

Enzymic synthesis of ether types of choline and ethanolamine phosphoglycerides by microsomal fractions from rat brain and liver

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Abstract The formation of product by ethanolaminephosphotransferases (EC 2.7.8.1) and cholinephosphotransferases (EC 2.7.8.2) in microsomal fractions from brains and livers of mature rats is increased several fold by 1,2-diacyl-*sn*-glycerols. With the addition of 1-alkyl-2-acyl-*sn*-glycerols, we have found an 11-fold increase with brain microsomes and a 20-fold increase with liver microsomes in the synthesis of choline ether lipids (1-alkyl-2-acyl- and 1-alk-1'-enyl-2-acyl-*sn*-glycero-3-phosphorylcholines). For the synthesis of ethanolamine ether lipids (1-alkyl-2-acyl- and 1-alk-1'-enyl-2-acyl-*sn*-glycero-3-phosphorylethanolamines), the stimulation of alkylacylglycerols was 7-fold for brain microsomes and 18-fold for liver microsomes. The alkylacyl glycerols (8 mM) also inhibited the synthesis of diacyl phosphoglycerides by 44 to 65%, indicating that the same ethanolaminephosphotransferases and cholinephosphotransferases are utilized for the synthesis of alkylacyl phosphoglycerides and diacyl phosphoglycerides. A desaturation of the alkyl groups may take place in the same reaction mixture. The rate of incorporation of phosphorylcholine into alkenylacyl glycerophosphorylcholines (choline plasmalogens) with alkylacylglycerols, cytidine diphosphate choline, and liver microsomes was 15 nmoles per mg protein per hour. The in vitro synthesis of choline plasmalogens with alkylacylglycerols had not been observed previously. The corresponding rate of incorporation of phosphorylethanolamine into ethanolamine plasmalogens was 10 nmoles per mg protein per hour, a value greater than any of the previously reported values for ethanolamine plasmalogen formation from alkylacyl glycerophosphorylethanolamines.

Supplementary key words cholinephosphotransferase (EC 2.7.8.2) · ethanolaminephosphotransferase (EC 2.7.8.1) · plasmalogen · phosphatidylethanolamine · phosphatidylcholine · cytidine-5'-diphosphate choline · cytidine-5'-diphosphate ethanolamine · 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylcholines · 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylethanolamines

Diacyl-GPE and diacyl-GPC are formed in liver and brain by the reactions catalyzed by CDP-ethanolamine:1,2-diacyl-*sn*-glycerol ethanolaminephospho-

transferase (EC 2.7.8.1) and CDP-choline:1,2-diacyl-*sn*-glycerol cholinephosphotransferase (EC 2.7.8.2) respectively (1–5). A similar reaction results in the synthesis of alkylacyl-GPE from CDP-ethanolamine and 1-alkyl-2-acyl-*sn*-glycerols with rat brain microsomes (6). Alkylacyl-GPE may be precursors of alkenylacyl-GPE, the ethanolamine plasmalogens (7–10). Neither the in vitro synthesis of alkylacyl-GPC, an ubiquitous trace component of mammalian tissues (10), nor the conversion of alkylacyl-GPC to alkenylacyl-GPC has been described. The incorporation of phosphorylcholine into both types of compounds has been demonstrated with microsomes from rat brain and rat liver in the present study.

METHODS

Rat brain and liver microsomes

Brains and livers were removed from rats with a body weight of 200–220 g after ether anesthesia and decapitation. The microsomes were prepared as previously described (6) except that the centrifugations were done in a MSE Superspeed 50 centrifuge (MSE Limited, London, England) with a 30 rotor. The microsomal preparations were placed in a freezer at –20°C for one month or less. The protein content

Abbreviations: GPC, *sn*-glycero-3-phosphorylcholines; GPE, *sn*-glycero-3-phosphorylethanolamines; TLC, thin-layer chromatography.

