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THE PROTEIN-MEDIATED NET TRANSFER OF PHOSPHATIDYLINOSITOL IN MODEL SYSTEMS

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Summary

The phospholipid monolayer technique has been used to study the transfer activity of the phospholipid exchange protein from beef brain. In measuring the transfer between a monolayer consisting of equimolar amounts of phosphatidylcholine and phosphatidylinositol and liposomes consisting of 98 mol% phosphatidylcholine and 2 mol% phosphatidylinositol, the beef brain protein demonstrates an 8-fold higher transfer activity for phosphatidylinositol than for phosphatidylcholine. Under similar conditions the phosphatidylcholine exchange protein from beef liver showed a great preference for phosphatidylcholine. Phosphatidylcholine liposomes devoid of phosphatidylinositol still functioned as receptors of phosphatidylinositol when the beef brain exchange protein was present. This indicates that this protein can catalyse a net transfer of phosphatidylinopsitol. Binding of both phosphatidylinositol and phosphatidylcholine to the beef brain protein was shown.

Introduction

It has been shown that the transfer of phospholipids from one membrane to an other can be mediated by specific proteins [1]. In rat liver most of the subcellular membrane phospholipids are synthesized in the endoplasmic reticulum. These newly synthesized phospholipids are very rapidly transferred to other subcellular membrane fractions such as mitochondria [2]. The exchange of several classes of phospholipids in vitro was shown between natural membranes, particularly mitochondria and microsomes from rat liver.

The crude 105 000 × *g* supernatant fraction from rat liver [3—5] and beef liver [8] mediated the transfer of phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine. With the beef heart [6,7] supernatant fraction the transfer of phosphatidylcholine, phosphatidylinositol, phosphatidylethanol-

amine and sphingomyelin was shown. The purified exchange protein from beef liver showed a highly specific affinity for phosphatidylcholine [8]. To date only an exchange of phosphatidylcholine between two membrane interfaces could be demonstrated and no net transfer. From bovine cerebral cortex two proteins have been isolated which both stimulate the transfer of phosphatidylinositol and phosphatidylcholine [9,10]. With these two proteins a net transfer of phosphatidylinositol from microsomes to phosphatidylcholine liposomes containing phosphatidylinositol or phosphatidic acid was demonstrated. The protein-mediated transfer of phospholipids can be measured continuously under well controlled conditions between a monomolecular film at the air-water interface and vesicles present in the subphase. In a previous study monomolecular layers have been used to demonstrate that the exchange protein from beef liver acts by a carrier mechanism [11]. In this paper the properties of the purified beef brain exchange proteins are studied with respect to the ability to catalyse the transfer of phosphatidylinositol and phosphatidylcholine between a monolayer and vesicles. It will be demonstrated that the protein has a great affinity for phosphatidylinositol.

Materials and Methods

Purification of bovine brain phosphatidylinositol exchange protein

The purification is based on the procedure as described by Helmkamp et al. [10,12]. To obtain larger quantities of exchange protein some changes were introduced.

All purification steps were performed at 0—4°C. (Table I)

(1) Eight bovine brains, stored 1 day at 4°C in the municipal slaughterhouse, were washed with ice-cold 0.32 M sucrose solution. After removal of as much white matter as possible, a 25% homogenate was prepared of the resulting cortical tissue (wet weight 2275 g) in 0.32 M sucrose, 10 mM Tris · HCl, and 1 mM EDTA, pH 7.4, with a Intern. Laborat. (Dottingen) type 40/34 homogenizer in 20 s. Cel debris and mitochondria were removed by centrifugation at 14 000 × *g* for 1 h in a WKF model G 50 K centrifuge.

(2) Ammonium sulphate was slowly added to the post-mitochondrial supernatant till 90% saturation. After stirring overnight the precipitate was collected on a Buchner funnel and dissolved in 1500 ml of 5 mM sodium phosphate buffer pH 7.2. The dispersion was centrifuged for 3 h at 35 000 × *g* in a Beckman ultracentrifuge to remove microsomes. The clear supernatant was dialysed against 2 × 40 l, 5 mM sodium phosphate buffer pH 7.2.

(3) This fraction was applied on a DEAE 52 cellulose column (7 × 46 cm), which was equilibrated with 5 mM sodium phosphate buffer pH 7.2. After an extensive wash with the buffer the column is eluted with 2 × 4 l of a linear gradient of 0—0.3 M NaCl in the same buffer at a flow rate of 180 ml/h. Fractions of 27 ml were collected and assayed for phosphatidylinositol transfer activity, which eluted at approximately 50 mM NaCl.

(4) After dialysis of the active fraction against 5 mM sodium phosphate buffer pH 6.8, it was applied on a hydroxyapatite (Biogel HTP, Biorad) column (2.5 × 10 cm), which was previously equilibrated with the same buffer. The column was eluted with 2 × 2 l of a linear gradient of 10—100 mM sodium

phosphate buffer pH 6.8 at a flow rate of 84 ml/h. 10 ml fractions were collected and assayed for phosphatidylinositol transfer activity. Two activity peaks eluted at 50 and 60 mM sodium phosphate, respectively. These peaks were combined (total vol. 490 ml) dialysed against a 20% sucrose and further concentrated to 55 ml with dry Ficoll (Pharmacia).

