Stimulation of Cholinephosphotransferase Activity by Phosphatidylcholine Transfer Protein

REGULATION OF MEMBRANE PHOSPHOLIPID SYNTHESIS BY A CYTOSOLIC PROTEIN*

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The effect of rat liver phosphatidylcholine transfer protein on the incorporation of CDP-choline and dioleoylglycerol into phosphatidylcholine catalyzed by rat liver microsomal CDP-choline:1,2-diacyl-sn-glycerol cholinephosphotransferase was studied. In the presence of phosphatidylcholine transfer protein, the incorporation of CDP-choline into phosphatidylcholine was markedly stimulated. Phosphatidylcholine transfer protein isolated from either rat or bovine liver was capable of this stimulatory effect; in contrast, phosphatidylinositol transfer protein from rat liver had no effect on phosphatidylcholine synthesis. Kinetic analysis showed that microsomal phosphatidylcholine synthesis increased 2.4-fold after 1 min and reached a maximum of approximately 10-fold within 10 min in the presence of phosphatidylcholine transfer protein; in the absence of this protein phosphatidylcholine synthesis stopped after 2–4 min. These results suggest that phosphatidylcholine transfer protein permits phosphatidylcholine synthesis to proceed further. With the addition of phospholipid vesicles, as an acceptor membrane in the reaction mixture, there was a significant amount of protein-mediated transfer of synthesized phosphatidylcholine to the vesicles. Measurable transfer of synthesized phosphatidylcholine to vesicles could only be detected after a lag of 2–4 min. The stimulation of cholinephosphotransferase could be nearly abolished by increasing the amount of added phospholipid vesicles; concurrently, a greater transfer to the vesicles was observed. These results describe a new property of phosphatidylcholine transfer protein which may be of physiological significance in the regulation of phosphatidylcholine synthesis in mammalian tissues.

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The majority of enzymes utilized in the biosynthesis of phospholipid in mammalian tissues is located on the endoplasmic reticulum (Vance and Vance, 1988). In rat liver PtdCho is mainly synthesized by the de novo CDP-choline pathway, which contributes about 70–80% of PtdCho formation (Sundler and Akesson, 1975; Vance and Choy, 1979). The final step of this pathway involves the reaction between CDP-choline and 1,2-diacyl-sn-glycerol to yield PtdCho and CMP (Weiss et al., 1958), a step catalyzed by CDP-choline:1,2-diacyl-sn-glycerol cholinephosphotransferase (EC 2.7.8.2). While there is considerable evidence for regulation of PtdCho synthesis at the level of CDP-choline, several recent reports suggest additional control at the terminal step. Lim et al. (1986) have suggested that the activity of cholinephosphotransferase may be limited by the availability of both CDP-choline and diacylglycerol in HeLa cells permeabilized with digitonin. Cholinephosphotransferase activity was stimulated by analogues of cAMP, such as chlorophenylthio-cAMP, and CTP:phosphocholine cytidylyltransferase activity was reduced in cultured rat hepatocytes (Pelech et al., 1981). Moreover, insulin increased cellular diacylglycerol content (Farese et al., 1997) and activated protein kinases (Cooper et al., 1987). Nair et al. (1988) reported that insulin provoked biphasic increases in the labeling of phosphocholine and PtdCho by [3H]choline in cultured BC3H-myocytes, whereas 12-O-tetradecanoylphorbol-13-acetate, a potent activator of protein kinase C, increased the labeling of phosphocholine but not PtdCho (Bocchino et al., 1987). An increase in the level of endogenous diacylglycerol led to a corresponding increase in PtdCho synthesis, supporting the idea that the availability of this substrate, under certain conditions, may be rate limiting for the synthesis of PtdCho in rat lung microsomes (Rustow and Kunze, 1987).

