Biochimica et Biophysica Acta, 946 (1988) 119–128
Elsevier

BBA 74219

Tissue distribution, purification and characterization of rat phosphatidylinositol transfer protein

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(Received 31 May 1988)

Key words: Phosphatidylinositol; Phospholipid transfer protein; Immunoblot; Microsome; (Rat brain)

Phosphatidylinositol transfer activity is measured in cytosol fractions prepared from 13 rat tissues; specific activity is highest in brain and lowest in adipose and skeletal muscle. Based upon electrophoretic analysis phosphatidylinositol transfer protein is purified to homogeneity from whole rat brain. The protein has a molecular weight of 36000 and exists as a mixture of species having isoelectric points of 4.9 and 5.3. In a vesicle-vesicle assay system, the intermembrane transfer rate is greatest for phosphatidylinositol and less by a factor of 2 for phosphatidylcholine; transfer of phosphatidylethanolamine, phosphatidylserine or sphingomyelin is not observed. Using a polyclonal rabbit antibody against bovine phosphatidylinositol transfer protein, immunologic cross-reactivity is noted between the rat protein and other mammalian phosphatidylinositol transfer proteins. A strong correlation is established between a tissue's capacity for phosphatidylinositol transfer and the amount of immunoreactive transfer protein seen in that tissue. Purified phosphatidylinositol transfer protein is capable of transporting newly synthesized phosphatidylinositol molecules from rat brain microsomes to small unilamellar phospholipid vesicles. The results are discussed within the context of cellular phosphoinositide metabolism and the maintenance of the metabolically responsive pool of phosphatidylinositol in the plasma membrane.

Introduction

Cytosolic proteins which are able to transport PtdIns molecules between membranes have been purified and characterized from a wide spectrum of eukaryotic cells, including yeast [1], bovine brain and heart [2,3], and human platelets [4]. With molecular weights in the range 34000–36000 and isoelectric points in the range pH 5.0–5.6, the proteins exhibit a limited substrate specificity toward PtdIns and PtdCho. The protein-mediated transfer of these and other phospholipids has also been detected in rat tissues [5–9]. Two proteins responsible for these transfers are PtdCho transfer protein and nonspecific lipid transfer protein, both of which have been purified and studied extensively [9,10]. The presence of a PtdIns transfer protein in rat tissues was first suggested by Possmayer [5]; a similarity to PtdIns transfer proteins described in other eukaryotic cells was based upon early reports of substrate specificity and isoelectric points [6,8]. We have undertaken a survey of rat tissues to identify and quantitate PtdIns transfer activity and protein levels. We describe the purification of the protein from rat brain, the tissue

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; LacCer, lactosylceramide; PMSF, phenylmethylsulfonyl fluoride.

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containing the highest specific activity; some structural and catalytic properties are reported. The ability of rat PtdIns transfer protein to interact with rat brain microsomes is assessed, and the transfer of newly synthesized PtdIns to small unilamellar vesicles is measured.

Materials and Methods

Materials. *myo*-[2-^{3}H]Inositol (15.0 Ci · mmol^{-1}) and [9,10-^{3}H]oleic acid (4.8 Ci · mmol^{-1}) were purchased from Amersham, Arlington Heights, IL; cholesteryl [1-^{14}C]oleate (56.6 mCi · mmol^{-1}) was purchased from New England Nuclear, Boston, MA. Bovine plasma albumin, PMSF, pepstatin A, LacCer and *Escherichia coli* lipid extract were purchased from Sigma (St. Louis, MO). CDP-dioleoylglycerol was a product of Serdary Research Laboratories (London, Ontario); egg phosphatidate was supplied by Avanti (Birmingham, AL). Bovine brain was purchased from a local slaughterhouse; human cerebral cortex was obtained at autopsy. All other reagents were the highest quality available.

Tissue homogenization and brain microsome isolation. Male Sprague-Dawley rats (Sasco, Omaha, NE), weighing 150–175 g and fed ad libitum, were used for tissue homogenization and brain microsome isolation. Immediately following CO_2 asphyxiation tissues were removed and placed in crushed ice. These tissues and other frozen tissues which had been rapidly thawed at 37°C were homogenized at 4°C in 0.25 M sucrose (0.32 M sucrose for brain)/10 mM Hepes-Na/1 mM Na_2EDTA (pH 7.4) at a tissue wet weight (in g): buffer volume (in ml) ratio of 1 : 4. Six strokes of a motor-driven (800 rpm), loose-fitting Teflon pestle in an appropriately sized Potter-Elvehjem apparatus were used to disrupt the sample. Homogenates were centrifuged at 1500 × *g* for 15 min (International swinging bucket rotor) and next at 11 500 × *g* for 20 min (Sorvall SS-34 rotor). The post-mitochondrial supernatant was centrifuged at 150 000 · *g* for 1 h (Beckman Ti 75 rotor) to yield a crude microsomal pellet and a cytosol fraction. The pellet was resuspended in 10 mM Hepes-Na/50 mM NaCl/1 mM Na_2EDTA (pH 7.4) and stored at −70°C prior to use.

