Base exchange reactions of the phospholipids in rat brain particles

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Abstract A particulate fraction from rat brain catalyzes the incorporation of [^{14}C]choline, [^{14}C]ethanolamine, and L-[^{14}C]serine into phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine, respectively. The reaction appears to be energy-independent since Mg^{2+}, CTP, ATP, and NaF have no stimulatory action. The incorporation is inhibited by EDTA and activated by Ca^{2+}. The pH optimum for the incorporation of choline is 9.5, for ethanolamine it is 9.0, and for L-serine it is 8.5. Tris, bicine, and imidazole buffers are inhibitory. The incorporations are inhibited by a variety of structurally related alcohols and are stimulated by isoserine (α-hydroxy,β-aminopropionic acid).

Supplementary key words phosphatidylserine · phosphatidylcholine · phosphatidylethanolamine · base incorporation · isoserine · Ca^{2+} stimulation · rat brain microsomes

THE BIOSYNTHESIS of phosphatidylserine in mammalian tissues appears to occur solely by an "exchange" of L-serine for the ethanolamine moiety of phosphatidylethanolamine (1), based on studies employing a rat liver homogenate. The products in this system were reported to be 33% phosphatidylserine and 66% phosphatidylethanolamine, presumably due to the presence of an active mitochondrial phosphatidylserine decarboxylase in the homogenate. The incorporation of ethanolamine into phosphatidylethanolamine was also reported in this system. This type of non-energy-dependent incorporation of bases into the corresponding phospholipid has been reported to occur in locust flight muscle (2), housefly fat bodies (3), *Tetrahymena* (4), several mammalian tissues (5–10), and pea seedlings (11). The present publication documents some properties of a particulate fraction from rat brain which catalyzes a similar "exchange" reaction.

Abbreviations: TLC, thin-layer chromatography.

MATERIALS AND METHODS

Rats of the Charles River strain were killed at approximately 1 month of age; the brains were removed and homogenized in 4 ml of 0.25 M sucrose/g wet wt of tissue. The homogenate was centrifuged at 10,000 *g* for 30 min and the supernate was saved. The pellet was rehomogenized in a volume of 0.25 M sucrose equal to that removed and the new homogenate was centrifuged at 10,000 *g* for 30 min. The supernate was decanted and pooled with the previous one, and the mixture was centrifuged at 35,000 *g* for 1 hr. The supernatant solution was discarded; the pellet was suspended in 1 ml of 0.25 M sucrose/g wet wt of brain and was used as the enzyme source.

The following incubation mixtures were routinely employed: for serine incorporation, L-[U-^{14}C]serine (New England Nuclear, spec. act. 210,000 cpm/nmole), 1.2×10^6 total cpm, 1 μmole of $CaCl_2$, 10 μmoles of *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPS) buffer, pH 8.5, and 1 μmole of glutathione; for ethanolamine incorporation, [1,2-^{14}C]ethanolamine (International Chemical & Nuclear, spec. act. 170,000 cpm/nmole), 7.5×10^5 total cpm, 0.2 μmole of $CaCl_2$, 10 μmoles of cyclohexylaminopropane sulfonic acid (CAPS) buffer, pH 9.0, and 1 μmole of glutathione; for choline incorporation, [*Me*-^{14}C]choline (Amersham/Searle, spec. act. 100,000 cpm/nmole) 1.1×10^6 total cpm, 10 μmoles of HEPS buffer, pH 9.5, and 0.2 μmole of $CaCl_2$. 100–250 μg of protein in a total volume of 0.2 ml was used in all incubations. These substrate concentrations were not always saturating. At the end of the 30-min incubation at 37°C, 3.5 ml of methanol was added and

the samples were processed as previously described (12). Boiled enzyme and zero-time controls carried through this procedure did not contain appreciable radioactivity in the final chloroform phase and these values were close to background. Radioactivity present in the final chloroform phase was determined in a Packard model 3380 scintillation counter. Quenching was monitored by using the automatic external standard on this instrument.

TLC was carried out either on Analtech silica gel G plates with chloroform–methanol–water 65:25:4 as solvent or on Brinkmann F plates with chloroform–methanol–acetic acid–water 25:15:4:2 as the solvent system. The radioactive materials were located with a Berthold thin-layer radioscanner (Varian).

Phosphatidic acid, phosphatidylserine, phosphatidylcholine, and phosphatidylethanolamine chromatographic standards were purchased from Supelco, Inc., Bellefonte, Pa., and gave only one spot when examined by TLC. Other reagents were of commercial origin. Phospholipases A and C were purchased from Sigma Chemical Co., St. Louis, Mo., and phospholipase D was from Koch-Light Labs Ltd., Colnbrook, England. D-[^{14}C]Serine was obtained from Amersham/Searle, [^{14}C]-dimethylethanolamine from both International Chemical & Nuclear and New England Nuclear, and *myo*-[^{3}H]inositol from New England Nuclear.