(5) The protein was applied to a Sephadex G 75 (fine) column (6.5 × 68 cm) and eluted with a 50 mM sodium phosphate buffer pH 7.4 containing 0.02% NaN_3 at a flow rate of 69 ml/h. Fractions of 12.5 ml were collected and assayed for phosphatidylinositol transfer activity. The active fractions (total vol. 253 ml) were dialysed against 20% sucrose and 1% glycine pH 7.0 and concentrated with dry Ficoll till a volume of 40 ml.

(6) Isoelectric focussing of the protein was performed on a LKB apparatus in a sucrose gradient containing a 2% (w/v) mixture of carrier ampholytes (44% pH 4—6; 33% pH 5—8; 23% pH 3.5—10) at 4°C as described by Vesterberg and Svensson [13]. The voltage was maintained for 15 h at 200 V and 48 h at 520 V. The fractions were tested for phosphatidylinositol transfer activity. The active fractions had an isoelectric point of 5.3 and 5.6, respectively.

(7) The two active fractions were pooled (12 ml) and chromatographed on a Sephadex G 50 column (2.5 × 48 cm) to remove the ampholines. The protein was eluted with 10 mM sodium phosphate buffer pH 6.8, 50 mM NaCl, 10 mM 2-mercaptoethanol, 0.02% NaN_3. The flow rate was 8 ml/h. 2 ml fractions were collected and assayed for phosphatidylinositol transfer activity. The active fraction was mixed with 10% glycerol (v/v) and subsequently dialysed against 65% glycerol. The resulting solution contained 0.45 mg/ml in 50% glycerol and was stored at −20°C.

Phospholipid preparations

Egg phosphatidylcholine was isolated from egg yolk and purified with silicic acid chromatography. 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine was synthesized according to established procedures [14]. A ^{14}C methyl group was introduced into this compound according to the method of Stoffel et al. [15] (specific activity 40 Ci mol^{-1}).

Phosphatidylinositol was prepared by Folch extraction of whole rat liver. The chloroform/methanol layer was washed twice with an upper phase of methanol/water (1 : 1, v/v) containing 2 mM inositol and once with methanol/water (1 : 1, v/v). Phosphatidylinositol was purified by preparative thin-layer chromatography on Silica Gel H (Merck) to which 1 mM Na_2CO_3 was added. The plates were prerun with pentane/petroleumether 40—60 (1 : 1, v/v) and chromatographed with chloroform/methanol/acetic acid/water (25 : 15 : 4 : 2, v/v). Phosphatidylinositol was eluted from the silica in a pasteur pipette with chloroform/methanol/water (50 : 50 : 4, v/v).

Phosphatidyl[^{14}C]inositol was prepared by the combined action of inositol exchange with microsomal phosphatidylinositol [16] and incorporation of *myo*-inositol in de novo synthesized phosphatidylinositol [17]. Microsomes were isolated from the liver of male rats, fasted overnight, in 0.25 M sucrose, 10 mM Tris · HCl pH 7.4 and 1 mM EDTA. After centrifugation at 105 000 × *g* for 90 min, the unwashed microsomal pellet is resuspended in 0.25 M sucrose, 2 mM Tris · HCl pH 7.4. 16.6 mg microsomal protein is incubated in 0.25 M

sucrose; 2 mM Tris · HCl pH 7.4; 1 mM ATP; 0.5 mM CTP; 2 mM NaN_3; 8 mM $MnCl_2$; 5 mM rac-glycerol-3-phosphate; 0.05 mM CoA; 0.1 mM reduced glutathione; 0.08 mM sodium palmitate; 0.016 mM sodium oleate and 5 μCi *myo*-[^{14}C]inositol (Radiochemical Centre, Amersham, U.K.) in a total volume of 3.4 ml for 1 h at 25°C and 1 h at 37°C under nitrogen. After incubation the microsomes were sedimented at 105 000 × *g* for 30 min and resuspended in 10 mM Tris · HCl pH 8.6 and 2 mM inositol. Phosphatidylinositol (specific activity 1 Ci mol^{-1}) was isolated as outlined above.

Interfacial measurements

Monomolecular layers are formed at the air-water interface in a Teflon trough 6 × 4.2 cm wide and 1 cm deep. A 1.5 × 1.5 cm extended corner with a hole of 0.8 cm was made for the injection of protein underneath the monolayer. The trough was filled with 10 mM 10^{-2} M Tris · HCl, 1 mM EDTA (pH 7.4). The subphase (26 ml) was stirred with a magnetic bar. The surface pressure was determined with a recording Beckman LM 500 electrobalance. The surface radioactivity was measured with a gas flow detector (Nuclear, Chicago 8731). The gas used was helium/3% butane, the window, micromil 150 $\mu g/cm^2$, 4.2 × 1.3 cm.

The trough was placed in a thermostated box filled with nitrogen. All experiments were performed at 37°C. Monomolecular films were spread from a chloroform/10% methanol solution from a capillary pipette till the desired interfacial pressure was reached. Subphase samples of 10 ml were collected by sucking through the injection hole into a round bottomed flask. The trough was then refilled and the surface pressure and radioactivity checked in order to establish that no interfacial material was lost during the collection.