Protein-catalyzed transport of phospholipids between membranes was first described in rat liver preparations by Wirtz and Zilversmit (1968). Intracellular phospholipid transfer proteins differ not only in physical and chemical properties but more dramatically in their catalytic activities (Helmkamp, 1985). Rat tissues contain the following three well-defined phospholipid transfer proteins: (a) PtdIns transfer protein, which transports both PtdCho and PtdIns (Helmkamp, 1985), (b) nonspecific lipid transfer protein, which facilitates the movement of most phospholipids and other amphiphilic lipid such as cholesterol and glycolipids (Bloj and Zilversmit, 1977), and (c) PtdCho transfer protein. Rat PtdCho transfer protein is a cytosolic protein that has been purified to homogeneity (Teerlink et al., 1983), has a molecular weight of 28,000 (Poorthuis et al., 1980), and is localized mainly in liver (Lutton and Zilversmit, 1976) and small intestinal mucosa (Yamada et al., 1978). Like other transfer proteins, it can mediate the transport of lipid between isolated cellular membranes, including endoplasmic reticulum, intact mitochondria, synaptosomes, and myelin (Wirtz, 1982); it also functions with a variety of artificial lipid membranes. PtdCho transfer protein is highly specific for PtdCho and does not transfer phospholipids of other polar head group classes (Poorthuis et al., 1980; Teerlink et al., 1981). Catalytically, it operates as a carrier of...
monomeric PtdCho molecules in the aqueous phase between membranes or vesicles.

An important event in membrane biogenesis is the movement of phospholipids from their sites of synthesis on the endoplasmic reticulum to the other subcellular membranes. While the ability of transfer proteins to effect intermembrane phospholipid transport in vitro has been clearly demonstrated, any direct evidence of a physiological function is lacking. Recognizing the substrate specificity of PtdCho transfer protein and its ability to interact with endoplasmic reticulum, we tested the hypothesis that this protein may, under the conditions of fetal liver development, effect partitioning of phospholipids from their sites of synthesis on the endoplasmic reticulum to the other subcellular membranes. We have shown that transfer proteins can be extracted from fetal liver, and these preparations can be used to determine phospholipid concentration (Rouser et al., 1970).

**EXPERIMENTAL PROCEDURES**

**Materials**—PtdCho was purified from crude egg PtdCho (Sigma) by dry column elution chromatography on silica Gel-G using chloroform/methanol/water (65:25:4, v/v) as a developing system (Welti and Silberr, 1982). Crude pig liver Ptdlsns (Serday Research Laboratories, London, Ontario) was similarly purified, using the solvent system chloroform/methanol/acetic acid/water (50:25:7:3, v/v). Phospholipid transfer protein was purified from crude pig liver Ptdlsns (Kasper and Helmkamp, 1981b). [3H]OleGro was prepared by hydrolysis of [H]dioleoyl phosphatidylcholine with Bacillus cereus venom (Wells, 1975), reacylated with [9,10-3H]oleic acid (4.8 Ci/mmol, Du Pont-New England Nuclear), and purified as described by Wirtz and Moonen (1977) and analytical procedures (Wirtz and Moonen, 1977) and analytical procedures (Wirtz and Moonen, 1977).

**Phospholipid Transfer Proteins**—Transfer protein was purified from rat liver essentially as described by Teerlink et al. (1983). Purity of protein was greater than 95% when analyzed by sodium dodecyl sulfate-polycrylamide gel electrophoresis and detected by silver staining. The specific activity was 2200 units mg⁻¹, a unit being defined as 1 nmol of PtdCho transferred/min from donor membranes (100 nmol of phospholipid) to acceptor membranes (300 nmol of phospholipid) using a small unilamellar vesicle assay system (Kasper and Helmkamp, 1981a). Protein (39 µg/ml) was stored at −20°C in 50% glycerol containing 50 mM sodium phosphate, pH 7.2. Bovine liver PtdCho transfer protein and rat liver Ptdlsns transfer proteins were purified as described by Welti and Helmkamp (Welti and Helmkamp, 1984; Venuti and Helmkamp, 1988). Protein concentrations were based on a molar absorption coefficient (Wirtz and Moonen, 1977) and analytical protein assay (Bradford, 1976).

**Isolation of Rat Liver Microsomes**—Male Sprague-Dawley rats (Sasco, Omaha, NE), weighing 175–200 g and fed ad libitum, were used for this study. Immediately following CO₂ asphyxiation, livers were removed and homogenized at 4°C in 0.25 M sucrose, 10 mM Tris-Cl, pH 7.4, using a Potter-Elvehjem apparatus. The 20% tissue homogenate was centrifuged at 15,000 x g for 30 min (Sorvall SS-34 rotor); the post-mitochondrial supernatant was centrifuged at 105,000 x g for 1 h (Beckman Ti 50.1 rotor) to yield a microsomal pellet. This pellet was exchanged, washed twice with 10 mM Tris-Cl, pH 7.4, and finally suspended in the same buffer and stored at −70°C.