RESULTS

Product identification

To obtain sufficient material for chromatographic identification of the products, incubations were increased 10-fold over that given in the section on Methods. Aliquots of the final chloroform phases were chromatographed on thin-layer plates and the results are presented in Fig. 1. These data indicate that the radioactive product obtained in each incubation was as follows. With [^{14}C]ethanolamine (lane *2*) the product cochromatographed with the phosphatidylethanolamine standard (lane *1*); with L-[^{14}C]serine (lane *4*) the product cochromatographed with the phosphatidylserine standard (lane *3*); and with [^{14}C]choline (lane *6*) the product cochromatographed with the phosphatidylcholine standard (lane *5*). It should be noted that there was no indication for the formation of phosphatidylethanolamine from incubation mixtures containing L-[^{14}C]-serine under these assay conditions. These separations have also been observed on silica gel G plates with chloroform–methanol–water 65:25:4 as solvent. However, with this system phosphatidylserine yields a diffuse band and does not give a sharp peak. Samples from the incubation mixtures with the different substrates were also subjected to a mild deacylation procedure (13), and virtually all of the original radioactivity was recovered in the aqueous phase. The water-soluble glycerylphosphoryl bases were then examined by paper chromatography, employing phenol-saturated water–acetic acid–ethanol 50:5:6 with standards prepared from the intact phospholipids. The radioactive material derived from the [^{14}C]choline, L-[^{14}C]serine, and [^{14}C]ethanolamine incubations cochromatographed with glycerylphosphorylcholine, glycerylphosphorylserine, and glycerylphosphorylethanolamine, respectively.

pH curve

The effect of varying pH of the buffer is shown in

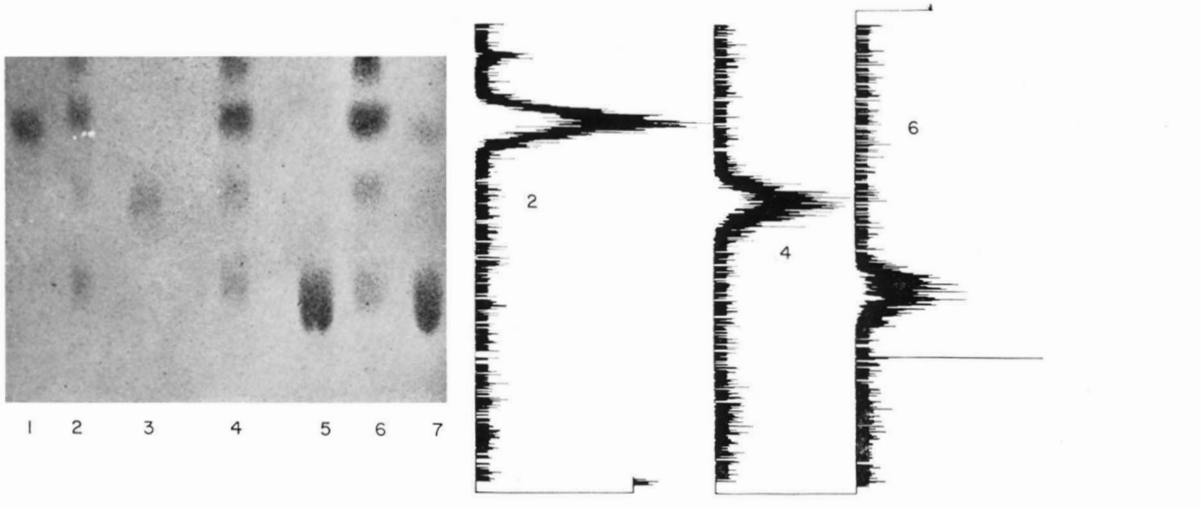

FIG. 1. TLC and radioactive scans of the lipid-soluble reaction products. Lane *1*, phosphatidylethanolamine standard; lane *2* and scan *2*, [^{14}C]ethanolamine incubation; lane *3*, phosphatidylserine standard; lane *4* and scan *4*, L-[^{14}C]serine incubation; lane *5*, phosphatidylcholine standard; lane *6* and scan *6*, [^{14}C]choline incubation; lane *7*, mixed standards. Brinkmann F plate, chloroform–methanol–acetic acid–water 25:15:4:2 as solvent.