Assay systems

Phosphatidylinositol transfer activity was determined using the in vitro transfer of phosphatidyl[^{3}H]inositol from microsomes to liposomes [10]. The microsomes contained 80 nmol of phosphatidylinositol per assay. The protein concentration was determined by following the method of Lowry et al. [18] using bovine serum albumin as standard.

Other preparative and analytical methods

Polyacrylamide-gel electrophoresis was carried out on 7% acrylamide gels at pH 8.6 according to Davis [19]. Samples in 50% glycerol were applied to the gels. The buffer front was indicated with bromophenol blue. Gels were stained with Coomassie brilliant blue.

Radioactivity was counted in 12 ml of toluene containing 0.5% 2,5-diphenyloxazole and 0.03% 1.4 bis-[2-(5-phenyloxazolyl)] benzene, in a Packard Tricarb liquid scintillation spectrometer and was corrected to 100% relative efficiency by the channel ratio method.

Liposomes were prepared by a 3-min sonication of a hand-shaken dispersion of phospholipids. Liposomes containing phosphatidylcholine and 2 mol% phosphatidylinositol gave a clear suspension. A liposomal suspension of only phosphatidylcholine was still turbid. Single bilayer vesicles of phosphatidylcholine were prepared as described by de Kruyff et al. [20].

Results

Protein purification (see Table I)

The purification described in Materials and Methods is a combination of the steps as described by Helmkamp et al. [10,12]. A modification was introduced in step 2 where ammonium sulphate precipitation, followed by filtration was used to avoid centrifugation and dialysis of relatively large volumes. Fractionation of the exchange protein on hydroxylapatite (step 4) resulted in two peaks with phosphatidylinositol exchange activity at 50 and 60 mM sodium phosphate, respectively. These values correspond with those given by Helmkamp [12] for protein I and protein II. Since little difference is found between protein I and protein II in their immunological, kinetic, specificity and specific activity properties both activities were pooled [10,12], also in the subsequent steps. Polyacrylamide gel electrophoresis of the final material showed two bands of equal intensity (Fig. 1). This appearance corresponds with the difference in isoelectric points (5.3 and 5.6, respectively) and indicates that the proteins are purified to homogeneity. The specific activity is 150 nmol · min^{-1} · mg^{-1}.

Comparison of the specificities of the exchange proteins isolated from beef liver and beef brain

It has been shown that the phosphatidylcholine exchange protein from beef liver is highly specific for phosphatidylcholine. No transfer of phosphatidylethanolamine, phosphatidylserine, phosphatidic acid or lysophosphatidylcholine from microsomes to liposomes was observed and only very low transfer of phosphatidylinositol [21]. Chemical modifications of the polar head group of phosphatidylcholine also reduces the interaction with the exchange protein sharply [22]. Monolayer studies where the transfer of ^{14}C-labelled lipid from the air-water interface to phosphatidylcholine/4% phosphatidic acid liposomes was studied confirmed the specificity [11]. With monolayers it was also shown

TABLE I

PURIFICATION OF PHOSPHATIDYLINOSITOL EXCHANGE PROTEIN FROM BOVINE BRAIN

Step	Volume (ml)	Protein (mg)	Activity (nmol · min^{-1})	Recovery (%)	Specific activity * (nmol · min^{-1} · mg^{-1})	Purification factor
1. Lowspeed centrifugation	5510	33515	9285	100	0.27	1
2. $(NH_4)_2SO_4$ precipitation	1420	30198	11397	100	0.41	1.3
3. DEAE cellulose	990	905	8130	87	8.9	33
4. Hydroxyl apatite	490	119	2613	28	21.8	81
5. Sephadex G75	253	18	1888	20	106.7	395
6. Isoelectric focusing	12		984	10.5		
7. Sephadex G50	14.3	6.4	960	10.3	149.3	553

* The specific activity was determined at 25° C and indicates the nmol of phosphatidylinositol transferred from the microsomes to the liposomes.

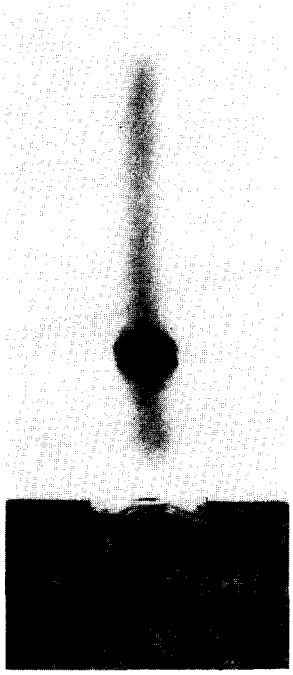

Fig. 1. Electrophoresis of phospholipid exchange protein in pH 8.6, 7% polyacrylamide gel.

that only exchange of phosphatidylcholine was apparent and no net transport since both monolayer and liposome had to contain phosphatidylcholine. For example the phosphatidylcholine exchange protein did not transport ^{14}C label from a ^{14}C-labelled palmitoyloleoyl phosphatidylcholine monolayer to sphingomyelin/4% phosphatidic acid liposomes [11]. The protein isolated from beef brain cortex stimulates preferentially the transfer of phosphatidylinositol from microsomes to mitochondria or liposomes but also of phosphatidylcholine [10]. It is however conceivable that the various phospholipids present in the microsomal membrane are not homogeneously distributed, making some phospholipids less accessible to the exchange protein. Therefore, the transfer activities of the protein from beef liver and brain have been determined with monomolecular films consisting of phosphatidylinositol and phosphatidylcholine.