Purification of rat PtdIns transfer protein. Rat brains were purchased from Pel-Freez (Rogers, AR) and stored at −75°C until use. Tissue (250 brains weighing 410 g) was thawed at 37°C and then chilled to 4°C, the temperature at which all subsequent stages of purification were performed. A 20–25% (w/v) homogenate was prepared in 0.32 M sucrose and 10 mM Tris-HCl containing 1 mM Na_2EDTA, 0.1 mM PMSF and 10 mg · l^{-1} pepstatin A and adjusted to pH 7.4. A 1 liter Waring Blendor was operated at medium speed for 2 min. After passing the homogenate through five layers of cheesecloth, it was centrifuged at 11 600 × *g* for 20 min (Sorvall GSA rotor). The loose pellet was discarded, and the supernatant was centrifuged at 50 000 × *g* for 3 h (Beckman 19 rotor). The high-speed supernatant was then dialyzed overnight against 40 l of 5 mM sodium phosphate, 5 mM 2-mercaptoethanol, and 0.1 mM Na_2EDTA (pH 7.4). Protein separation steps in the purification scheme are summarized in Table I; protocols for these steps are essentially identical

TABLE I

PURIFICATION SUMMARY OF PtdIns TRANSFER PROTEIN FROM RAT BRAIN

Step	Protein (mg)	Total activity (nmol min^{-1})	Recovery (%)	Specific activity (nmol · min^{-1} · mg^{-1})	Purification factor
High-speed supernatant	7410	5310	(100)	0.71	(1)
DEAE-cellulose	698	2234	41	3.2	4.4
Sephadex G-75	120	1465	27	12.2	17
Hydroxyapatite	17.5	963	18	55	77
Isoelectric focusing	2.1	342	7	163	228
DEAE-Sephacel	0.5	139	3	277	388

to those employed in the isolation of PtdIns transfer protein from bovine brain [2,11].

Phospholipid transfer activity. Phospholipid transfer activities were measured between two populations of small unilamellar vesicles, as described previously [12]. The radiolabelled phospholipid substrates, phosphatidyl[^{3}H]inositol and 2-[^{3}H]oleoyl-PtdCho, were prepared with rat liver microsomes and purified by thin-layer chromatography. Routine assay system mixtures contain 100 nmol donor vesicles (PtdCho/PtdIns/LacCer, 87 : 5 : 8, mol%, and a trace of radiolabelled phospholipid), 300 nmol acceptor vesicles (PtdCho/PtdIns; 95 : 5, mol%, and a trace of cholesteryl [^{14}C]oleate), 0.1 mg bovine plasma albumin, 5–100 μg cytosolic protein, 50 mM NaCl, 1 mM Na_2EDTA, and 10 mM Hepes-Na (pH 7.4) in a total volume of 0.5 ml. After incubating the mixture for 30 min at 37 °C, the donor vesicles were agglutinated and precipitated in the presence of 0.25 mg *Ricinus communis* agglutinin; the supernatant, containing the acceptor vesicles, was analyzed by liquid scintillation spectrometry. Control incubations were carried out in the absence of cytosolic protein. Activity is calculated as nmol phospholipid transferred per h. In some cases activity is more simply expressed as the percent of the donor pool of phospholipid transferred. An alternative assay system was developed in which the donor and acceptor membranes contained equal molar proportions of PtdIns and PtdCho; in these membranes the bulk phospholipid was unfractionated *Escherichia coli* lipids, consisting of PtdEtn/PtdGro/cardiolipin (75 : 15 : 10, mol%). Further details have recently been described [13].

CDP-diacylglycerol: inositol phosphatidyltransferase activity. The standard system for de novo PtdIns synthesis employed 150 μg microsomal protein which was added to 0.4 mM CDP-dioleoylglycerol, 2 mM [^{3}H]inositol ((5–8) · 10^6 dpm), 2 mM $MgCl_2$, 2 mM $MnCl_2$, 0.5 mM Na_2EGTA, 5 mM 2-mercaptoethanol, 2.5 mg · ml^{-1} bovine plasma albumin, and 50 mM Tris-HCl (pH 8.5) in a volume of 0.25 ml, adapted from the protocols of Kumara-Siri and Gould [14] and Parries and Hokin-Neaverson [15]. When indicated, phospholipid vesicles and/or transfer protein were also added. After incubating this mixture for 30 min at 37 °C, the reaction was stopped by adding 5 ml of chloroform/methanol/12 M HCl (200 : 100 : 1, v/v). The phospholipid product was partitioned into the organic phase, mixed with carrier PtdIns, and separated by thin-layer chromatography, using silica gel H plates developed in chloroform/methanol/acetic acid/water (65 : 40 : 1 : 4, v/v). In some assays, the microsomes were re-isolated by differential centrifugation prior to phospholipid extraction. Boiled microsomes served as the blank. Phosphatidyltransferase activity is expressed as nmol PtdIns formed per h.