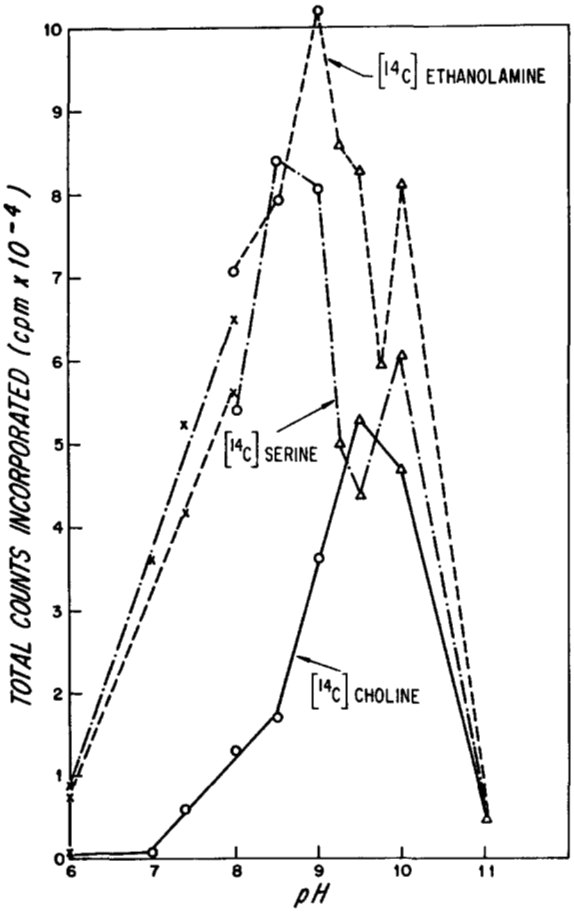

FIG. 2. pH curve for incorporation of [14C]ethanolamine, L-[14C]serine, and [14C]choline. ×, phosphate buffer; O, HEPS buffer; △, CAPS buffer.

Fig. 2. [14C]Ethanolamine incorporation was found to have a biphasic curve, with a maximum at pH 9.0 and a smaller peak at pH 10.0; for incorporation of L-[14C]-serine there were peaks at pH 8.5 and pH 9.75, while [14C]choline incorporation had a single peak at pH 9.5. The products were identical at all pH values examined, as judged by TLC. Tris, bicine, and imidazole buffers at the same pH and concentration were found to be much less effective, giving incorporation values of from 20 to 50% of the value found with HEPS or CAPS, depending upon the substrate employed.

Role of metals

The incorporation of the labeled bases was appreciably reduced when EDTA was added to the incubation mixture; this suggested the presence of a metal in the enzyme preparation. Addition of Ca^{2+} caused a stimulation (Table 1). In order to further document this observation, studies were undertaken to remove metals from the enzyme system. Samples of the particulate preparation were dialyzed for either 2 consecutive days against 0.25 M sucrose or 1 day against 0.25 M sucrose–1.25 mM EDTA, followed by dialysis for 1 day against 0.25 M sucrose at 4°C. It is evident from the data presented in Table 2 that L-[14C]serine incorporating activity is completely restored by the addition of Ca^{2+} to the sucrose–EDTA-dialyzed particles, while incorporation of [14C]ethanolamine and [14C]choline is restored to only 25% and 50%, respectively, of the nondialyzed $+Ca^{2+}$ activity. Addition of Mn^{2+}, Cd^{2+}, or Mg^{2+} to these incubations caused no recovery of activity. The data thus suggest a specific role of Ca^{2+} in this system.

TABLE 1. Effects of EDTA and Ca^{2+} on incorporation of L-[14C]serine, [14C]ethanolamine, and [14C]choline into phospholipids[a]

	L-[14C]-Serine	[14C]Ethanolamine	[14C]-Choline
	cpm	*cpm*	*cpm*
Incubation mixture − Ca^{2+}	51,016	208,208	12,964
+ EDTA (2.5 μmoles/ml) − Ca^{2+}	770	5,264	238
+ Ca^{2+} (5 μmoles/ml)	99,176	247,646	48,384

[a] Details are provided in the text.

Effect of phospholipase treatment

The effect of several phospholipases on the incorporation of L-[14C]serine and [14C]ethanolamine into lipid is presented in Table 3. Phospholipase A treatment caused a significant loss of incorporation with both substrates, while phospholipases C and D resulted in an increase of [14C]ethanolamine incorporation. L-[14C]Serine incorporation was reduced by phospholipase D treatment and unaffected by phospholipase C treatment. TLC examination revealed major reductions of the parent endogenous phospholipids present in these particles. The effects of adding phosphatidic acid, phosphatidylserine, phosphatidylethanolamine, and phosphatidylcholine to either the phospholipase-treated or untreated preparations were variable and inconsistent.

TABLE 2. Effect of dialysis of the particulate preparation against 0.25 M sucrose–1.25 mM EDTA and 0.25 M sucrose on incorporation of L-[14C]serine, [14C]ethanolamine, and [14C]choline into phospholipids[a]

	L-[14C]-Serine	[14C]Ethanolamine	[14C]-Choline
	cpm	*cpm*	*cpm*
Nondialyzed − Ca^{2+}	46,295	183,066	23,147
Nondialyzed + Ca^{2+}	119,346	247,406	72,356
Sucrose-dialyzed	41,006	209,360	2,748
Sucrose-dialyzed + Ca^{2+}	96,721	263,114	51,930
EDTA–sucrose-dialyzed	1,556	1,925	471
EDTA–sucrose-dialyzed + Ca^{2+}	111,332	51,997	31,657

[a] Details are provided in the text.

TABLE 3. Effect of phospholipase treatment of brain particles on L-[14C]serine and [14C]ethanolamine incorporation

	L-[14C]-Serine	[14C]Ethanolamine
	nmoles	
Untreated	0.252	0.66
Phospholipase A-treated[a]	0.054	0.30
Phospholipase C-treated[b]	0.26	1.23
Phospholipase D-treated[c]	0.097	0.98

The mixtures were incubated at 25°C for 20 min, at which time 30 ml of 0.25 M sucrose was added. The samples were well mixed and centrifuged for 1 hr at 30,000 *g* and the particles were suspended in 2.0 ml of 0.25 M sucrose. Aliquots were assayed for the ability to incorporate the radioactive substrates as described in the text.