For the beef liver phosphatidylcholine exchange protein a surface activity of 12.5 dynes/cm was found [11]. The exchange protein isolated from beef brain showed a higher surface activity and gave surface pressures up to 20 dynes/cm. Therefore, the monolayer experiments were performed at pressures above the collaps pressure of the pure protein. Monomolecular films with a surface pressure of approximately 30 dynes/cm are formed of an equimolar mixture of phosphatidylinositol and phosphatidylcholine where either the phosphatidylinositol or the phosphatidylcholine was ^{14}C-labelled. Liposomes are formed of egg phosphatidylcholine containing 2% phosphatidylinositol. The injection of the liposomes under the monolayer hardly affected the film pressure (Fig. 2). Injection of phosphatidylcholine exchange protein from beef liver also did not affect the surface pressure (Fig. 1). The surface radioactivity however is sharply

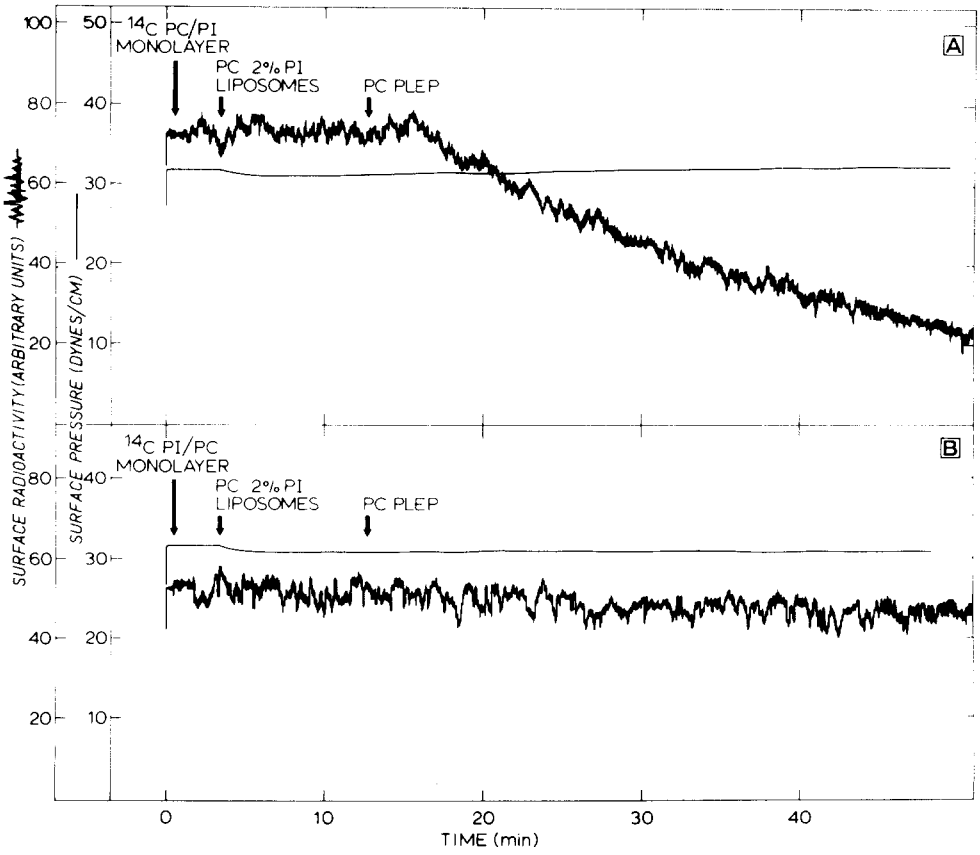

Fig. 2. (A) Exchange of phosphatidylcholine (PC) between monolayer and liposomes. The monolayer consisted of a mixed monolayer of 6 nmol 16 : 0/18 : 1 phosphatidyl[*Me*-^{14}C]choline and rat liver microsomal phosphatidylinositol (PI), molar ratio 1 : 1. The liposomes consisting of egg yolk phosphatidylcholine and 2% phosphatidylinositol. The subphase contained 1 μmol liposomal phosphatidylcholine and 1.25 nmol exchange protein isolated from beef liver in a 10 mM Tris · HCl buffer, 1 mM EDTA (pH 7.4). (B) Exchange of phosphatidylinositol between monolayer and liposomes. The procedure was similar to that described in (A), except that phosphatidylinositol was ^{14}C-labelled instead of phosphatidylcholine.