Preparation of rabbit anti-PtdIns transfer protein antibody. PtdIns transfer protein, purified to homogeneity from bovine brain by published procedures [2], was injected subcutaneously into a New Zealand white rabbit as a mixture with Freund's complete adjuvant. When the titer had increased to a reasonable level, blood was collected from an ear vein. The serum was treated with $(NH_4)_2SO_4$ to 45% saturation and allowed to stand overnight at 4 °C; the immunoglobulin-enriched precipitate was collected by centrifugation, dialyzed against water, lyophilized to dryness, and stored at −20 °C [11]. Preimmune serum was handled in a similar manner.

Electrophoresis and immunoblotting. Protein samples (1–200 μg) were subjected to electrophoresis on gels of 12 or 14% polyacrylamide (0.75 mm thick) in the presence of sodium dodecylsulfate and 2-mercaptoethanol. Molecular weight standards (pre-stained and unstained, Bio-Rad Laboratories, Richmond, CA) were applied to the gels. For the general detection of proteins, gels were fixed in methanol/acetic acid/water (5 : 1 : 4, v/v) and analyzed by silver staining [16]. For immunological detection of PtdIns transfer protein, the electrophoresed proteins were transferred to nitrocellulose membranes (grade BA80, Schleicher & Schull, Keene, NH) for 1.5–3 h and 190 mA at room temperature [17]. Reconstituted non-fat dried milk (22% by wt.) was used in the blocking step [18]. The nitrocellulose blot was incubated with primary antibody (1 : 200 dilution of a 0.3 mg · ml^{-1} solution) overnight at 4 °C. Following extensive washing and incubation for 1 h with goat anti-rabbit IgG antibody-alkaline phosphatase conjugate (product A-7539, Sigma Chemical Co., St. Louis, MO), used at a 1 : 2500 dilution, the blot was developed at pH 9.5 with

nitro blue tetrazolium (3.3 mg · ml^{-1}) and 5-bromo-4-chloro-3-indolyl phosphate (1.7 mg · ml^{-1}) [19].

Other analytical methods. Protein was determined according to Lowry et al. [20] with bovine plasma albumin as standard. Lipid phosphorus was quantitated according to Rouser et al. [21].

Results

Tissue distribution of PtdIns transfer activity

Membrane-free cytosolic fractions of various rat tissues were analyzed for protein quantity and transfer activity. PtdIns transfer activity was detected in all 13 tissues examined (Fig. 1). Brain cytosolic fraction exhibited the highest specific activity; the cytosolic fractions of adipose, skeletal muscle and heart (cardiac muscle) exhibited the lowest transfer activity. The rates of PtdIns transfer were linear with respect to protein concentration in the range 5–40 μg for the brain and liver preparations using the routine assay system (data not shown). We observed no significant difference between specific activities of PtdIns transfer for cytosolic fractions prepared from fresh or frozen–once-thawed brain or liver.

Purification of PtdIns transfer protein from rat brain

Based on the high specific activity found in brain, this tissue was selected for large-scale purification of PtdIns transfer protein. Steps in the purification scheme are summarized in Table I. From 410 g of frozen tissue was obtained approx. 0.5 mg of homogeneous protein. PtdIns transfer protein was purified nearly 400-fold to a specific activity of 277 nmol · min^{-1} · mg^{-1}. This value of this specific activity is comparable to that found for PtdIns transfer protein purified from bovine brain and somewhat larger than that found for PtdIns transfer protein purified from human platelets, using the vesicle–vesicle routine assay system [22]. The final purification step, an anion-exchange chromatography column, is described in Fig. 2. PtdIns transfer activity was coincident with the protein peak eluting at 60 mM NaCl; fractions 32–38 contained a single protein species by electrophoretic analysis and silver staining. Long-term storage of the purified protein was at −20 °C in the presence of 50% glycerol (by vol.); stability was maintained for at least 3 months. Compared with the isolation of PtdIns transfer proteins from other mammalian tissues [2,4], the overall recovery of the rat protein was considerably smaller, in the range of 1–3% for several purification attempts.