**PtdCho Synthesis**—Cholinephosphotransferase activity was assayed as described by Tsao (1986). The reaction mixture contained 0.5 mM CDP-choline, 0.5 mM OleGro, 20 mM MgCl₂, 1 mM dithiothreitol, 50 µg of microsomal protein in 50 mM Tris-Cl, pH 8.2, and a total volume of 0.5 ml. Either CDP-[methyl-14C]choline (2000 cpm) or [3H]OleGro (1.0–1.1 x 10⁵ dpm) was added to the incubation mixture. When indicated, PtdCho transfer protein (0.78 µg) and phospholipid vesicles (300 nmol) were also included. A sonicated suspension of diacylglycerol in 50 mM Tris-Cl, pH 8.2, was prepared as described by Weinhold (1981); however, neither Tween 20 nor phosphatidylglycerol was added. The reaction was conducted at 37°C. At different time points, 100 µl of 0.2 M sodium acetate, pH 5.0, was added to stop the reaction. After 15 min at 4°C, the reaction mixtures were centrifuged at 15,000 x g for 15 min (Sorvall SH-MM rotor) to separate aggregated microsomes from phospholipid vesicles. The supernatants were taken directly for lipid extraction (Bligh and Dyer, 1959); the washed pellet was also processed for the extraction of lipids (Folch et al., 1957). Phospholipids were fractionated on 0.25-mm silica Gel-G thin layer plates developed unidimensionally in chloroform, methanol, 7 N ammonium hydroxide (330:90:14, v/v) (Abraham and Blecher, 1964). PtdCho zones were scraped into scintillation vials for radioactivity measurement.

**Veal Preparation and Characterization**—Solvent injection vesicles were prepared by the method of Batzri and Korn (1973) from 30 mM phospholipid solutions in ethanol/dimethyl sulfoxide (3:1, v/v). In all cases acceptor vesicles contained PtdCho/PtdIns (95:5, mol%). These vesicles were similar in size, as measured by chromatography on Sepharose 4B, and in participation in assays as the small, sonicated, single bilayer vesicles previously used in this laboratory (Helmkamp, 1980). Each assay was performed in triplicate and reported as a mean ± S.D.

**RESULTS**

**Cholinephosphotransferase Activity in Absence and Presence of Phospholipid Transfer Proteins**—The synthesis of PtdCho catalyzed by isolated rat liver microsomes is summarized in Table I. Upon addition of rat liver PtdCho transfer protein, cholinephosphotransferase activity was stimulated about 10.4-fold. The combined addition of transfer protein and small unilamellar vesicles reduced the fold stimulation to 7.6; under these conditions, significant protein-mediated transfer of PtdCho to the acceptor vesicles was observed. Bovine liver PtdCho transfer protein exhibited a comparable stimulation of basal cholinephosphotransferase activity (Table I). In marked contrast, however, PtdIns transfer protein from rat liver was incapable of effecting any stimulation. A similar experiment was performed with non-radioactive CDP-choline and OleGro in the presence and absence of rat liver PtdCho transfer protein. PtdCho content was chemically analyzed by extraction, thin layer chromatography, and lipid phosphorus determination. The endogenous PtdCho in 50 µg of microsomes was 22.7 ± 1.3 nmol. After 30 min of incubation, total microsomal PtdCho increased by 0.38 nmol. In the presence of PtdCho transfer protein, however, the quantity of PtdCho increased by 3.9 nmol. These data confirm the findings in Table I.

**Table I**

<table>
<thead>
<tr>
<th>Addition to assay</th>
<th>Remaining with microsomes</th>
<th>Transferred to vesicles</th>
<th>Total</th>
<th>PtdCho synthesized</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>12 ± 3 (7)</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vesicles</td>
<td>12.5 ± 3.1 (4)</td>
<td>0.5 ± 0.2 (3)</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>PC-TP (rat liver)</td>
<td>123 ± 18 (5)</td>
<td>154 ± 22 (5)</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>PC-TP (rat liver) + vesicles</td>
<td>94 ± 16 (5)</td>
<td>3.8 ± 0.5 (3)</td>
<td>97.8</td>
<td></td>
</tr>
<tr>
<td>PC-TP (bovine liver) + vesicles</td>
<td>82 ± 0.2 (1)</td>
<td>15 ± 2 (1)</td>
<td>97.8</td>
<td></td>
</tr>
<tr>
<td>PI-TP (rat liver) + vesicles</td>
<td>8.7 ± 0.8 (1)</td>
<td>N/D</td>
<td>8.7</td>
<td></td>
</tr>
</tbody>
</table>