[a] Particles were suspended in 1.6 ml of 0.25 M sucrose, 0.8 ml of 0.05 M Tris, pH 7.5, 8.0 μmoles of $CaCl_2$, 1 mg of serum albumin, and 1.5 mg of phospholipase A.

[b] Particles were suspended in 2 ml of 0.154 M KCl–0.5 M Tris pH 7.4–0.5 mM $CaCl_2$ and 1.5 mg of phospholipase C.

[c] Particles were suspended in 1.5 ml of 0.2 M acetate buffer pH 5.5, 0.3 ml of 1 M $CaCl_2$, 5 mg of phospholipase D, and 0.2 ml of ether.

TABLE 4. Effects of certain structural analogs of L-[14C]serine, [14C]ethanolamine, and [14C]choline on incorporation into phospholipids[a]

	L-[14C]-Serine	[14C]Ethanolamine	[14C]-Choline
	percentage change[b]		
L-Serine	−92.3	−91	−87
D-Serine	−59	−67	−65
Ethanolamine	−78	−88	−72
Choline	−50	−96	−92
Monomethylethanolamine	−86	−67	−93
Dimethylethanolamine	−70	−15	−87
Isoserine	+50	+70–100	+67
Serinol	−84	−63	−80
Threonine	−26	0	−22

[a] Details are provided in the text. The final concentration of all compounds added was 2.5 mM.

[b] Compared with control values.

Effect of certain structural analogs

The data presented in Table 4 indicate that several alcohols structurally related to the substrates possess the capacity to inhibit the incorporation of the radioactive precursors into phospholipids. Choline inhibits the incorporation of [14C]ethanolamine more than that of L-[14C]serine, while the reverse situation was found with dimethylethanolamine. Isoserine (α-hydroxy,β-aminopropionic acid) consistently stimulated ethanolamine, serine, and choline incorporation; the effects of various concentrations of isoserine are presented in Fig. 3. It is apparent that even at relatively high concentrations there is no inhibition.

Exchange reaction

In order to examine the possibility that the reaction studied was an exchange of the polar portion of the lipid with the labeled alcohols, a two-stage incubation was carried out. Particles were incubated with radioactive L-serine, ethanolamine, or choline for 10 min under standard conditions. The samples were then diluted with 0.25 M sucrose and centrifuged at 100,000 *g* for 30 min, and the supernates were discarded. The pellets were then rediluted with 0.25 M sucrose and recentrifuged at 100,000 *g* for 30 min. Each of the three preparations was then suspended separately in sucrose and was found to contain 4.4×10^4 counts as phosphatidylserine, 1×10^4 counts as phosphatidylcholine, or 3×10^4 counts as phosphatidylethanolamine. The preparations were then incubated under the standard conditions in the absence of isotope, alone or in the presence of nonradioactive L-serine, choline, or ethanolamine at concentrations of 2.5 μmoles/ml. The amount of radioactivity present in the aqueous layer after the partitioning was then quantitated; these results are presented in Table 5. It is readily apparent that water itself is very effective in catalyzing the release of radioactivity from the prelabeled particles. L-Serine appears to be a bit more active than ethanolamine in releasing radioactivity from the [14C]ethanolamine-labeled particles. Ethanolamine, however, appears slightly more effective than L-serine in releasing radioactivity from the L-[14C]serine-labeled particles. Choline, however, appears to inhibit the release of radioactivity by water alone.

These observations were further pursued in the following manner. Young rats, 10–12 days of age, were injected intracranially with L-[14C]serine, [14C]choline, or [14C]ethanolamine. 4 hr later brains were removed and the particles were isolated as usual. Standard incubations were carried out comparing the release of water-soluble radioactivity by water alone or by the addition of nonradioactive L-serine, choline, and ethanolamine. The results of these studies also indicated that the augmentation of release of radioactivity by serine and ethanolamine was only slightly more than water itself, and choline depressed this release.

Other properties

Lineweaver-Burk plots are presented for [14C]ethanolamine (Fig. 4), [14C]choline (Fig. 5), and L-[14C]serine (Fig. 6) incorporation. The calculated K_m values for the individual substrates, as well as K_i values for their respective inhibitions of each other's incorporation, are presented in Table 6.

Proportionality of substrate incorporation with time is shown in Fig. 7. In Fig. 8 is presented the effect of varying the quantity of enzyme protein in the presence and absence of isoserine on incorporation of labeled substrates.