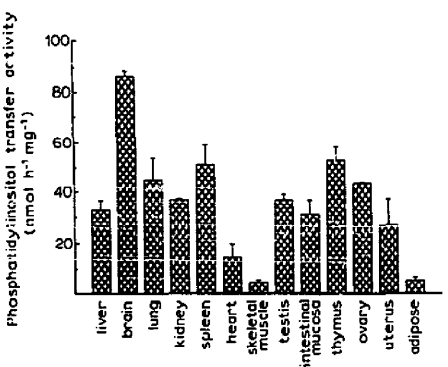

Fig. 1. Distribution of PtdIns transfer activity in rat tissues. Cytosolic fractions were prepared from tissue homogenates, as described in Materials and Methods. PtdIns transfer activity was measured using the routine assay system and 20–50 μg of protein. Bar heights represent the mean ± S.D. of 3–8 determinations.

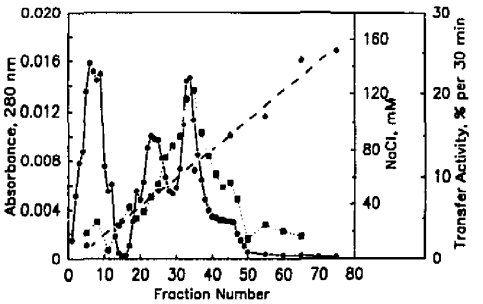

Fig. 2. Chromatography of rat brain PtdIns transfer protein on DEAE-Sephacel. In the final step of purification protein is adsorbed to and eluted from a column of DEAE-Sephacel. The two peaks of phosphatidylinositol transfer activity, between pH 4.9 and 5.3 of the preparative isoelectric focussing step, were combined and dialyzed against 4 l of 10 mM Tris-HCl (pH 7.2). The sample was applied to a 1.5×10 cm column of DEAE-Sephacel (Pharmacia, Piscataway, NJ) which had been prepared in the same buffer. After briefly washing, a 500 ml linear gradient from 0 to 200 mM NaCl (flow rate 25 ml·h^{-1}) was used to elute several major proteins. PtdIns transfer activity was coincident with the protein peak at 60 mM NaCl. PtdIns transfer activity (■) was determined on 50-μl aliquots. The solid curve indicates protein absorbance; the dashed line shows the gradient of NaCl in the elution buffer.

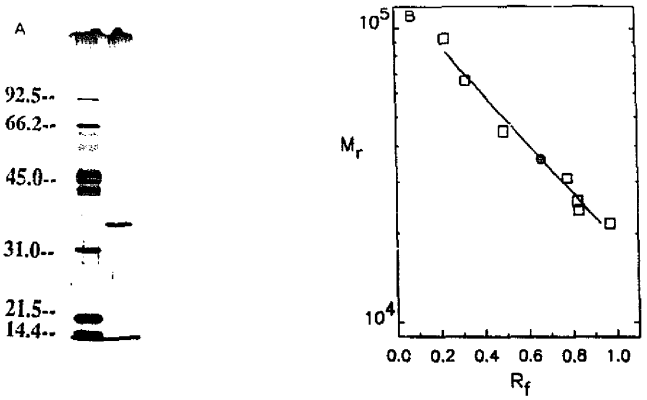

Fig. 3. Electrophoresis of rat PtdIns transfer protein on polyacrylamide gel. (A) 1 μg of purified rat PtdIns transfer protein, representing pooled fractions 32–38 from the DEAE-Sephacel column chromatographic step (Fig. 2), is electrophoresed on a 12% polyacrylamide gel in the presence of sodium dodecylsulfate and 2-mercaptoethanol and detected by silver staining; molecular weight markers include phosphorylase, *B* subunit (92500) bovine plasma albumin (66200), ovalbumin (45000), bovine erythrocyte carbonic anhydrase (31000), soybean trypsin inhibitor (21500), and hen lysozyme (14400). (B) A determination of molecular weight was made from the relative mobility of rat PtdIns transfer protein (filled circle) on a 14% polyacrylamide gel; additional protein standards include chymotrypsinogen (25800) and trypsinogen (24000). The correlation coefficient of the plot of molecular weight standards was 0.988.

Estimation of molecular weight and isoelectric point of rat PtdIns transfer protein

The electrophoretic migration of purified PtdIns transfer protein on a polyacrylamide gel in the presence of sodium dodecylsulfate and 2-mercaptoethanol is shown in Fig. 3A, where a single protein species is seen on the silver-stained gel. From the data in Fig. 3B a molecular weight of 36000 was calculated. The employment of preparative isoelectric focusing during the purification scheme allowed a determination of the transfer protein's isoelectric point. Two isoforms were separated (Fig. 4): a protein species with a p*I* of 4.9 accounted for approx. 65% of the total PtdIns transfer activity and a protein species with a p*I* of 5.3 accounted for the remainder. Analysis of the two isoforms by electrophoresis and immunoblotting indicated no detectable difference between them (data not shown).