reduced when the phosphatidylcholine is labelled (Fig. 2A). The rate of surface radioactivity decrease is 3% min^{-1} which is in agreement with previous experiments [11]. In the mixed monolayer, containing [^{14}C]phosphatidylinositol there is a very small reduction in surface radioactivity after injection of the exchange protein from beef liver (Fig. 2B). The rate is less than 0.25% min^{-1}. In a similar system as described in Fig. 2 the activity of the beef brain exchange protein is tested. The injection of this protein underneath a mixed monolayer of phosphatidylcholine and phosphatidylinositol causes an increase in surface pressure of 2—3 dynes/cm. When phosphatidylcholine is ^{14}C-labelled the decrease in surface radioactivity is limited to 0.64% min^{-1} (Fig. 3A). The decrease in surface radioactivity is more pronounced after injection of beef exchange protein underneath a mixed monolayer where phosphatidylinositol is ^{14}C-labelled (Fig. 3B). The rate of surface radioactivity decrease amounts to 5.0% min^{-1}. When the transfer of [^{14}C]phosphatidylinositol is studied from a

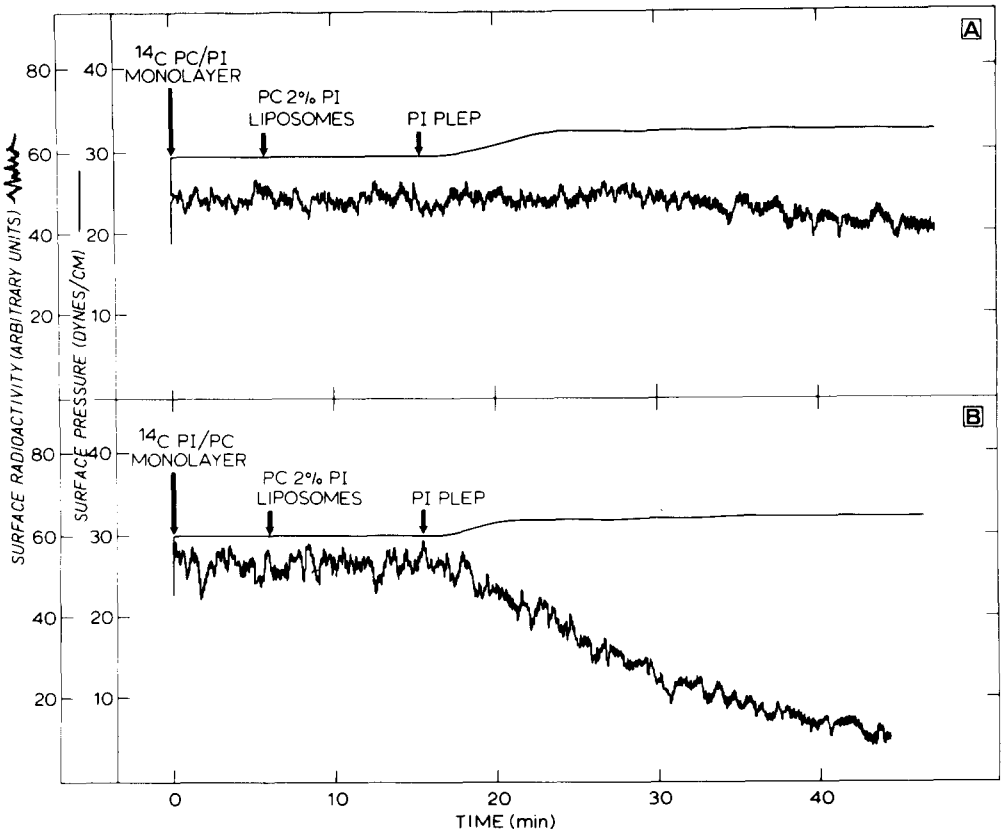

Fig. 3. (A) Exchange of phosphatidylcholine (PC) between monolayer and liposomes. The monolayer consisted of a mixed monolayer of 6 nmol 16 : 0/18 : 1 phosphatidyl[*Me*-^{14}C]choline and rat liver microsomal phosphatidylinositol (PI), molar ratio 1 : 1. The liposomes consisting of egg yolk phosphatidylcholine and 2% phosphatidylinositol. The subphase contained 1 μmol liposomal phosphatidylcholine and 6 nmol exchange protein isolated from beef brain in a 10 mM Tris · HCl buffer, 1 mM EDTA (pH 7.4). (B) Exchange of phosphatidylinositol between monolayer and liposomes. The procedure was similar to that described in Fig. (A), except that phosphatidylinositol was ^{14}C-labelled instead of phosphatidylcholine.

pure phosphatidylinositol monolayer to phosphatidylcholine/2% phosphatidylinositol liposomes after injection of the beef brain exchange protein, the rate of surface radioactivity decrease is reduced to 2.2% min^{-1}. However, no decrease in surface radioactivity of a [^{14}C]phosphatidylinositol monolayer can be observed under the influence of the beef brain exchange protein, when pure phosphatidylinositol liposomes are used.