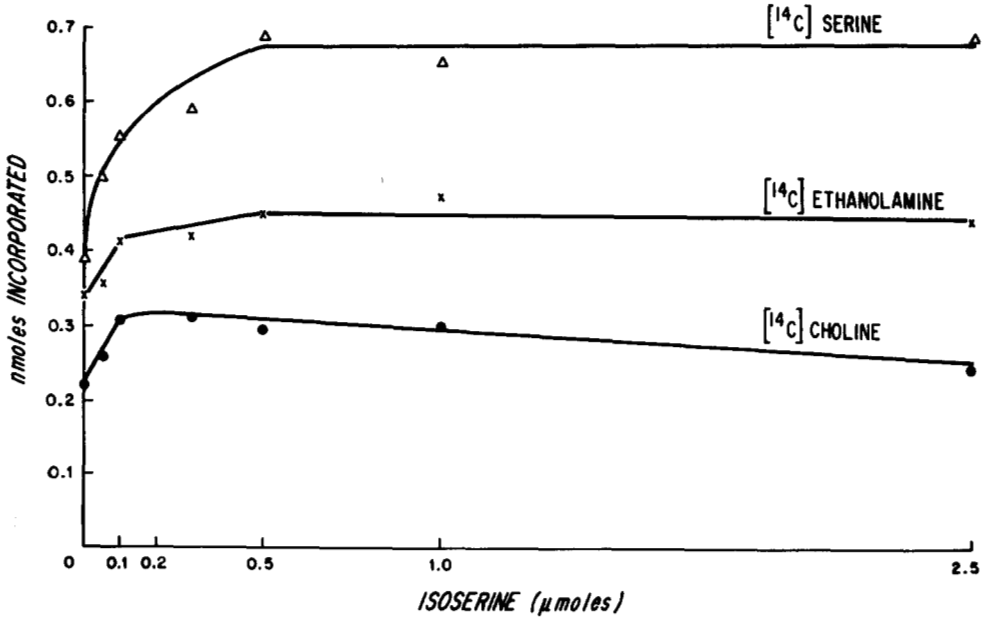

FIG. 3. Effect of varying isoserine concentration on incorporation of [14C]ethanolamine, L-[14C]serine, and [14C]choline.

The L-[14C]serine-incorporating activity was found to be stable for several weeks after freezing and thawing. However, [14C]choline incorporation was rapidly lost, 50% in 3–4 days, while [14C]ethanolamine incorporation decreased 50% in 7–10 days. The [14C]ethanolamine activity was found to be stabilized by freezing the particles in glycerol–water 1:1, while the [14C]choline activity was retained by particles lyophilized from an aqueous suspension.

The inhibition of the incorporation of labeled precursors into phospholipids by several detergents is presented in Table 7. It is apparent that [14C]ethanolamine incorporation is less sensitive than that of [14C]-choline and L-[14C]serine to all the materials employed.

D-Serine, as indicated in Table 4, inhibits the incorporation of L-[14C]serine, [14C]ethanolamine, and [14C]choline into phospholipids. When incubations were carried out with D-[14C]serine as the lipid precursor, the radioactive product cochromatographed with the phosphatidylserine standard as well as with the product obtained with L-[14C]serine incorporation. [14C]Dimethylethanolamine, which is an inhibitor as shown in Table 4, was incubated with the particles. A material cochromatographing with phosphatidylethanolamine was observed when this incubation mixture was subjected to chromatography on silica gel G employing chloroform–methanol–7 N NH_4OH 60:35:5 as solvent. In addition, a second radioactive peak was found in the area corresponding to phosphatidylcholine. Chromatographic examination of the starting material

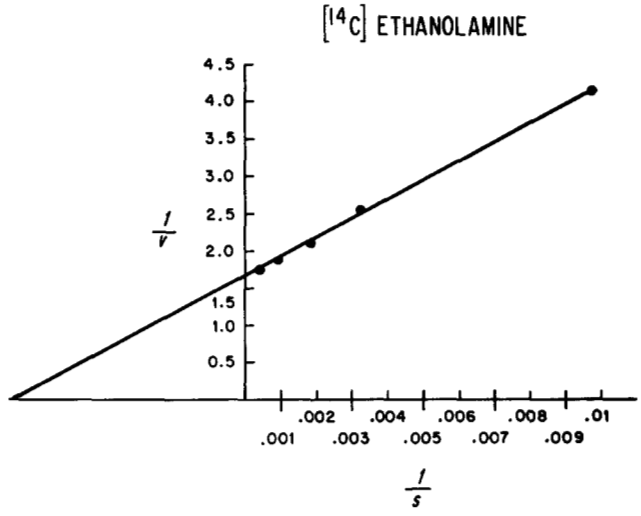

FIG. 4. Lineweaver-Burk plot of effect of varying substrate concentration on [14C]ethanolamine incorporation.

TABLE 5. Base "exchange" reaction of phospholipids[a]

	Particles from 1st Incubation with:		
	L-[14C]-Serine	[14C]-Choline	[14C]Ethanolamine
	counts liberated into aqueous phase		
Alone	4872	1106	4662
+ L-Serine (0.5 μmole)	6272	532	6412
+ Choline (0.5 μmole)	28	514	266
+ Ethanolamine (0.5 μmole)	7686	316	5726

[a] Details are provided in the text.