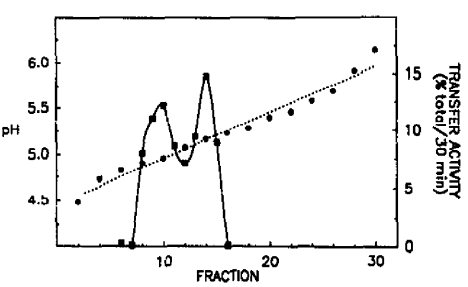

Fig. 4. Isoelectric focussing of rat PtdIns transfer protein. Partially purified rat brain cytosolic proteins is subjected to preparative isoelectric focussing on a horizontal bed of dextran with a mixture of ampholytes between pH 4.5 and 6.0, as described in Materials and Methods. Following the measurement of pH (●), fractions are adjusted to pH 7.2 and 10 ml before transfer activity (■) is determined.

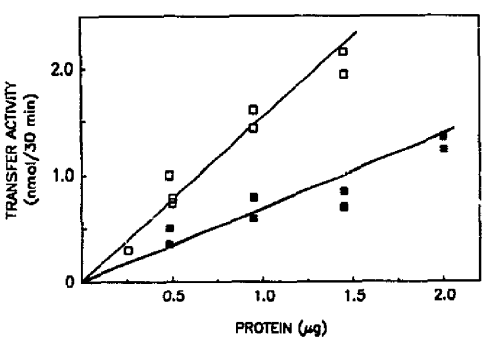

Fig. 5. Phospholipid specificity of rat brain PtdIns transfer protein. The indicated amounts of purified protein are assayed for intermembrane transfer activity using the alternative *E. coli* lipid vesicle system; phospholipids measured are PtdIns (□) and PtdCho (■).

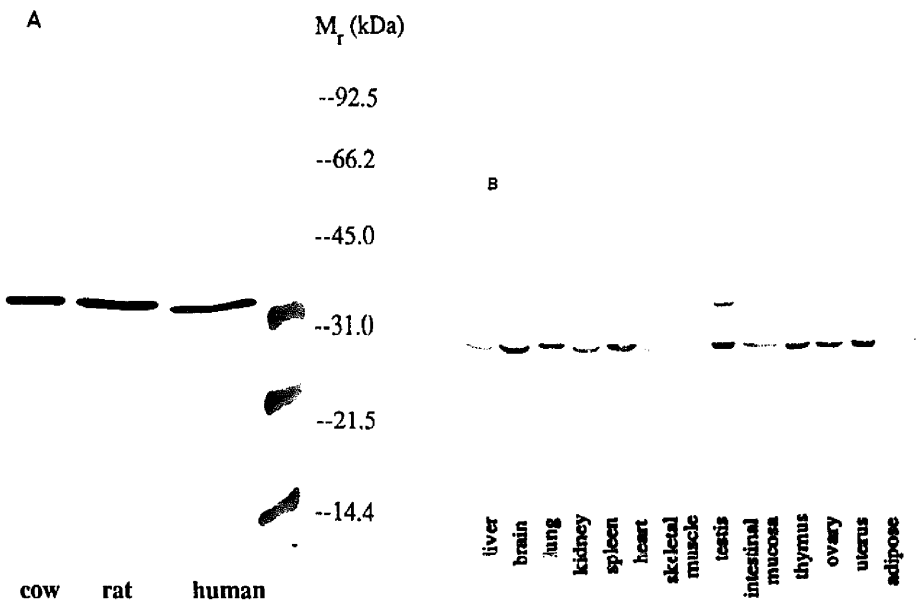

Fig. 6. Immunoblot of mammalian brain and rat tissue cytosolic preparations. Following electrophoresis on polyacrylamide gels and electroblotting onto nitrocellulose membranes, PtdIns transfer protein is detected with a polyclonal rabbit anti-bovine PtdIns transfer protein antibody and a goat anti-rabbit immunoglobulin antibody-alkaline phosphatase conjugate. Samples include 100 μg of the indicated mammalian brain cytosol (A) or 35 μg of the indicated rat tissue cytosol (B).

Catalytic activity of rat PtdIns transfer protein

Determinations of catalytic activity and substrate specificity were performed at various stages of purification. Specific activity was highly dependent upon the lipid composition of the donor and acceptor membranes. Using aliquots of unfractionated brain cytosol, the ratio of PtdIns to PtdCho transfer activities was 0.1 in the routine assay system, which employs vesicles containing 5 mol% PtdIns and 87–95 mol% PtdCho. In contrast, this ratio was greater than 2.2 in the alternative assay system with limiting proportions of PtdIns and PtdCho (no more than 5 mol%) in the two vesicle populations. Transfer activity was also compared for PtdIns and PtdCho for the purified protein (Fig. 5). Based on initial rates of transfer with the alternative assay system, a 2-fold preference for PtdIns is readily apparent. Catalytic turnovers for the purified protein were calculated to be 2970 min^{-1} for PtdIns and 1390 min^{-1} for PtdCho. If the PtdIns component of the donor vesicle population in the routine assay system were replaced with radiolabelled PtdEtn, phosphatidylserine or sphingomyelin, no detectable transfer of these phospholipids could be attributed to the presence of purified rat PtdIns transfer protein (data not shown). The substrate specificity of rat PtdIns transfer protein appears to be limited to PtdIns and PtdCho.