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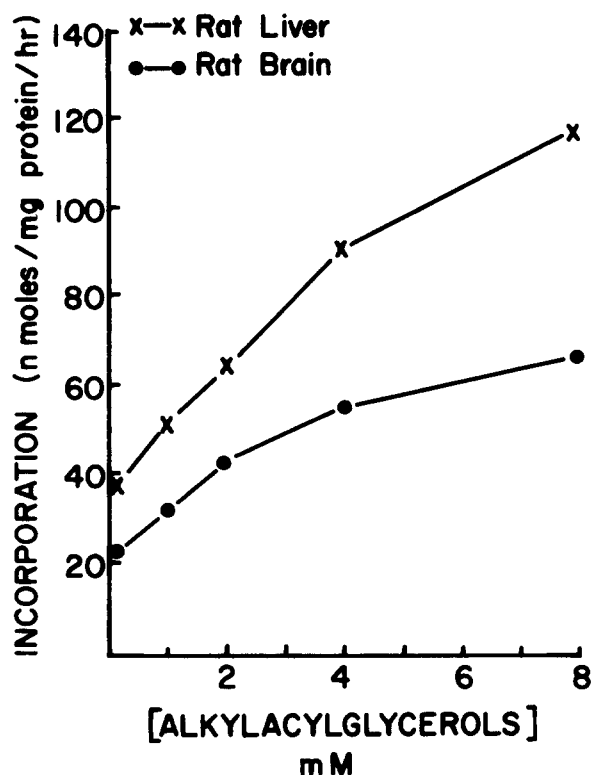


Fig. 1. Effect of alkylacylglycerol concentrations on the incorporation of radioactivity from CDP-[^{14}C]choline into lipid by microsomes from rat liver and rat brain. Assays were performed as described in Table 1, except that the alkylacylglycerol concentrations varied as indicated in the figure.

was determined (11) with bovine serum albumin as the standard.

Alkylacylglycerols and diacylglycerols

Chimera monstrosa (ratfish) liver oil (Western Chemical Industries, Ltd., Vancouver, Canada) was subjected to lipolysis as previously described (6). The bile salts were from Polfa Laboratories, Warsaw, Poland, and the pancreatic lipase was obtained from Sigma Chemical Company, St. Louis, MO. The 1-alkyl-2-acyl-*sn*-glycerols were separated by TLC on a 0.5 mm layer of silica gel G (E. Merck A. G., Darmstadt, Germany) by development with 90:10:8 (by v/v) toluene-acetone-trimethyl borate (K. Light, Colnbrook, Bucks, England). Trace amounts of alkenylacylglycerols were also present in this product. The 40 mM alkylacylglycerol emulsion was prepared in 0.1 M Tris-HCl pH 7.4, containing 0.1% Tween 20, sonicated (MSE Ultrasonic Disintegrator, London, England) for 2 min and used for the experiments reported in Tables 1 and 2 and Figs. 1 and 2.

Another portion of ratfish liver oil was placed in a round-bottom flask which was then filled with HCl gas. After 5 min, the flask was flushed with N_2 and the

liver oil was subjected to lipolysis (6). Another portion of ratfish liver oil was saponified. The alkylglycerols were separated by preparative TLC and reduced with tritium (New England Nuclear, Boston, MA). The [^3H]alkylglycerols were acylated with linoleoyl chloride in the presence of pyridine. The alkyldiacylglycerols were then subjected to lipolysis (6). No alkenylacylglycerols were present in these products. The 6.6 mM alkylacylglycerol emulsions were prepared in 50 mM Tris-HCl pH 7.6 containing 0.05% Tween 20, sonicated (Biosonik IV, Bronwill, Rochester, NY) for 2 min and used for incubations reported in Table 3.

The 1,2-diacyl-*sn*-glycerols were prepared from egg lecithin (E. Merck A. G., Darmstadt, Germany) by treatment with phospholipase C (*Clostridium welchii*, Calbiochem, Los Angeles, CA) according to Renkonen (12). The isolation by preparative TLC and the preparation of the 40 mM emulsion were as described above for alkylacylglycerols.

Preparation of labeled compounds

[Me- ^{14}C]Choline chloride (2.1 mCi/mmmole) was obtained from the Institute of Nuclear Research,

