INFLUENCE OF MEMBRANE PHOSPHATIDYLINOSITOL CONTENT ON THE ACTIVITY OF BOVINE BRAIN PHOSPHOLIPID TRANSFER PROTEIN

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1. Introduction

Two proteins have recently been isolated from homogenates of bovine cerebral cortex both of which stimulated the transfer of phosphatidylinositol (PI) and phosphatidylcholine (PC) between artificial and biological membranes [1]. Upon purification these proteins exhibited similar molecular weights and specific activities of phospholipid transfer but differed significantly in isoelectric points and sensitivity toward tryptic digestion. The molecular properties and phospholipid transfer specificity and activity of these brain proteins are distinct from those reported for phospholipid exchange proteins isolated from bovine liver [2] and bovine heart [3]. In the present study the ability of these proteins to catalyze the net transfer of PI into membranes initially deficient in this phospholipid is investigated. Results further suggest that transfers of phospholipids are controlled in part by the composition of the membranes involved.

2. Materials and methods

Phospholipid transfer protein (Protein I) was purified according to Helmkamp et al. [1]. The transfer of phospholipids from rat liver microsomes containing specifically labelled phospholipids to sonicated dispersions of PC containing varying amounts of PI or phosphatidic acid (PA) was assayed as previously described [1,2]. The converse transfer of PI and PC from liposomes containing these specifically labelled phospholipids to microsomes was assayed under similar conditions; in these experiments microsomes were separated from liposomes by centrifugation through a sucrose solution (15%, w/v) at 105 000 *g* for 1 hr and analysed for transferred lipid radioactivity. Phospholipid transfers into the microsomal or liposomal 'acceptor' membranes were corrected for total recovery and contamination by the 'donor' membrane.

3. Results and discussion

The transfer of [^{3}H]PI from microsomes to liposomes containing 2 mole% PI or 2 mole% PA was studied in the presence of increasing amounts of phospholipid transfer protein (fig. 1). It is seen that there is a direct relationship between the amount of PI of microsomal origin transferred to the liposomes and the quantity of transfer protein. Similar results were obtained for the transfer of [^{14}C]PC from microsomes to liposomes. Protein-mediated net, i.e., unidirectional, transfer of PI was apparent in that this phospholipid could be recovered from liposomes which initially contained only PC and PA. However, these liposomes were less effective participants in the phospholipid transfer process than liposomes containing 2 mole% PI, suggesting that the activity of the phospholipid transfer protein is affected by the phospholipid composition of the membrane involved.

With increasing amounts of transfer protein it is seen that there is a fall-off in the expected linear relationship between protein and PI transfer, in contrast to that observed for PC transfer. These results

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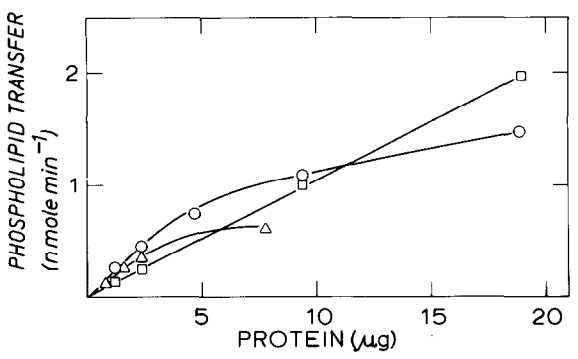

Fig. 1. Transfer of phospholipids from rat liver microsomes to liposomes of various composition as a function of phospholipid transfer protein. Incubations were performed at 25°C for 30 min in 2.5 ml 0.25 M sucrose, 10 mM Tris–HCl, and 1 mM Na_2 EDTA, pH 7.4, in the presence of the indicated quantity of purified Protein I. PI transfer was followed from microsomes (1.25 mg protein, 75 nmoles [^{3}H]PI) to 1 μmole egg PC liposomes containing 2 mole% PI (0–0) or 2 mole% PA (△–△). PC transfer was followed from microsomes (1.0 mg protein, 554 nmoles [^{14}C]PC) to 1 μmole PC liposomes containing 2 mole% PA (□–□).

reflect differences in the initial donor microsomal phospholipid composition, namely 56 nmoles PI and 554 nmoles PC per mg protein, and the more rapid equilibration of the radioactive PI pool. Nevertheless, in comparing the initial rates of PI and PC transfer at low protein concentration, the brain protein demonstrated a marked preference for PI.

The preceding experiments predict an absolute decrease in the microsomal PI pool during the net transfer of this phospholipid to liposomes. In an attempt to show such a depletion of endogenous PI, 1 mg microsomal protein was incubated for 30 min with 10 μmoles PC liposomes containing 2 mole% PA and 17,5 μg transfer protein. Since the transfer protein used in this study does not catalyse the transfer of phosphatidylethanolamine (PE) [1], quantitation of PI was made relative to PE in the recovered donor microsomes. After incubation with transfer protein the PI/PE mass ratio of the microsomes decreased from 0.66 to 0.49, indicating a 25% loss in PI. This result corroborates the above experiment in which a net movement of [^{3}H]PI from microsomes to liposomes was demonstrated.