Exchange experiments between microsomes and liposomes have shown that a net transfer of phosphatidylinositol to phosphatidylcholine liposomes containing phosphatidylinositol or phosphatidic acid is feasible [9]. With the monolayer technique it can be determined whether phosphatidylinositol is transferred to an uncharged interface. For this reason the protein-mediated transfer of phosphatidylinositol from the air-water interface to pure phosphatidylcholine membranes was studied (Fig. 4). When underneath a [^{14}C]phosphatidylinositol monolayer, beef brain exchange protein is injected in the

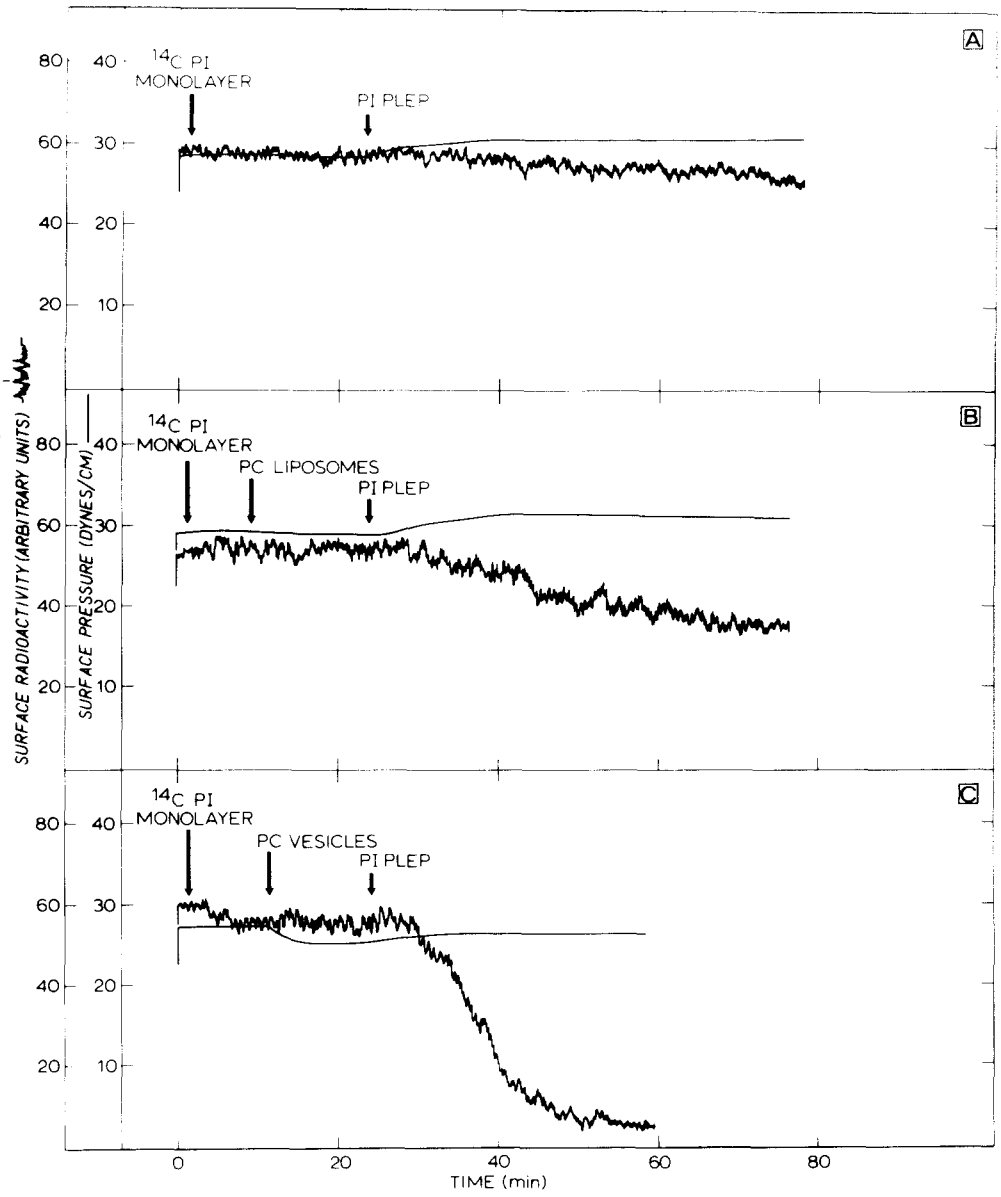

Fig. 4. (A) Exchange of phosphatidylinositol (PI) between monolayer and protein. The monolayer consisted of 6 nmol ^{14}C-labelled rat liver microsomal phosphatidylinositol. The subphase contained 6 nmol exchange protein isolated from beef brain in a 10 mM Tris · HCl buffer, 1 mM EDTA (pH 7.4). (B) Exchange of phosphatidylinositol between monolayer and liposomes. The monolayer consisted of 6 nmol ^{14}C-labelled rat liver microsomal phosphatidylinositol. The liposomes consisted of egg yolk phosphatidylcholine. The subphase containe 1 μmol liposomal phosphatidylcholine and 6 nmol exchange protein isolated from beef brain in a 10 mM Tris · HCl buffer, 1 mM EDTA (pH 7.4). (C) Exchange of phosphatidylinositol between monolayer and vesicles. The procedure was similar to that described in (B) except that phosphatidylcholine vesicles were used.

absence of liposomes, a surface radioactivity decrease with 0.25% min^{-1} and a concomitant increase of the surface pressure with 2.2 dynes cm^{-1} is observed (Fig. 4A).