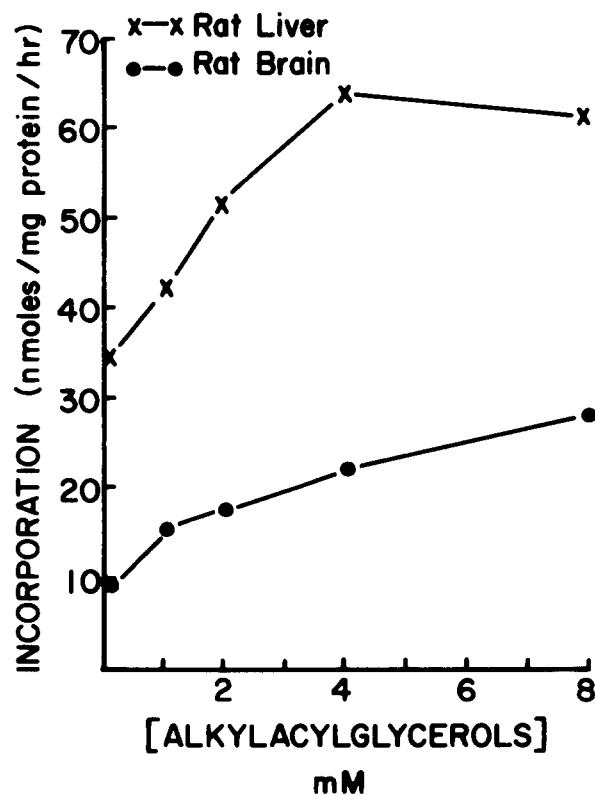


Fig. 2. Effect of alkylacylglycerol concentrations on the incorporation of radioactivity from CDP-[^{14}C]ethanolamine into lipid by microsomes from rat liver and rat brain. Assays were performed as described in Table 2, except that the alkylacylglycerol concentrations varied as indicated in the figure.

Swierk, Poland, and [1,2-¹⁴C]ethanolamine (3.19 mCi/mmol) was obtained from the Radiochemical Centre, Amersham, Bucks, England. The preparation of [¹⁴C]phosphorylcholine and [¹⁴C]phosphorylethanolamine was as described by Ansell and Chojnacki (13). Cytidine-5'-diphosphate-[Me-¹⁴C]choline was prepared using diacylcyclohexylcarbodiimide (Ralph Emanuel Ltd., Wembley, England) as the coupling reagent according to Kennedy (14). Cytidine-5'-diphosphate-[1,2-¹⁴C]ethanolamine was prepared by the method of Chojnacki and Metcalfe (15) with CTP (Sigma Chemical Company). The purification of the product was performed in all cases by chromatography on Dowex 1 × 8 (formate form, Serva, Feinbiochemica, Heidelberg, Germany). The amounts of CDP-[¹⁴C]choline and CDP-[¹⁴C]ethanolamine were assayed spectrophotometrically at 260 and 280 nm. For the incubations in Table 3, these nucleotides were purchased (ICN, Irvine CA).

Assay of cholinephosphotransferase and ethanolaminephosphotransferase activities

Assay mixtures were incubated as indicated in the tables and figures. Reactions were stopped after 30 min of incubation by the addition of 3.0 ml of chloroform-methanol 2:1 (v/v). Lipids were extracted as described previously (6). The radioactivity was assayed either with the total lipids or after separation-reaction-separation TLC as described previously (6). For the TLC, carrier lipids from rat brain or rabbit heart were added for the separation of ethanolamine phosphoglycerides or choline phosphoglycerides. Samples were counted in a Nuclear-Chicago ISOCAP/

TABLE 1. Stimulation of incorporation of radioactivity from CDP-[¹⁴C]choline into choline phosphoglycerides by addition of diradylglycerols

Addition	Diacyl-GPC	Alkenyl-acyl-GPC	Alkylacyl-GPC	nmol/mg protein/hr			
				Brain			
None	12.4	4.4	0.8				
8 mM Alkylacylglycerols	4.3	9.7	47.4				
8 mM Diacylglycerols	57.6	4.6	1.7				
				Liver			
None	31.4	3.4	1.5				
8 mM Alkylacylglycerols	17.6	14.8	83.8				
8 mM Diacylglycerols	127.5	3.6	1.1				

Incubation mixture: 75 mM Tris-HCl, pH 7.6; 30 mM MgCl₂; 0.01% Tween 20; 3.00 mM CDP-[¹⁴C]choline (1.20 × 10⁵ dpm); 0.10 mM dithiothreitol and 0.50 mg of microsomal protein from Wistar rat brain or liver. Incubation was for 30 min at 37°C in a final volume of 0.20 ml. Each value represents the mean of four experiments, each with a different microsomal preparation and emulsions.

TABLE 2. Stimulation of incorporation of radioactivity from CDP-[¹⁴C]ethanolamine into ethanolamine phosphoglycerides by addition of diradylglycerols

Addition	Diacyl-GPE	Alkenyl-acyl-GPE	Alkylacyl-GPE	nmol/mg protein/hr			
				Brain			
None	7.1	2.1	0.1				
8 mM Alkylacylglycerols	2.9	3.5	11.9				
8 mM Diacylglycerols	32.2	2.7	0.3				
				Liver			
None	26.9	2.9	0.3				
8 mM Alkylacylglycerols	12.6	10.5	46.6				
8 mM Diacylglycerols	54.1	3.9	0.6				

Assays were performed as described in Table 1 except that the nucleotide substrate was 3.50 mM CDP-[¹⁴C]ethanolamine (1.02 × 10⁵ dpm). Each value represents the mean of two experiments.