In order to study in greater detail the effects of liposomal composition and concentration on the rate of PI transfer, liposomes were prepared which initially contained 2,4,8 or 12 mole% PI. At concentrations less than 0.1 μmole liposomal phospholipid per assay, the rate was independent of the liposomal composition (fig. 2). At higher concentrations, however, the rate of PI transfer into 4, 8 and 12 mole% PI liposomes decreased. For these species maximal transfer activity occurred at about 0.2 μmole liposomal phospholipid. Using 2 mole% PI liposomes the transfer exhibited a hyperbolic concentration dependence, showing no decreased activity at the highest level tested (2 μmole per assay). In an analogous series of experiments using the phosphatidylcholine exchange protein from bovine liver and an assay system containing two liposome populations, Hellings et al. [4] have observed similar effects of phospholipid composition on transfer. With increasing PI content of the donor liposomes, less PC was transferred from these liposomes to the acceptor liposomes; PA was also inhibitory, but to a lesser extent. Thus, the effects of specific membrane components, in this case acidic phospholipids, on phospholipid transfer protein activity may be a rather general phenomenon.

The above results describe the movements of PI and PC only in the direction microsome to liposome. Experiments were therefore undertaken to compare simultaneously phospholipid transfer in both directions between the two different membranes at various

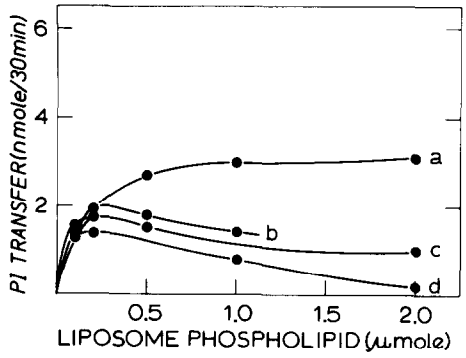

Fig. 2. Transfer of phosphatidylinositol from microsomes to liposomes as a function of liposomal phospholipid composition and concentration. Incubations were carried out as described in fig. 1 and includes 1.17 μg purified Protein I, microsomes (1.36 mg protein, 35 nmoles [^{3}H]PI) and the indicated amount of PC liposomes containing the following levels of PI: a) 2 mole%; b) 4 mole%; c) 8 mole%; and d) 12 mole%.

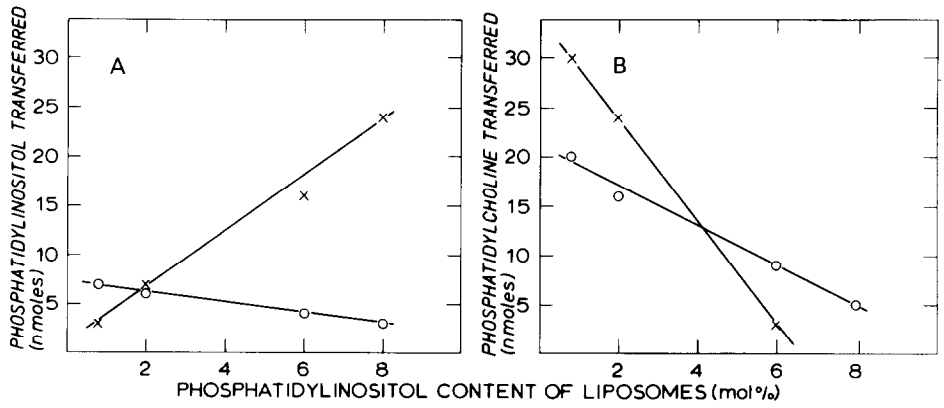

Fig. 3. Effect of liposomal phosphatidylinositol content on transfer of phosphatidylinositol and phosphatidylcholine between microsomes and liposomes. Experimental conditions are described in fig. 1; incubations included 1.75 μg purified Protein I, 1.37 mg microsomal protein and 1 μmole liposomal phospholipid with the indicated content of PI. Transfer of PI is measured from [^{3}H]PI-labelled microsomes to liposomes (o—o) and from [^{3}H]PI-labelled liposomes to microsomes (X—X). Transfer of PC is measured from [^{14}C]PC-labelled microsomes to liposomes (o—o) and from [^{14}C]PC-labelled liposomes to microsomes (X—X).

liposomal PI content. It can be seen from fig. 3A that a direct and linear relationship exists between increasing liposomal PI content and the PI mass transported to the microsomes. Conversely, and in agreement with fig. 2, as liposomal PI increases from 0.8 to 8 mole%, the transfer of this phospholipid from microsomes to liposomes is inhibited. Whether a net transfer of PI occurs to liposomes *or* microsomes is determined by the PI content of the liposomes.

On the other hand, the transfer of PC from microsomes to liposomes and concomitantly from liposomes to microsomes decreases in both instances with increasing liposomal PI (fig. 3B). The intersecting behaviour of the curves shown in fig. 3 indicates net fluxes of PI and PC which differ in not only magnitude but also direction. Thus, for liposomes containing 6 mole% PI there is a net movement of 12 nmoles PI to the microsomes and a net movement of 6 nmoles PC to the liposomes. With these limited data, however, we cannot ascertain whether the transfer of a phospholipid molecule in one direction is necessarily compensated for by the transfer of a second phospholipid molecule in the opposite direction. Nevertheless, these results clearly demonstrate that protein-catalyzed movements of phospholipids into and out of biological and artificial membranes can be directed by the phospholipid composition and concentration of these membranes. Such a control mechanism may be of profound importance in vivo during the metabolism and distribution of phospholipid between sites of de novo synthesis and sites of utilization [5] and during the increased turnover of PI in a variety of exogeneously stimulated tissues [6].

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