* PC-TP, PtdCho transfer protein.
* PI-TP, PtdIns transfer protein.
ND, none detected.
Cholinephosphotransferase and Phosphatidylcholine Transfer Protein

Substrate Utilization by Microsomal Cholinephosphotransferase—To determine how much each substrate contributed to the synthesis of PtdCho, CDP-[\(^{14}\)C]choline, and \([\text{H}]\)Ole\(_2\)Gro were compared in the microsomal assay. As shown in Table II, the ratio of \(^{14}\)C/\(^{3}\)H in newly synthesized PtdCho molecule is essentially unity. The incorporation of CDP-[\(^{14}\)C] choline into PtdCho was activated from a basal level of 11.2 nmol (mg protein\(^{-1}\) h\(^{-1}\)) to 85.2 nmol (mg protein\(^{-1}\) h\(^{-1}\)) in the presence of PtdCho transfer protein; similarly, the contribution of \([\text{H}]\)Ole\(_2\)Gro to PtdCho synthesis was stimulated from 11.6 nmol (mg protein\(^{-1}\) h\(^{-1}\)) to 83.7 nmol (mg protein\(^{-1}\) h\(^{-1}\)) (Table II). These levels of isotope incorporation are consistent with a strong reliance on exogenous substrate utilization. In contrast, the isotopic distribution in PtdCho which was transferred to vesicles yielded a mole ratio of CDP-choline to Ole\(_2\)Gro (\(^{14}\)C/\(^{3}\)H) of 1:2.2, a value which may reflect a preference of PtdCho transfer protein for transfer of certain molecular species (Welti and Helmkamp, 1984).

Kinetics of PtdCho Synthesis and Transfer—The foregoing experiments clearly describe two functions of PtdCho transfer protein: stimulation of cholinephosphotransferase and transfer of PtdCho from microsomes to vesicles. These properties were examined in greater detail by kinetic analysis (Fig. 1).

In the presence of PtdCho transfer protein, the maximum synthetic activity was attained within 3-4 min and was followed by a slower rate for 10 min (Fig. 1A). On the other hand, microsomes in the absence of PtdCho transfer protein reached a maximum level of PtdCho synthesis within 2-4 min and thereafter stopped. Thus, PtdCho transfer protein not only enhances cholinephosphotransferase activity but stabilizes it for an extended period of time as compared with microsomes alone. At times as early as 1 min, the fold stimulation is 2.4 (Fig. 1A); this parameter increases to approximately 8 within 10 min, approaching the maximum level of PtdCho transfer protein-derived activation. The fold stimulation increased with time because the reaction rate without PtdCho transfer protein fell to zero after 2 min. The transfer of newly synthesized PtdCho from microsome donor membranes to vesicle acceptor membranes could not be detected at early times. Only after a reproducibly observed lag of 2-4 min was significant transfer noted (Fig. 1B), and the transfer activity of PtdCho transfer protein remained a linear phenomenon for at least 30 min. A higher concentration of PtdCho transfer protein was employed in this experiment. We observed a linear increase in PtdCho synthesis with increasing concentration of PtdCho transfer protein in the reaction mixture (Fig. 2).

Effect of Vesicle Concentration on PtdCho Synthesis—In the above experiments, we have consistently observed a reduced stimulation of cholinephosphotransferase activity in the presence of PtdCho transfer protein and phospholipid vesicles, compared with the addition of PtdCho transfer protein alone (Table I). To pursue this further, the concentration of vesicles in the reaction mixture was varied; the results are presented in Fig. 3A. As the vesicle concentration increased, the stimulation of cholinephosphotransferase activity became less pronounced and finally, at 200 nmol of phospholipid vesicles, approached the basal level of PtdCho synthesis observed for microsomes and transfer protein. Concomitant with the loss of stimulated cholinephosphotransferase activity, there was a proportional increase in transfer of newly synthesized PtdCho to the vesicles (Fig. 3B). The reduction in transfer-activity of PtdCho to the vesicles (Fig. 3B).
subsequently used for the synthesis of PtdCho as described with [methyL3H]choline were isolated (Wirtz et al., 1972) and protein, rat liver microsomes which had been labeled in vivo in the presence and absence of 1.56 pg of rat liver PtdCho transfer protein. Various concentrations of phospholipid vesicles are added to the reaction mixture for a 15-min incubation period.

Experiments are performed as described in Fig. 1, except that no vesicles are added to the assay mixtures. The indicated quantity of rat liver PtdCho transfer protein is present during the 3-min incubation period.

