TRANSFER OF PHOSPHATIDYLINOSITOL FROM MICROSOMES TO LIPOSOMES MEDIATED BY PHOSPHOLIPID TRANSFER PROTEINS

The inability of phosphatidylserine and sphingomyelin to replace phosphatidylcholine in stimulating this process

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1. Introduction

During recent years a number of phospholipid transfer proteins have been isolated from animal and plant tissues. Some of these proteins are highly specific toward a certain class of phospholipids, e.g., PtdCho, whereas other proteins can mediate the transfer of two or more classes of phospholipids [1–3] (reviewed [4,5]).

Two proteins have been isolated from bovine cerebral cortex [6] that both stimulate the transfer of PtdIns and PtdCho. With these two proteins a net transfer of PtdIns from microsomes to PtdCho-liposomes [7] and from phospholipid monolayers to liposomes [8] was demonstrated. We have also shown a net transfer of PtdIns from microsomes and mitochondria to PtdCho-liposomes mediated by rat liver cytosol [9]. All these data, however, do not enable us to decide whether the transfer of PtdIns is or is not accompanied by a concomitant transfer of another phospholipid in the opposite direction. This paper shows that the net transfer of PtdIns from microsomal membranes is stimulated when PtdCho is present in the recipient membrane and that the PtdCho cannot be replaced by PtdSer or sphingomyelin. The preliminary results were presented at the 12th FEBS Meeting [10].

Abbreviations: PtdCho, phosphatidylcholine; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine

2. Materials and methods

Rat liver microsomes and the cytosol were prepared by the conventional procedure [11]. In some experiments the cytosol used was made free of lipoproteins by sedimenting them at pH 5.1 [12]. Phosphatidylinositol-exchange protein from beef brain was purified up to step 4 by the procedure in [6]. Microsomes labeled in PtdIns were obtained by in vitro preincubation with myo-[^{3}H]inositol as in [11]. Liposomes were made by sonication of phospholipid suspension for 1–2 min. PtdCho used for this purpose was extracted from rat liver and purified as in [9]. PtdSer from beef brain was prepared by a combination of the extraction method [13] and column chromatography on DEAE-cellulose [14] as modified [15]. To ensure that all PtdSer was in the acid form, the phospholipid was washed with HCl by the procedure in [16]. Sphingomyelin and tridecylamine were commercial products of Koch-Light Lab. (Colnbrook) and Fluka AG (Buchs SG), respectively.

Transfer of PtdIns was measured as follows. Labeled microsomes were incubated at 37°C with liposomes of various phospholipid composition in the presence of rat liver cytosol or partially purified PtdIns-exchange protein from beef brain in the medium containing 250 mM sucrose, 1 mM EDTA and 2 mM Tris–HCl (pH 7.4) in 2.0 ml total vol. In experiments with the purified protein the incubation was terminated by addition of ice-cold 0.2 M sodium

acetate (pH 5.0) in 250 mM sucrose, followed by sedimenting microsomes at 13 000 X *g* for 15 min [6]. In experiments with rat liver cytosol, the microsomes labeled in PtdIns were pretreated with $CaCl_2$ according to Kamath and Rubin [17] in order to facilitate their separation from liposomes by low speed centrifugation. The resulting supernatant of each sample was used for lipid extraction by the method in [18]. The samples of lipid material were taken for liquid scintillation counting.

Phospholipid phosphorus was determined as in [19]. Protein content was estimated with the biuret reagent after solubilizing the material with deoxycholate, or by the Lowry method as modified [20].