When in a similar experiment the decrease in surface radioactivity is measured from a [^{14}C]phosphatidylinositol monolayer after injection of beef brain exchange protein, but now in the presence of phosphatidylcholine liposomes, a rate of 1% min^{-1} is found (Fig. 4B). Again a slight increase in surface pressure is observed. The transfer of phosphatidylinositol from the air-water interface to membranes in the subphase can be greatly increased when single bilayer phosphatidylcholine vesicles are used instead of liposomes (Fig. 4C). When phosphatidylcholine vesicles are injected underneath a phosphatidylinositol monolayer a decrease in surface pressure of 2 dynes/cm is observed. This decrease is partly compensated when the beef brain exchange protein is injected. The surface radioactivity decreases very drastically with a rate of 6.5% min^{-1}.

The increase in surface pressure of approximately 2 dynes/cm which is observed after injection of beef brain exchange protein underneath a phosphatidylinositol or mixed phosphatidylinositol/phosphatidylcholine monolayer is also observed for phosphatidylcholine monolayers with an initial surface pressure of 30 dynes/cm.

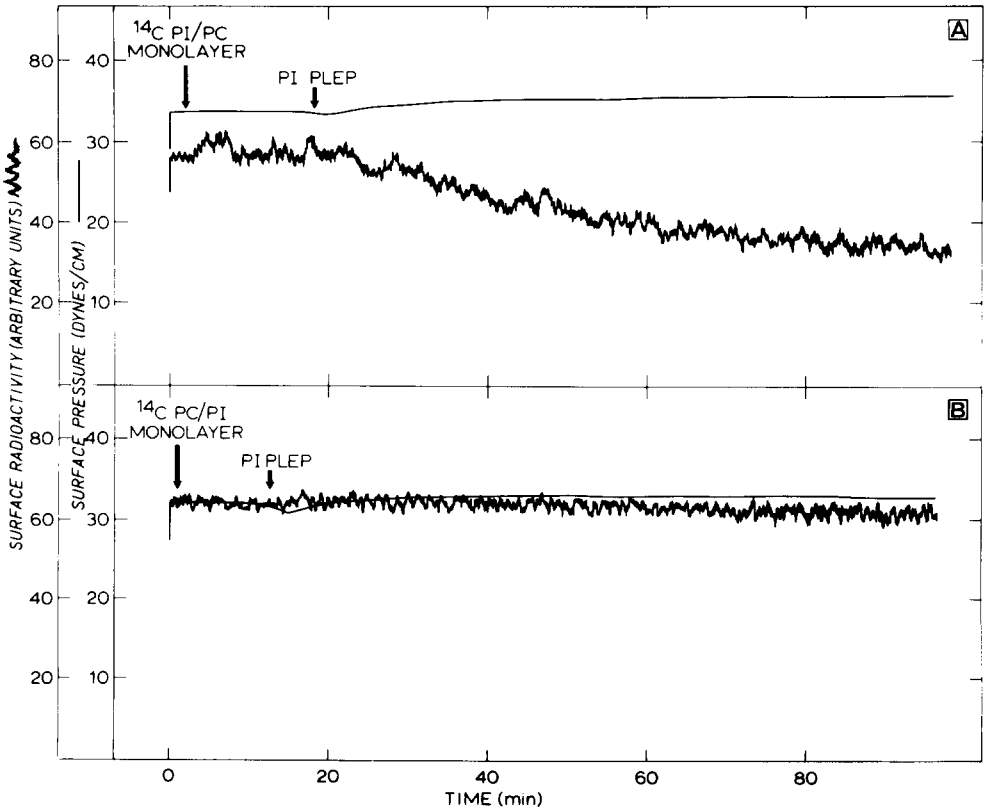

Fig. 5. (A) Binding of phosphatidylinositol from the monolayer by beef brain exchange protein. The monolayer consisted of a mixed monolayer of 6 nmol *myo*-[^{14}C]inositol-labelled phosphatidylinositol (PI) and palmitoyloleoyl/phosphatidylcholine molar ratio 1 : 1. The subphase contained 15 nmol exchange protein isolated from beef brain in a 10 mM Tris · HCl buffer, 1 mM EDTA (pH 7.4). (B) Binding of phosphatidylcholine from the monolayer by beef brain exchange protein. The procedure was similar to that described in (A) except that phosphatidylcholine was ^{14}C-labelled instead of phosphatidylinositol.

TABLE II

BINDING OF [^{14}C]PHOSPHATIDYLCHOLINE OR PHOSPHATIDYLINOSITOL BY THE EXCHANGE PROTEIN FROM BEEF BRAIN

Monolayer	Protein injected (nmol)	Radioactivity found in subphase (%)	Radioactivity bound by protein (%)
[^{14}C] Phosphatidylinositol/phosphatidylcholine	—	4.8	
[^{14}C] Phosphatidylinositol/phosphatidylcholine	15	49.8	45.0
Phosphatidylinositol/[^{14}C] phosphatidylcholine	—	1.1	
Phosphatidylinositol/[^{14}C] phosphatidylcholine	15	4.9	3.8

The binding of phosphatidylinositol and phosphatidylcholine by beef brain exchange protein