300 liquid scintillation system in Bray's scintillation fluid (16). The results in Table 3 were obtained as described previously (6) or by counting of the ¹⁴CO₂ recovered after combustion of samples in a Model 306 sample oxidizer (Packard Instruments Co., Downers Grove, IL). Radioautograms were prepared with No-Screen X-ray film (Wicor-XRP Ceaverken AB, Strängnäs, Sweden). *K_m* values were determined as described by Eisenthal and Cornish-Bowden (17).

RESULTS

Synthesis of ether types of EPG or CPG

The addition of alkylacylglycerols increased both ethanolaminephosphotransferase and cholinephosphotransferase activities (Figs. 1 and 2). For the brain ethanolaminephosphotransferase, the results agree with Fig. 4 in ref. 6. In that study the brain enzyme had a *K_m* of about 2 mM and was saturated by 8 mM alkylacylglycerols. The liver enzyme appears to be saturated by 4 mM alkylacylglycerols. In the case of the cholinephosphotransferases, the liver and brain enzymes are saturated by 8 mM alkylacylglycerols. The *K_m* values for CDP-choline are 0.36 mM for liver and 0.30 mM for brain. Cholinephosphotransferase activities were linear with time for at least 30 min.

The effects of alkylacylglycerols and diacylglycerols on the synthesis of different types of choline phosphoglycerides and ethanolamine phosphoglycerides were compared (Tables 1 and 2). As found previously for the brain ethanolaminephosphotransferase (6), the addition of 8 mM alkylacylglycerols inhibited the formation of diacyl phosphoglycerides, increased the

TABLE 3. Stimulation of incorporation of radioactivity from [¹⁴C] nucleotides into choline and ethanolamine phosphoglycerides by addition of purified alkylacylglycerols

Addition	Diacyl-GPC	Alkenylacyl-GPC	Alkylacyl-GPC
None	3.15 ± 0.60	0.77 ± 0.20	0.19 ± 0.07
1.1 mM Alkylacylglycerols	8.40 ± 1.02	1.68 ± 0.41	15.65 ± 3.42
Addition	Diacyl-GPE	Alkenylacyl-GPE	Alkylacyl-GPE
None	3.85 ± 1.29	2.43 ± 0.32	0.51 ± 0.06
1.1 mM Alkylacylglycerols	6.02 ± 1.91	3.33 ± 1.46	12.26 ± 3.14

Incubation mixture: 58 mM Tris-HCl, pH 8.0; 20 mM MgCl₂ and 0.67 mM CDP-[¹⁴C]choline (4.84 × 10⁵ dpm) or 10 mM MnCl₂ and 0.78 mM CDP-[¹⁴C]ethanolamine (5.39 × 10⁵ dpm); 0.008% Tween 20; 0.08 mM dithiothreitol and 0.70 mg of microsomal protein from Sprague-Dawley rat brain. Incubation was for 30 min at 39°C in a final volume of 0.30 ml. Each value represents the mean ± SD of five incubations of which three were done with synthetic alkylacylglycerols and two were done with alkylacylglycerols from HCl-treated ratfish liver oil.

formation of alkenylacyl phosphoglycerides, and greatly increased the formation of alkylacyl phosphoglycerides. Since alkenylacyl-GPE may be formed from alkylacyl-GPE (10), we have calculated the stimulation by alkylacylglycerols from the sum of the rates for these two ether lipids. With brain microsomes, alkylacylglycerols stimulated choline ether lipid formation 11-fold and ethanolamine ether lipid formation 7-fold with inhibitions of 65% and 59% for the formation of diacyl-GPC and diacyl-GPE, respectively. Diacylglycerols stimulated the formation of diacyl-GPC and diacyl-GPE 4.6-fold and 4.5-fold, respectively. Ether lipid formation by the phosphotransferases in liver microsomes was stimulated 20-fold and 18-fold, respectively, for choline and ethanolamine phosphoglycerides by the addition of alkylacylglycerols. For the formation of diacyl-GPC and diacyl-GPE, the inhibition by alkylacylglycerols was 44% and 53% and the stimulation by diacylglycerols was 4.1-fold and 2.0-fold, respectively.