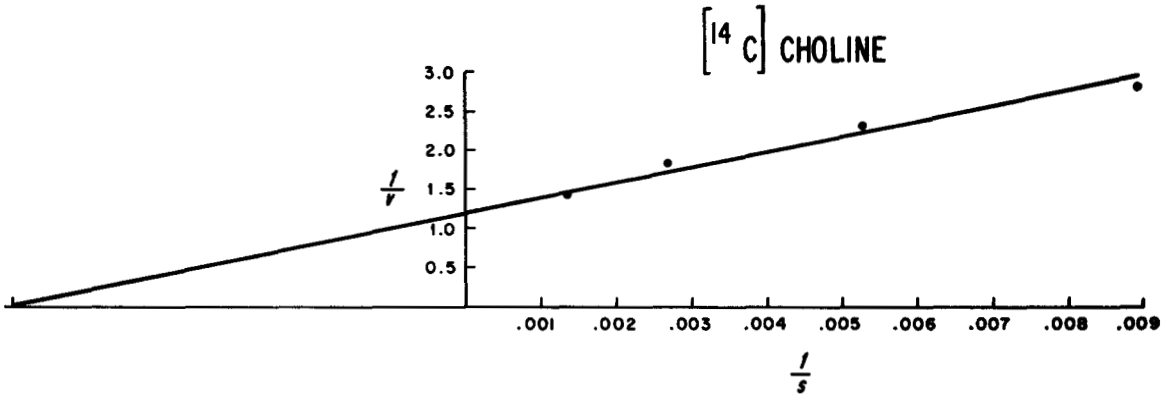

FIG. 5. Lineweaver-Burk plot of effect of varying substrate concentration on [^{14}C]choline incorporation.

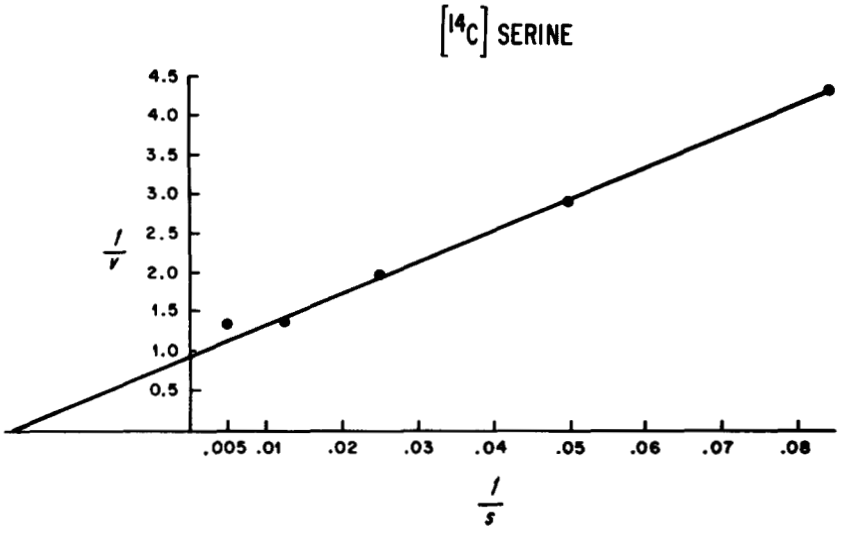

FIG. 6. Lineweaver-Burk plot of effect of varying substrate concentration on L-[^{14}C]serine incorporation.

revealed the presence of a material migrating with choline standards. Under similar conditions, *myo*-[^{3}H]inositol was not incorporated into phospholipid.

DISCUSSION

The particulate fraction from rat brain described has the capacity to catalyze the direct incorporation of [^{14}C]ethanolamine into phosphatidylethanolamine, [^{14}C]-choline into phosphatidylcholine, and L-[^{14}C]serine into phosphatidylserine. Under these present experimental conditions there was no effect by the addition of sodium fluoride, ATP, or CTP to the incubation mixtures. Phosphatidylethanolamine formation was not observed with L-[^{14}C]serine as substrate, indicating that phosphatidylserine decarboxylase activity is absent under these experimental conditions. The pH optimum of the incorporation was slightly different for each substrate, and Tris and imidazole buffers were found to be inhibitory for this system.

This system is somewhat similar to that recently reported for chick brain microsomes (10). L-[^{14}C]Serine and [^{14}C]ethanolamine were found to be incorporated directly into their corresponding phospholipids. The authors presented data on the stimulation by Ca^{2+} and slight inhibition by Mg^{2+} in the presence of Ca^{2+}. However, [^{14}C]choline was reported not to be utilized by their preparations. There are several possible reasons for this observation. These authors employed Tris or

TABLE 6. K_m and K_i values for incorporation of [^{14}C]choline, [^{14}C]ethanolamine, and L-[^{14}C]serine into phospholipids

	Radioactive Substrate		
	[^{14}C]Choline	[^{14}C]Ethanolamine	L-[^{14}C]Serine
K_m	1.9×10^{-4} M	1.5×10^{-5} M	4.2×10^{-5} M
K_i ethanolamine	5.7×10^{-4} M		5.7×10^{-5} M
K_i L-serine	1.0×10^{-3} M	1.0×10^{-3} M	
K_i choline			4.0×10^{-5} M