Protein-mediated Transfer of Newly Synthesized and Endogenous PtdCho Transfer protein for a 20-min incubation period. The transfer of endogenous PtdCho (3H-labeled) and newly synthesized PtdCho (14C-labeled) to acceptor phospholipid vesicles could then be measured simultaneously. The total transfer from microsomes to acceptor membranes was 15.2 ± 1.9 nmol/mg protein/20 min, of which 7.4 ± 1.0 nmol was newly formed PtdCho. Using the endogenously labeled microsomes as donor membranes in standard transfer assay procedure (Helmkamp et al., 1974), we found that transfer of [3H]PtdCho from microsomes to small unilamellar vesicles was 14.9 ± 1.2 nmol. These results indicate that the transfer catalytic activity of PtdCho transfer protein was not altered by our synthetic reaction system. On the basis of chemical analysis, the endogenous pool of PtdCho in 1 mg of microsomal protein was 463 ± 25 nmol. In addition, newly synthesized PtdCho represented an approximately 11-16% increase over the endogenous PtdCho pool. Notably, the mole ratio of endogenous to newly formed PtdCho in microsomes is 6:1, but in the transferred population it becomes 1:1. These findings again may be attributed to the substrate selectivity of PtdCho transfer protein. This single time point assay provides an estimation of total PtdCho transferred, without adjustment for the lag seen in the transfer of newly synthesized species (Fig. 1B).

**DISCUSSION**

Previous studies on the regulation of de novo PtdCho synthesis strongly indicated a control point at the formation of CDP-choline from CTP and choline phosphate (Pelech and Vance, 1984). A cytidylyltransferase-regulated pathway implies that the rate of the reaction catalyzed by cholinephosphotransferase, the next and last enzyme in the pathway, should be limited by the supply of CDP-choline, but not necessarily by the level of diacylglycerol. A variety of agents which increased the levels of CDP-choline also stimulated PtdCho synthesis (Vance et al., 1980; Sleight and Kent, 1980; Whitton et al., 1985). In this investigation the activity of cholinephosphotransferase was measured, and the incorporation of both CDP-choline and OlePGro into PtdCho was monitored. The basal cholinephosphotransferase activity of rat liver microsomes measured in our assay system is low when compared with values reported in literature (Vance and Vance, 1988). This difference can most likely be attributed to the absence of detergent and anionic phospholipid in the preparation of the OleGro substrate. Miller and Weinhold (1981) have reported that cholinephosphotransferase activity was drastically reduced in rat lung when diacylglycerol was used without Tween 20 and phosphatidylglycerol. We would, in fact, suggest that the mixed micelles formed from detergent and phospholipid may function in other assay systems to release "product inhibition" in much the same way as PtdCho transfer protein.

CDP-choline and diacylglycerol are required substrates for PtdCho synthesis catalyzed by microsomal cholinephosphotransferase. In the presence of rat liver PtdCho transfer protein, the incorporation of either (radioisotopically labeled) substrate into PtdCho was approximately 10-fold higher (Table I). Activation of membrane-bound cholinephosphotransferase exhibited a linear dependence on the amount of PtdCho transfer protein added (Fig. 2), regardless of reaction incubation period (data not shown). PtdCho transfer protein from rat and bovine liver is able to stimulate de novo PtdCho synthesis (Table I). While these two proteins differ significantly in chemical properties, molecular size, isoelectric point, and immunogenicity (Poorthuis et al., 1980; Westerman et al., 1983; Teerlink et al., 1983), each catalyzes exclusively the transfer of PtdCho. Notably, rat PtdIns transfer protein failed...
to stimulate PtdCho synthesis, even though this protein can also facilitate the intermembrane movement of PtdCho (Venuti and Helmkamp, 1988). Cholinephosphotransferase activation is, therefore, highly specific for PtdCho transfer protein.

Relative contributions of substrates to PtdCho synthesis in stimulated and unstimulated microsomes revealed that the ratio of CDP-choline and OleGro were equivalent and close to one (Table II). In the presence of PtdCho transfer protein, however, the transferred PtdCho displayed a contribution of 1 mol of CDP-choline for each 2.2 mol of OleGro. This difference may arise from acyl chain remodeling of PtdCho molecules. It has been reported that the majority of newly formed PtdCho in the hamster heart is subsequently remodeled by deacylation-reacylation processes (Arthur and Choy, 1984). Alternatively, the difference may suggest a selective acyl chain specificity of PtdCho transfer protein. Indeed, Welti and Helmkamp (1984) have clearly demonstrated that dioleoyl phosphatidylcholine was among the best substrates for bovine PtdCho transfer protein.