Correlation of PtdIns transfer activity and protein by immunoblot

Immunochemical comparison of PtdIns transfer proteins from three mammalian species is depicted in Fig. 6A. Cytosolic fractions of rat, cow, and human cerebral cortex were separated electrophoretically, transferred to nitrocellulose, and treated with antibody raised to the purified bovine PtdIns transfer protein. A single immunoreactive band is seen in each preparation; the identical mobilities are indicative of a common molecular weight, calculated to be 36000. It should be noted that purified PtdIns transfer protein from rat and cow brain also yield a single immunoreactive band. The pronounced cross-reactivity of the bovine antibody with rat PtdIns transfer protein permitted

further analysis of rat tissues for the detection of this specific protein. In the 13 tissues examined (Fig. 6B), a common immunoreactive band was observed, corresponding to a protein with a molecular weight of 36000. The amount of protein applied to the polyacrylamide gel (35 μg) was intentionally kept low in order to establish a qualitative correlation between immunostaining and transfer activity. The correlation is, in fact, quite respectable: brain is the most intensely stained, while heart, skeletal muscle, and adipose are the weakest (compare with Fig. 1). Thus, we are confident that the measured PtdIns transfer activity in most, if not all, rat tissues is a direct reflection of the quantity of PtdIns transfer protein present in a given tissue. A most unexpected observation was that of an additional intensely staining protein in testis, with a molecular weight of 41000. We are currently investigating the structural and functional properties of this unique testicular protein. We have also obtained immunoblot evidence for the presence of PtdIns transfer protein in 4 additional rat tissues: whole adrenal, lactating mammary, pancreas, and placenta.

Transfer of newly synthesized PtdIns

The biosynthesis of PtdIns from CDP-diacylglycerol and inositol is a reaction that is localized to cellular membranes [23]. The incubation of rat brain microsomes with the appropriate substrates led to a linear synthesis of PtdIns during the indicated time period; nearly 90% of all de novo synthesized PtdIns was found in the re-isolated membrane fraction (Fig. 7A). The distribution of de novo synthesized PtdIns between the membrane and supernatant fractions was not altered by the addition to the incubation system of phospholipid vesicles or purified PtdIns transfer protein (data not shown). Furthermore, the rate of PtdIns synthesis was linear with respect to the amount of microsomes used in the incubation, a refection of the membrane localization of CDP-diacylglycerol: inositol phosphatidyltransferase. When both vesicles and transfer protein were added to the microsomes to generate a complete phospholipid transfer system (Fig. 7B), more of the de novo synthesized PtdIns was found in the supernatant fraction. As the quantity of protein catalyst increased from 0 to 7.2 μg, the proportion of de novo synthesized PtdIns associated with the vesicle increased from 6 to 54%; on the other hand, the total synthesis of PtdIns was essentially unchanged. Thus, PtdIns transfer protein was capable of transporting PtdIns molecules from the membrane where synthesis occurred to a suitable acceptor membrane. While these data show that de novo synthesized PtdIns is a substrate for

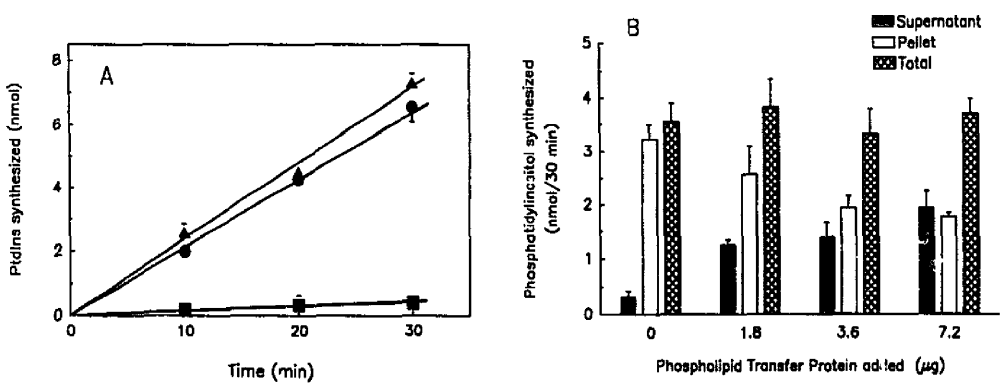

Fig. 7. Kinetics of PtdIns synthesis by rat brain microsomes. Rat brain microsomes are prepared and assayed for CDP-diacylglycerol: inositol phosphatidyltransferase activity, as described in Materials and Methods. Microsomal protein is 60 μg. Vesicles are prepared from egg PtdCho and phosphatidate (95:5, mol%); transfer protein is purified to homogeneity. Following the incubation of microsomes alone, with neither transfer protein nor vesicles, the distribution of de novo synthesized, radiolabelled PtdIns in microsomal pellet (filled circles), supernatant (filled squares), and total (filled triangles) is indicated (A). PtdIns synthesis is measured in the presence of 100 nmol vesicles and the indicated amounts of rat brain PtdIns transfer protein; product distribution is shown by the different bars, each of which represents the mean ± S.D. of three determinations (B).