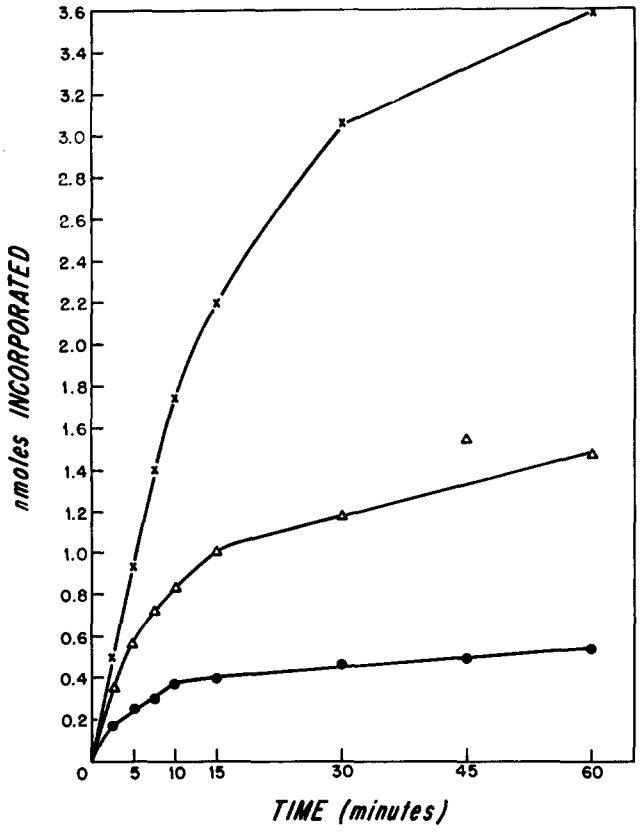

FIG. 7. Proportionality of [^{14}C]ethanolamine (△), L-[^{14}C]serine (●), and [^{14}C]choline (×) incorporation with time of incubation.

Veronal buffer at pH 8.0; however, in the present report this measures only 20% of the total [^{14}C]choline incorporation since the optimum is 9.0 (Fig. 2). The K_m for [^{14}C]choline in the particles employed in the present study is nearly 10-fold that for ethanolamine. In the other study (10) cysteine, which is a very potent inhibitor of [^{14}C]choline incorporation but stimulates L-[^{14}C]-serine and [^{14}C]ethanolamine incorporation of rat brain particles, was present in the incubations.

Several compounds related to the substrates were tested for their inhibitory activity (Table 4). Similar inhibitions were found with serinol, mono- and dimethylethanolamine, and D-serine. Isoserine was found to stimulate the incorporation of the three substrates 50–100%. The cause of this stimulation is unexplained, since several structural analogs of isoserine, such as lactic acid and diaminopropionic acid, were found to have little effect on this system.

It was found that phospholipase A treatment caused loss of enzymatic activity for both L-serine and ethanolamine, as shown in Table 3. This effect may be due to the formation of lysophosphatides which possess detergent properties. As indicated in Table 7, the presence of several detergents resulted in decreased enzymatic

TABLE 7. Effect of detergents on incorporation of [^{14}C]choline, [^{14}C]ethanolamine, and L-[^{14}C]serine into phospholipids[a]

	[^{14}C]-Choline	[^{14}C]-Ethanolamine	L-[^{14}C]-Serine
	% inhibition		
Sodium cholate (1 μmole)	74	44	85
Sodium deoxycholate (1 μmole)	98	38	97
Sodium taurocholate (1 μmole)	97	72	97
Tween 20 (100 μg)	55	8	50

[a] Details are provided in the text.

activity. Phospholipase C treatment resulted in a stimulation of ethanolamine incorporation without any effect on L-serine activity. Phospholipase D resulted in an increased incorporation of ethanolamine and a decrease in L-serine incorporation. Porcellati et al. (10) reported that phospholipase D was without effect when added to their incubation mixture. It is tempting to speculate that these changes are due to alterations of the endogenous phospholipid acceptors. However, further data are required to prove this.

The mechanism of direct incorporation of these substrates into their respective phospholipids is a matter of speculation. It has been hypothesized that this activity may merely represent the reversal of phospholipase D activity (7). This hypothesis is based largely upon the common Ca^{2+} (6) stimulation as well as the demonstrated transphosphatidylation reaction catalyzed by this enzyme (14–16). However, this mechanism is not thought to be probable, largely due to the reported absence of phospholipase D in mammalian tissues (17). Therefore, these incorporations are thought to be specific "exchanges" or "interchanges" of the polar portion of nitrogen-containing phospholipids. The possibility does exist, however, that this is a reflection of phospholipase D activity under circumstances where it is necessary for both the enzyme and the substrate to be part of the same architectural or structural unit.