The binding of phosphatidyl[^{14}C]choline to the beef brain exchange protein was demonstrated upon incubation of the protein with [^{14}C]phosphatidylcholine liposomes [23]. This method was not suitable to show the binding of phosphatidylinositol. In this study the monolayer system is used to demonstrate not only the binding of phosphatidylinositol to the beef brain exchange protein but also to establish the relative affinities of phosphatidylinositol and phosphatidylcholine for this protein. Binding could be directly related to the decrease of surface radioactivity as the subphase was devoid of liposomes. The protein concentration was 2.5-times higher than in the previous experiments where liposomes were present. When underneath a mixed monolayer of [^{14}C]phosphatidylinositol/phosphatidylcholine (molar ratio 1 : 1), beef brain exchange protein is injected, the surface radioactivity decreases with 1% min^{-1} (Fig. 5A). In a second experiment beef brain exchange protein is injected underneath an equimolar phosphatidylinositol/[^{14}C]phosphatidylcholine monolayer (Fig. 5B). Then the rate of surface radioactivity decrease is 0.1% min^{-1}. In order to confirm the preferential extraction of phosphatidylinositol from the mixed monolayer by the exchange protein, the subphase has also been analysed for the presence of radioactivity. In both cases 10 ml of subphase solution was collected after 90 min. The exact volume was measured by weight. The sample was taken to dryness by evaporation at 45°C and dispersed with Biosolve (Beckman) and counted. The blanc experiments in the absence of beef brain exchange protein showed that after 90 min only a small amount of radioactivity was present in the subphase (Table II). In the presence of the exchange protein 45% of the [^{14}C]phosphatidylinositol and 3.8% of the [^{14}C]phosphatidylcholine were transported from the monolayer to the subphase. Since no liposomes were present these percentages indicate that the ratio of phosphatidylinositol/phosphatidylcholine bound by the exchange protein of beef brain was approximately 12.

Discussion

Comparing the exchange proteins from beef liver and beef brain it is evident that the exchange proteins from the two sources behave completely different towards phosphatidylcholine and phosphatidylinositol. In measuring the trans-

fer from monomolecular films of phosphatidylcholine/phosphatidylinositol (1 : 1 molar ratio) to phosphatidylcholine/2% phosphatidylinositol liposomes in the subphase, the exchange protein from beef liver shows a very high transfer activity for phosphatidylcholine and a very minor activity for phosphatidylinositiol. On the other hand the exchange protein from beef brain shows the highest transfer activity for phosphatidylinositol and about 8-times less transfer activity for phosphatidylcholine. As is demonstrated by immunological comparison the exchange protein isolated from beef brain is not contaminated with a phosphatidylcholine exchange protein similar to that isolated from beef liver [12]. At this stage we have to assume that both transfer activities reside in the same protein. Measuring the transfer from microsomes to phosphatidylcholine liposomes, containing 2% phosphatidic acid, the beef brain exchange protein transferred approximately eleven times as much of the microsomal phosphatidylinositol pool than the phosphatidylcholine pool [12]. Although the ratio of the phosphatidylinositol over phosphatidylcholine pool is quite different in microsomes and monolayer (0.15 and 1, respectively) the ratio at which phosphatidylinositol and phosphatidylcholine are transferred to the liposomes in both instances is approximately the same (11 and 8, respectively). It is not yet clear how the transfer of phosphatidylinositol and phosphatidylcholine by the beef brain exchange protein are related.

Liposome-microsome exchange experiments have shown that when the liposomes contain more than 12 mol% phosphatidylinositol and the liposome concentration is higher than 2 μmol no phosphatidylinositol transfer can be measured [9]. It has been shown that the dissociation constant of the exchange protein · liposome complex decreases with an increasing content of liposomal phosphatidylinositol [23]. The above inhibition of transfer is probably due to the fact that at the liposome concentration used all exchange protein is bound. This agrees with the present study where transfer of phosphatidylinositol between monolayer and liposomes was completely inhibited when the liposomes consisted of only phosphatidylinositol. Transfer, however was observed when the monolayer consisted of only phosphatidylinositol. This is probably due to the fact that the monolayer phosphatidylinositol pool is small as compared to the liposomal phosphatidylcholine pool, so that still sufficient exchange protein is freely available. As is demonstrated in Fig. 4 the beef brain exchange protein can catalyse a net transfer of phosphatidylinositol to membranes deficient in this phospholipid. Injection of only beef brain exchange protein under a [^{14}C]phosphatidylinositol monolayer results in a very small decrease in surface activity (Fig. 4A). When liposomes consisting of only phosphatidylcholine, are also present in this subphase, transfer of [^{14}C]phosphatidylinositol to the subphase is very much enhanced; even more so when vesicles are present. As noted before the extent of sonication affects the transfer [23] because of the increase in membrane surface area and/or difference in phospholipid packing. Transfer of phosphatidylinositol to the subphase did not result in a substantial change of the surface pressure. It will be a subject for further investigation whether this due to an increased adsorption of the exchange protein and/or incorporation of phosphatidylcholine.

The phosphatidylcholine exchange protein from beef liver contains one molecule of non cavalently bound phosphatidylcholine [11]. Upon injection of this

protein under a [^{14}C]phosphatidylcholine monolayer exchange of the protein-bound phosphatidylcholine for a molecule from the monolayer was observed. Injection of the beef brain exchange protein under a mixed monolayer of equimolar amounts of phosphatidylinositol and phosphatidylcholine demonstrates that the protein binds both phosphatidylinositol and phosphatidylcholine (Fig. 5, Table II). From these results we conclude that the protein has a 12-fold higher affinity for phosphatidylinositol than for phosphatidylcholine.

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