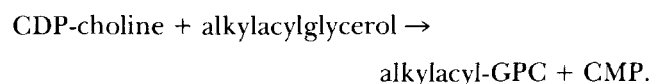
Alkylacylglycerols lacking the trace impurity of alkenylacylglycerols were prepared by two different methods in order to determine if the alkenylacylglycerols were responsible for the increased incorporation of [¹⁴C]ethanolamine or [¹⁴C]choline into ethanolamine or choline plasmalogens (Table 3). In these incubations, the alkylacylglycerol concentrations were much lower. The addition of alkylacylglycerols greatly increased the incorporation of radioactivity into alkylacyl phosphoglycerides, but was not sufficient to inhibit the incorporation of radioactivity into diacyl phosphoglycerides. The 37% increase in incorporation into alkenylacyl-GPE in the presence

of alkylacylglycerols was not significant, but the 118% increase in incorporation into alkenylacyl-GPC with alkylacylglycerols was significant ($t = 4.22, P < 0.01$).

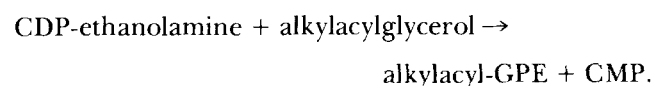
DISCUSSION

Ether lipid synthesis

We have found an enzyme in the microsomal fraction from rat brains and liver that catalyzes the reaction:




In addition, the corresponding ethanolamine enzyme was found in rat liver microsomes:



The presence of both reactions in preputial gland tumor microsomes was demonstrated indirectly (18) and the latter was found previously in rat brain microsomes (6). In reference 6, the reported enzyme activities were twice the correct value. Thus, the present value of 15.4 nmoles/mg protein per hr for the formation of ethanolamine ether lipid with alkylacylglycerols is in excellent agreement with the earlier value (6) of 15.8 nmoles/mg protein per hr. These enzyme activities were greater with liver microsomes than with brain microsomes even though the concentration of ether phosphoglycerides in rat liver is very low. Only 2–3% of liver phospholipids are plasmalogens (10). The small amount of liver plasmalogens, together with the high synthetic capability of the liver, may indicate a rapid turnover of ether lipids, particularly choline plasmalogens, or a lower availability of alkylacylglycerols in the liver.

A dehydrogenation of both alkylacyl-GPE and alkylacyl-GPC to alkenylacyl-GPE and alkenylacyl-GPC, respectively, is suggested by the increase of plasmalogen labeling with added alkylacylglycerols. When alkylacylglycerols were not added, more than two-thirds of ether lipid radioactivity was in the plasmalogen form. Under these conditions, the supply of alkylacyl phosphoglycerides may be rate-limiting for plasmalogen formation. When alkylacylglycerols were added, alkylacyl phosphoglycerides accumulated and less than one-fourth of the ether lipid radioactivity was in the plasmalogen form. Call and Rubert reported similar results for formation of ethanolamine ether lipids with platelet dispersions (19). In the presence of alkylacylglycerols, the alkylacyl phosphoglyceride desaturase may be saturated,

because the proportion of ether lipid radioactivity in the plasmalogen form was much less. Small amounts of plasmalogens were probably formed directly from alkenylacylglycerols (20, 21) that were present in the alkylacylglycerol substrate (22). The relative incorporation of radioactivity from CDP-choline or CDP-ethanolamine into alkenylacyl phosphoglycerides in the absence and presence of exogenous alkylacylglycerols was very similar with all preparations of alkylacylglycerols (Tables 1–3). Since the alkylacylglycerols used in Table 3 did not contain alkenylacylglycerols, a preferential incorporation of the alkenylacylglycerols can be ruled out as an explanation for the increased labeling of plasmalogens when alkylacylglycerols were added. The similarity of ethanolamine phosphotransferase results with CDP-ethanolamine labeled with [¹⁴C]ethanolamine (this paper) and with ³²P (6) refutes the suggestion (23) that the incorporation of radioactivity into the alkenylacyl-GPE is due to a Ca²⁺-stimulated base-exchange reaction. An exchange of phosphorylcholine or phosphorylethanolamine moieties is a possible explanation for the incorporation of radioactivity into alkenylacyl phosphoglycerides. However, the increased incorporation into plasmalogens was found only with exogenous alkylacylglycerols and not with exogenous diacylglycerols (Tables 1, 2).

The rate for ethanolamine plasmalogen formation with liver microsomes, CDP-ethanolamine, and alkylacylglycerols in this study is higher than any values previously reported for ethanolamine plasmalogen synthesis in vitro with the immediate precursor, alkylacyl-GPE (7–9, 23–28). The desaturase reaction may be stimulated by CDP-ethanolamine and/or the alkylacyl-GPE may be more available for dehydrogenation if it has been synthesized at a microsomal site than if it is added to the reaction mixture in micellar solution. 

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