3. Results and discussion

The incubation of microsomes containing radioactive PtdIns with rat liver cytosol led to the redistribution of the labeled phospholipid. The process was time-dependent and fast during first minutes of incubation. The transfer of a considerable part of PtdIns from microsomes to the soluble fraction (fig.1)

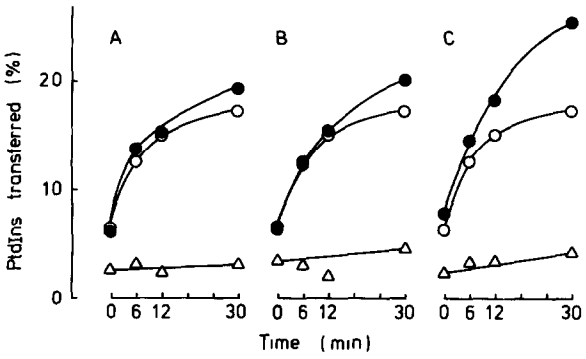

Fig.1. Effect of phospholipid composition of liposomes on the transfer of microsomal phosphatidylinositol mediated by rat liver cytosol.
Ca^{2+}-treated microsomes (8.0 mg protein) containing [^{3}H]-PtdIns were incubated in the presence of rat liver cytosol (16.0 mg protein) and liposomes consisting of phospholipids and tridecylamine, if indicated (0.83 μmol/incubation in total). The composition of liposomes was as follows: (A) PtdSer (100 mol%); (B) PtdSer (60 mol%) plus tridecylamine (40 mol%); (C) PtdSer (60 mol%) plus tridecylamine (20 mol%) plus PtdCho (20 mol%). (•) Complete system; (○) liposomes omitted; (△) cytosol omitted but liposomes present.

was apparently due to a specific binding of this phospholipid to the transfer proteins, since the solubilization of microsomes under similar experimental conditions was found to be negligible [9]. Addition of liposomes made of PtdSer had little, if any, effect on the transfer of PtdIns (fig.1A). Some stimulation of PtdIns transfer might have occurred when liposomes contained tridecylamine (introduced in order to neutralize, at least partly, the negative charge) (fig.1B), but this effect was small in comparison to that observed with liposomes containing PtdCho (fig.1C). In the absence of the cytosol but in the presence of different preparations of liposomes the amount of PtdIns transferred was low and did not change significantly with time.

Since the cytosolic fraction used in the experiments depicted in fig.1 contained several phospholipid exchange proteins [2,21], it seemed important to follow the net transfer of PtdIns using an individual PtdIns-specific transfer protein. The results of such experiments in which a partially purified PtdIns-exchange protein from beef brain [6] was used are presented in table 1. It is evident (expt. 1) that the addition of PtdSer-liposomes did not significantly affect the PtdIns transfer, whereas PtdCho-liposomes did. According to [7,9], the introduction of ≥10 mol% of PtdIns into PtdCho-liposomes had an inhibitory effect on PtdIns transport. By analogy, one could expect an inhibition of PtdIns transport by PtdSer due to negative charge of this phospholipid. In order to decide whether the PtdIns-exchange protein requires liposomes without a net negative charge or, rather, liposomes of a definite phospholipid composition, further experiments were performed with liposomes made of sphingomyelin. As shown in table 1, expt. 2, no significant difference was observed in the amount of PtdIns transferred when labeled microsomes were incubated in the presence of the exchange protein with or without liposomes made entirely of sphingomyelin. By increasing the amount of PtdCho in sphingomyelin-liposomes an increase in the stimulation of PtdIns transfer was observed. Although these results do not exclude the importance of the negative surface charge in preventing PtdIns transfer, they clearly indicate that a specific phospholipid composition of the recipient liposomes is also required. In agreement with this are recent data of Dr R. A. Demel (personal communication) showing

Table 1
Transfer of phosphatidylinositol to liposomes of various phospholipid composition by the phosphatidylinositol-exchange protein from beef brain

Expt. No.	Composition of liposomes added	PtdIns transferred (%)
1.	PtdSer (100 mol%)	0.23
	PtdCho (100 mol%)	0.70
	No liposomes added	0.17
2.	Sphingomyelin (100 mol%)	0.71
	Sphingomyelin (90 mol%) plus PtdCho (10 mol%)	0.94
	Sphingomyelin (70 mol%) plus PtdCho (30 mol%)	1.40
	PtdCho (100 mol%)	2.05
	No liposomes added	0.62