PtdIns transfer protein, the relative activities of the de novo synthesized and existing pools of this phospholipid cannot be compared. By analogy to bovine PtdIns transfer protein in a microsome-vesicle assay system [12,22], rat PtdIns transfer protein should be able to transfer approximately 24 nmol PtdIns $\cdot h^{-1} \cdot \mu g^{-1}$ when PtdIns exchange is possible and 16 nmol PtdIns $\cdot h^{-1} \cdot \mu g^{-1}$ for the net transfer conditions used in the present experiment. The amount of PtdIns associated with rat brain microsomes is 36 nmol $\cdot$ (mg protein)$^{-1}$ based on lipid extraction and quantitation. These observations would suggest that transfer protein should not be rate-limiting; however, the limited data in Fig. 7B are insufficient to support such a conclusion.

Discussion

A survey of rat tissues for PtdIns transfer activity, using a vesicle–vesicle assay, and PtdIns transfer protein, using an immunoblot procedure, demonstrated clearly that whole brain exhibited the highest specific transfer activity and protein content. Extensive purification from a brain cytosolic fraction yielded a homogeneous protein which possessed catalytic and structural properties similar to those reported for PtdIns transfer proteins isolated from cow and human tissues. An ability to transfer both PtdIns and PtdCho between populations of single bilayer phospholipid vesicles was observed for the most crude as well as the purest protein preparations. A distinct preference for PtdIns was apparent in assay systems designed to present to the protein nearly equivalent quantities of transferable phospholipid molecules.

Rat PtdIns transfer protein could be separated into two isoforms, with isoelectric points of 4.9 and 5.3. These isoelectric points are essentially identical to those observed in partially purified homogenates of rat brain [8] and liver [6]. The values may be compared with isoelectric points of 5.2 and 5.5 for bovine PtdIns transfer protein and 5.6 and 5.9 for human PtdIns transfer protein. The molecular basis of these isoforms was recently clarified by Van Paridon et al. [24], who showed that PtdIns was non-covalently associated with the more acidic protein, presumably at the catalytic site, and PtdCho was non-covalently associated with the more basic protein. Such phospholipid-protein complexes function as catalytic intermediates in the intermembrane transport of PtdIns and PtdCho [22]. Further structural similarity among PtdIns transfer proteins is seen in the immunologic cross-reactivity of the rat, bovine, and human proteins. The calculated size of rat PtdIns transfer protein (36000) is virtually indistinguishable from that of the cow and human proteins when the three are compared by electrophoresis and immunoblotting. Of further significance is the finding that PtdIns transfer protein purified from the yeast *Saccharomyces cerevisiae* has a molecular weight of 35000 [1]. These structural features suggest strongly that PtdIns transfer proteins from a wide spectrum of eukaryotic cells form an evolutionarily conserved family. One can only hypothesize that the physiological function of these proteins is likewise conserved among these cells.

To this latter point we directed our efforts to the participation of PtdIns transfer protein in de novo phospholipid synthesis. Rat brain microsomes contained CDP-diacylglycerol:inositol phosphatidyltransferase activity which could be assayed with a linear dependence on time. The specific activity of our preparation was 135 nmol $\cdot h^{-1} \cdot mg^{-1}$, somewhat lower than values of 309 and 192 nmol $\cdot h^{-1} \cdot mg^{-1}$ reported by Benjamins and Agranoff [25] and Ghalayini and Eichberg [26], respectively. The addition of PtdIns transfer protein and phospholipid vesicles to the microsomes prompted a dramatic redistribution of PtdIns from the microsomal membrane to the supernatant which contained the small unilamellar vesicles. This experiment demonstrates that molecules of PtdIns, newly synthesized from CDP-diacylglycerol and inositol, become available for intermembrane transfer. Whether these molecules must first enter another microsomal pool of PtdIns before they are transferred cannot be determined from the present data; nevertheless, no detectable lag in the transfer kinetics was noted. This experiment also shows the capacity of rat PtdIns transfer protein to effect a net transfer of PtdIns to an acceptor membrane initially devoid of PtdIns. In keeping with the dual specificity of rat PtdIns transfer protein, we suggest that PtdCho is being transported in the opposite direction during the

net transfer of PtdIns. The equivalence of these bidirectional fluxes has been described for bovine PtdIns transfer proteins [27].