Attempts to demonstrate a specific displacement of base-labeled particles prepared either in vitro or in vivo by nonradioactive substrates have been equivocal. This is due to the efficient displacement of radioactivity by water itself; this could be interpreted as phospholipase D activity (Table 5). Ethanolamine was a bit more effective than L-serine in releasing radioactivity from L-[^{14}C]-serine-labeled particles. Similarly, L-serine was slightly more effective than ethanolamine in releasing radioactivity from [^{14}C]ethanolamine-labeled particles. Choline depressed the release of radioactivity from prelabeled particles. It should be noted that similar experiments carried out with both *Tetrahymena* (4) and chick brain (10) have indicated a specific release of [^{14}C]-

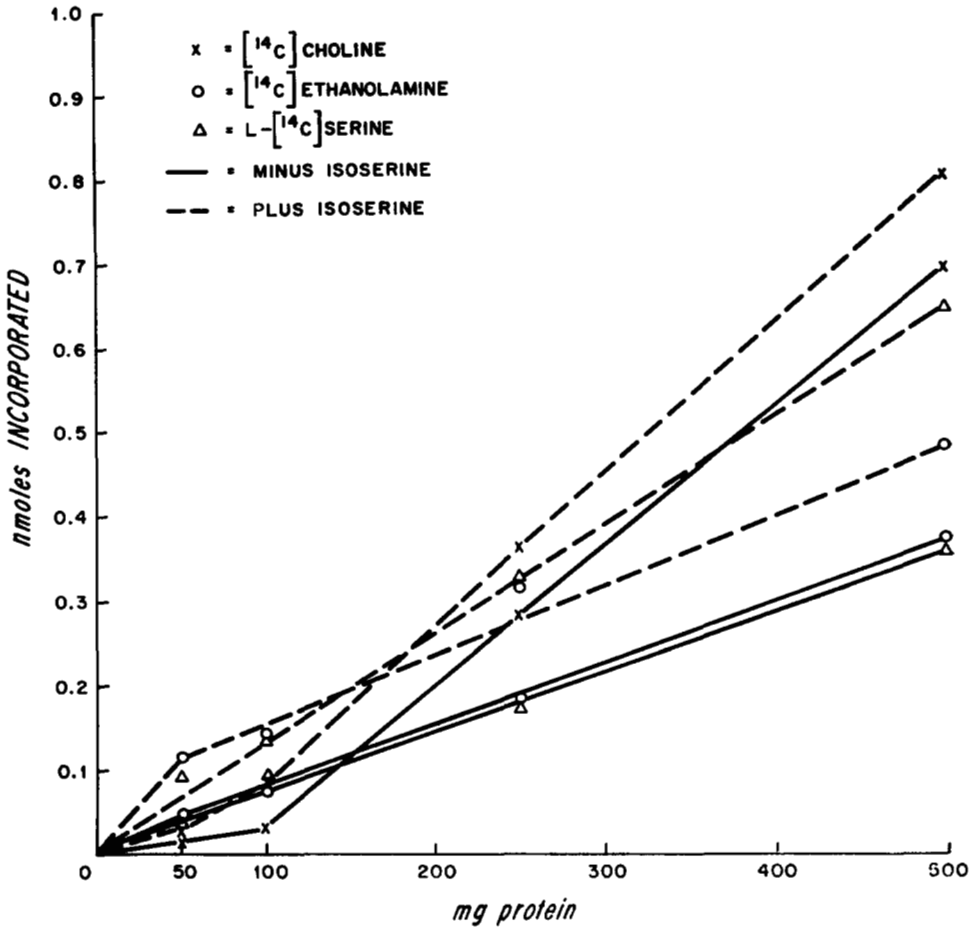

FIG. 8. Proportionality of [14C]ethanolamine, L-[14C]serine, and [14C]choline incorporation in the presence and absence of isoserine with increasing amounts of enzyme protein.

ethanolamine, presumably from phosphatidylethanolamine-labeled particles by L-serine.

The K_m values obtained for the individual substrate would suggest that ethanolamine has the strongest affinity and choline the weakest for the enzyme (Table 6) (5). Inhibition experiments indicated that all three substrates are competitive inhibitors of each other. Difficulty has been encountered with the kinetics of choline inhibition of [14C]ethanolamine incorporation since stoichiometry between the degree of inhibition and amount of inhibitor added was not attained.

There are similarities in the properties of the incorporation reactions: all three are inhibited by EDTA and stimulated by Ca^{2+} (Tables 1 and 2), all three are stimulated by isoserine (Table 4), and except for the effect of choline on ethanolamine incorporation the bases are competitive inhibitors (Table 6). Certain differences have been observed for the three substances: pH optimum, degree of inhibition by structural analogs (Table 4) and detergents (Table 7), effect of phospholipase treatment (Table 3), a 10-fold range in K_m values (Table 6), and stability on storage. Glutathione inhibited choline incorporation but stimulated L-serine and ethanolamine incorporation. The question as to whether these incorporations represent three distinct catalytic proteins or a single protein with different properties for three substrates will be solved only by further purification of the enzyme(s).

In vivo studies from two laboratories have indicated that the specific activity of hepatic lecithin is greater than that of phosphorylcholine and CDP choline after administration of [14C]choline (18, 19). These results have been interpreted as an in vivo demonstration of the direct "exchange" or "interchange" of the nitrogen-containing portion of the phospholipids.

The physiological significance, if any, of this reaction is obscure. It is tempting to speculate that this may function in the transport of the bases into the cell. Alternately, this may be a mechanism to either alter or maintain the phospholipid composition of biological structures in response to different physiological states.

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