Rat liver microsomes labeled in PtdIns were incubated with PtdIns-exchange protein in the presence of liposomes of various phospholipid composition (1.0 μmol phospholipid phosphorus/sample). The amount of [^{3}H]PtdIns found in the supernatant after sedimentation of microsomes is expressed as a percentage of [^{3}H]PtdIns initially present in microsomes. In expt. 1, the microsomes (5.8 mg protein) and PtdIns-exchange protein (342 μg) were incubated for 40 min; in expt. 2, the microsomes (7.6 mg protein) and PtdIns-exchange protein (557 μg) were incubated for 15 min. In expt. 1 and 2 two separate preparations of the protein were used

that the transfer of PtdIns from a phospholipid monolayer to liposomes does not occur when the latter are made of sphingomyelin alone.

On the basis of the data presented in this paper two explanations can be proposed for the effect of PtdCho in the recipient liposome:

1. PtdCho has to be exchanged for PtdIns in order that the PtdIns can be released by the transport protein;
2. PtdCho is required in the membrane in order that the transport protein can interact with it and deposit PtdIns.

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References

[1] Kamp, H. H., Wirtz, K. W. A. and Van Deenen, L. L. M. (1973) Biochim. Biophys. Acta 318, 313–325.
[2] Bloj, B. and Zilversmit, D. B. (1977) J. Biol. Chem. 252, 1613–1619.
[3] Dyatlovitskaya, E. V., Timofeeva, N. G. and Bergelson, L. D. (1978) Eur. J. Biochem. 82, 463–471.
[4] Zilversmit, D. B. and Hughes, M. E. (1976) in: Methods in Membrane Biology (Korn, E. D. ed) vol. 7, pp. 211–259, Plenum, London, New York.
[5] Kader, J. C. (1977) in: Dynamic Aspects of Cell Surface Organization (Poste, G. and Nicolson, G. L. eds) Cell Surface Reviews, vol. 3, pp. 127–204, Elsevier/North-Holland, Amsterdam, New York.
[6] Helmkamp Jr., G. M., Harvey, M. S., Wirtz, K. W. A. and Van Deenen, L. L. M. (1974) J. Biol. Chem. 249, 6382–6389.
[7] Harvey, M. S., Helmkamp, G. M., jr Wirtz, K. W. A. and Van Deenen, L. L. M. (1974) FEBS Lett. 46, 260–262.
[8] Demel, R. A., Kalsbeek, R., Wirtz, K. W. A. and Van Deenen, L. L. M. (1977) Biochim. Biophys. Acta 466, 10–22.
[9] Zborowski, J. and Wojtczak, L. (1975) FEBS Lett. 51, 317–320.
[10] Zborowski, J. (1978) 12th FEBS Meet., Dresden, abstr. no. 3469.
[11] Strunecká, A. and Zborowski, J. (1975) Comp. Biochem. Physiol. 50 B, 55–60.
[12] Wirtz, K. W. A. and Zilversmit, D. B. (1969) Biochim. Biophys. Acta 193, 105–116.
[13] Folch, J. (1942) J. Biol. Chem. 146, 35–44.
[14] Rouser, G., Bauman, A. J., Kritchevsky, G., Heller, D. and O'Brien, J. S. (1961) J. Am. Oil Chem. Soc. 38, 544–555.
[15] Papahadjopoulos, D. and Miller, N. (1967) Biochim. Biophys. Acta 137, 624–638.
[16] Rathbone, L., Magee, W. L. and Thompson, R. H. S. (1962) Biochem. J, 83, 498–502.
[17] Kamath, S. A. and Rubin, E. (1973) Arch. Biochem. Biophys. 158, 312–322.
[18] Bligh, E. G. and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917.
[19] Rouser, G., Fleischer, S. and Yamamoto, A. (1970) Lipids 5, 494–496.
[20] Hartree, E. F. (1972) Anal. Biochem. 48, 422–427.
[21] Lumb, R. H., Kloosterman, A. D., Wirtz, K. W. A. and Van Deenen, L. L. M. (1976) Eur. J. Biochem. 69, 15–22.