Kinetic analysis of de novo PtdCho synthesis and intermembrane transfer revealed distinct temporal differences. An immediate stimulation of PtdCho synthesis occurred in the presence of PtdCho transfer protein, but transfer of PtdCho from microsomes to acceptor vesicles was delayed for several minutes. The lag observed in protein-catalyzed transfer may reflect the transition of de novo synthesized PtdCho from a non-transferable to a transferable pool in the microsomal membrane. It should be stressed that it is experimentally difficult to quantitate the protein-catalyzed intra- and intermembrane transport of synthesized PtdCho.

We have routinely used small unilamellar vesicles as model membranes in phospholipid transfer systems. The activation of cholinephosphotransferase in rat liver microsomes by PtdCho transfer protein could be effectively blocked by the addition of such vesicles. We interpret this finding as a competitive inhibition by a limiting amount of PtdCho transfer protein for different membrane surfaces. In the present experiments the small unilamellar vesicle would function as an acceptor membrane for PtdCho transferred from a microsome donor membrane. Although there was a drastic reduction in the stimulation of PtdCho synthesis, we found a near-linear increase in protein-mediated transfer of PtdCho as more vesicles were added (Fig. 3B). Such a result could be anticipated from previous kinetic analysis of PtdCho transfer protein activity (Welti and Helmkamp, 1984; Yoshimura et al., 1988).

Phospholipid transfer proteins may play a role in determining the phospholipid composition of eukaryotic cell membranes (Harvey et al., 1974), in membrane biogenesis (Wirtz, 1974), and in synaptic transmission of nerve impulses (Michell, 1975). The activation of cholinephosphotransferase is specific for PtdCho transfer protein and is diminished by competing small unilamellar vesicles. It is likely that the stimulatory effect of PtdCho transfer protein can be attributed to a direct interaction of cholinephosphotransferase with PtdCho transfer protein at the cytoplasmic surface of the endoplasmic reticulum. In consideration of the immediate stimulation of PtdCho synthesis, the association between cholinephosphotransferase and PtdCho transfer protein to form a complex should be of moderate affinity. The formation of this hypothetical complex is clearly reversible, since significant transfer of microsomal PtdCho to vesicles is observed and PtdCho transfer protein functions catalytically through a water-soluble, protein-PtdCho intermediate (Wirtz, 1982). Several consequences may result from the protein-protein interaction: control of substrate availability through an alteration of the \( K_m \) or \( V_{max} \) for one or both substrates, or, as is more probable, relief of end-product inhibition. It has been conclusively demonstrated that the cholinephosphotransferase reaction is reversible (Kanoh and Ohno, 1973; Infante, 1984; Tsao, 1986). Therefore, removal of the reaction end-products, PtdCho and CMP, is critical to continued synthetic activity. The newly synthesized PtdCho molecule may be transported through the intervention of PtdCho transfer protein either laterally within the cytoplasmic face, where it becomes available for transfer to other cell membranes, or transversely to the luminal face of the endoplasmic reticulum, where it could participate in lipoprotein assembly and secretion. These possible mechanisms of enhanced synthetic rates are to be addressed in future experiments.

It is significant that the phospholipid transfer protein with the most restrictive substrate specificity, PtdCho transfer protein, is present primarily in liver and small intestinal mucosa (Lutton and Zilversmit, 1976; Yamada et al., 1978; Teerlink et al., 1982), two tissues with pronounced lipid secretory activity. That the stimulation of PtdCho synthesis resides solely with the level of PtdCho transfer protein in a tissue is further demonstrated by the observation that microsomal cholinephosphotransferase prepared from rat brain, a tissue with extremely low PtdCho transfer protein, could nevertheless be stimulated by rat liver PtdCho transfer protein. Recently, Venuti and Helmkamp (1986) reported that rat brain CDP-diacylglycerol:inositol phosphatidyltransferase, the terminal enzyme in the de novo synthesis of PtdIns, was unaffected by the presence of rat brain PtdIns transfer protein. In conclusion, we have provided evidence that cholinephosphotransferase, an enzyme localized to the endoplasmic reticulum, can be stimulated by cytosolic PtdCho transfer protein. This represents a previously unrecognized activity of PtdCho transfer protein and constitutes a novel regulatory step in the synthesis of PtdCho in selected mammalian tissues.

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