The dual specificity of rat PtdIns transfer protein also permits the metabolic coupling of two significant eukaryotic membrane phospholipids: PtdCho, abundant and widely distributed, and PtdIns, particularly rich in the plasma membrane and sensitive to stimulus processing. The maintenance of operational stimulus-response networks linked to phosphoinositide hydrolysis would depend upon the efficient regeneration of plasma membrane pools of PtdIns [28,29]. A highly specialized, protein-mediated transport of PtdIns from the endoplasmic reticulum to the plasma membrane, first suggested by Michell [30], still remains an attractive component of cellular PtdIns metabolism. The widespread tissue distribution of rat PtdIns transfer protein is consistent with such a proposed function. Indeed the stimulus-coupled hydrolysis and turnover of inositol-containing phospholipids is a phenomenon which is also widely distributed among animal tissues and other eukaryotic cells [29]. Although not investigated in the present study, we assume that rat PtdIns transfer protein, like PtdIns transfer protein isolated from bovin brain [31], does not catalyze an intermembrane translocation of PtdIns 4-phosphate or PtdIns 4,5-bisphosphate. The kinases responsible for the phosphorylation of PtdIns and PtdIns 4-phosphate are thought to be membrane-bound in general and localized to the plasma membrane in many cells [29]. These enzymes would, therefore, be in a position to take advantage of a PtdIns transfer protein-catalyzed flux of de novo synthesized PtdIns from the endoplasmic reticulum to the plasma membrane.

Rat tissues have yielded considerable information on the occurrence and distribution of lipid transfer proteins. PtdCho transfer protein has been purified to homogeneity [10]; this protein has a molecular weight of 28000. Its isoelectric point is reported to be 8.4 for liver preparations [6] or between 8 and 9 for small intestine preparations [7]. Like its acidic counterpart in bovine liver, rat PtdCho transfer protein exhibits catalytic activity toward PtdCho, but to neither PtdIns nor PtdEtn. A nonspecific lipid transfer protein was initially described in liver by Bloj and Zilversmit [9] and then purified to homogeneity by Poorthuis et al. [32]. This protein has a molecular weight of 14800 and, like PtdCho transfer protein, has a basic isoelectric point of 8.3–8.7. Substrate specificity extends to PtdCho, PtdIns, PtdEtn, sphingomyelin and cholesterol.

The structural and catalytic properties of PtdCho transfer protein and nonspecific lipid transfer protein distinguish these proteins from PtdIns transfer protein. The dual specificity of PtdIns transfer protein toward PtdIns and PtdCho, and the concomitant lack of activity toward other glycerophospholipids and sphingolipids, is, on the one hand, broader than that of PtdCho transfer protein and, on the other hand, more restrictive than that of nonspecific lipid transfer protein. In the purification schemes of these three proteins only PtdIns transfer protein remained bound to anion-exchange media at neutral pH and low salt concentration. Another significant difference among these proteins is their tissue distribution. PtdIns transfer protein is ubiquitous in the rat: measurable activity is detectable in all tissues investigated, with a range in specific activity of approx. 15. A similarly widespread distribution of nonspecific lipid transfer protein has been reported in rat tissues [33], with the highest concentration was found in liver and small intestine and the lowest in testis. PtdCho transfer protein also was most abundant in rat liver and small intestine, with lesser amounts in several other tissues; none was detected in brain or heart [34].

Several important conclusions may be drawn from the present study. First, based on structural and catalytic properties, PtdIns transfer proteins constitute a highly conserved family of proteins. This conservation is most apparent for the rat, bovine, and human proteins; it may eventually be extended to single-cell organisms, such as yeast. Of all phospholipid transfer proteins, the PtdIns transfer proteins appear to be most structurally related from species to species. Secondly, PtdIns transfer activity was measurable in diverse tissues of the rat, with an excellent correlation being observed between activity and immunoreactive PtdIns transfer protein. This distribution is more widespread than that found for other lipid transfer proteins and is consistent with a role of PtdIns transfer protein in the agonist-stimulated metabo-

lism of PtdIns and its phosphorylated analogs characteristic of most tissues. Finally, the transfer-protein-mediated transport of PtdIns molecules, newly synthesized by microsomal membranes, to phospholipid vesicles was demonstrated. Such a unidirectional flux would be consistent with a role of PtdIns transfer protein in stimulus-coupled phosphoinositide metabolism.

Acknowledgements

The authors gratefully acknowledge the assistance of Dr. F. Cuppage, Department of Pathology and Oncology, University of Kansas Medical Center, for the specimen of human cerebral cortex. This research was supported in part by grant GM 24035 from the National Institutes of Health, U.S. Public Health Service.

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