid exchange proteins do neither exert lytic effects on membranes nor do they affect the permeability properties of membranes [11–13]. Brush border membrane vesicles prepared by this procedure [3] or by a related one [4] have been shown to be impermeable to molecules as large as glutathione [14] and metoxyinulin [4,15] and, furthermore, that with the kind of membrane preparation used in this work >95% of the membrane vesicles are sealed and right side out [14]. However, the membrane integrity of brush border vesicles in the presence of liposomes was checked by measuring the ability of the brush border vesicles to take up D-glucose [4]. As evident from table 1, the amount of D-glucose entrapped in brush border vesicles was not significantly affected by the presence of liposomes. The same was true when brush border membrane vesicles were incubated with liposomes in the presence of exchange protein (M. Spiess, unpublished). This suggests that the integrity of the brush border membrane is preserved under the conditions of intermembrane phospholipid exchange and lipid exchange protein has no access to the inner surface of brush border membrane.

The result that most of the phosphatidylcholine present in brush border membrane is exchangeable together with the finding that the membrane integrity is preserved under the experimental conditions used can be interpreted as follows:

(1) Either >90% of phosphatidylcholine is located on the outer surface of the membrane readily accessible to the exchange protein; or
(2) A significant proportion of phosphatidylcholine is distributed over the two halves of the membrane and is involved in rapid transverse movement (flip–flop).

We tend to favour the latter interpretation because:

(1) A wholly asymmetric distribution of phosphatidylcholine does not look very likely;
(2) Preliminary ^{13}p NMR results show that part of the membrane phospholipids is involved in isotropic motion as would be expected from rapid flip–flop. NMR work relating to the structure and dynamics of the phospholipids in brush border membrane vesicles is in progress.

Similar rapid transverse movement has been reported for liver microsomes [12,13,16], but for plasma membranes flip–flop is a relatively slow process [17–19]. The membrane studied in this work is, however, the first epithelial plasma membrane investigated in this respect. Rapid phospholipid flip–flop in brush border plasma membrane could be related to the process of fat absorption

Table 1
D-[^{3}H]Glucose content of brush border vesicles before and after incubation with sonicated egg phosphatidylcholine liposomes

Addition to brush border membrane vesicles	Incubation time (min)	Amount of D-[^{3}H]glucose associated with brush border membrane vesicles	
		cpm	%
None	0	5824	100
None	40	5755	99
Liposomes	0	5562	96
Liposomes	40	5460	94

The D-glucose uptake of brush border membrane vesicles was measured under equilibrium conditions [4]. Vesicles in buffer (1 mg protein/ml) were equilibrated with D-[1-^{3}H]glucose at 20°C for 80 min. The D-glucose associated with the brush border vesicles was determined after 80 and 120 min. In a parallel experiment, sonicated phosphatidylcholine liposomes were added to brush border membrane vesicles pre-equilibrated with D-[1-^{3}H]glucose and the amount of D-glucose associated with the brush border vesicles was measured right after the addition of liposomes (zero time) and after 40 min incubation. Control experiments showed that D-[1-^{3}H]glucose uptake by liposomes was <2% of that measured for brush border vesicles

being a possible mechanism by which phospholipid molecules in the intact form are transported across the membrane. This transport would be supplementary to that assumed to involve phospholipid hydrolysis prior to uptake [20].

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