Enzymic synthesis of 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylethanolamines by the CDP-ethanolamine:1-radyl-2-acyl-*sn*-glycerol ethanolaminephosphotransferase from microsomal fraction of rat brain

Anna Radominska-Pyrek* and Lloyd A. Horrocks

Department of Physiological Chemistry, The Ohio State University, Columbus, Ohio 43210

Abstract The incorporation of radioactivity from cytidine-5′-phosphate-[^{32}P]phosphorylethanolamine into 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylethanolamines and 1,2-diacyl-*sn*-glycero-3-phosphorylethanolamines was stimulated more than fourfold by 1-alkyl-2-acyl-*sn*-glycerols and 1,2-diacyl-*sn*-glycerols, respectively, with an ethanolaminephosphotransferase (EC 2.7.8.1) present in the microsomal fraction from brains of mature rats. The K_m values, 0.28 mM for CDP-ethanolamine and 1.9 mM for 1-alkyl-2-acyl-*sn*-glycerols, were similar to those obtained by other investigators with other 1-radyl-2-acyl-*sn*-glycerols. The formation of 1,2-diacyl-*sn*-glycero-3-phosphorylethanolamines from endogenous 1,2-diacyl-*sn*-glycerols was inhibited by 1-alkyl-2-acyl-*sn*-glycerols. These properties indicate that the ethanolaminephosphotransferase lacks specificity for the type of group at the 1-position of the lipid substrate. The synthesis of 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylethanolamines from 1-alkyl-2-acyl-*sn*-glycerols and CDP-ethanolamine by an enzyme from rat brain supports the inclusion of this reaction in the metabolic pathway for the synthesis of 1-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphorylethanolamines.

Supplementary key words 1,2-diacyl-*sn*-glycero-3-phosphorylethanolamines · 1-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphorylethanolamines · 1-alkyl-2-acyl-*sn*-glycerols · CDP-ethanolamine:1,2-diacyl-*sn*-glycerol ethanolaminephosphotransferase (EC 2.7.8.1) · *Chimera monstrosa* liver oil · 1,2-diacyl-*sn*-glycerols · ethanolamine phosphoglycerides · ethanolamine plasmalogens · glycerol ethers

THE PATHWAY for the biosynthesis of alkenylacyl-GPE (1, 2) includes the incorporation of fatty alcohols into alkyl dihydroxyacetone phosphates (3–9). In the presence of a phosphatase and CDP-ethanolamine, microsomes from preputial gland tumors can convert alkylacyl-GP to alkylacyl-GPE (10). The enzymic synthesis of alkenylacyl-GPE has been described in cell-free systems from tumors with fatty alcohol, dihydroxyacetone phosphate, coenzyme A, ATP, Mg^{2+}, $NADP^+$ or NAD^+, microsomes, and a soluble fraction (11, 12) or with alkylacyl-GP substituted for the first three components (13). The incorporation of fatty alcohols into alkenyl groups was first shown with a heart–lung preparation (14) and rat brain dispersions (15). From the above in vitro studies and from in vivo studies in brain tissue (16–26), the most likely metabolic sequence (1) is alkylacyl-GP, alkylacylglycerol, alkylacyl-GPE, alkenylacyl-GPE. A possible sequence of alkylacylglycerol, alkenylacylglycerol, alkenylacyl-GPE is unlikely because in vivo studies with [^{14}C]ethanolamine have shown that the labeling of alkenylacyl-GPE is not proportional to the labeling of alkylacyl-GPE at short times after injection (25) and a labeled pool of alkenylacylglycerols cannot be detected after intracerebral injections of [^{14}C]hexadecanol (26). Alkylacyl-GPE accounts for

Abbreviations: alkenylacylglycerols, 1-alk-1′-enyl-2-acyl-*sn*-glycerols; alkenylacyl-GPE, 1-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphorylethanolamines; alkylacylglycerols, 1-alkyl-2-acyl-*sn*-glycerols; alkylacyl-GP, 1-alkyl-2-acyl-*sn*-glycero-3-phosphate; alkylacyl-GPE, 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylethanolamines; CDPE, cytidine-5′-diphosphate ethanolamine; CMP-^{32}PE, cytidine-5′-phosphate-[^{32}P]phosphorylethanolamine; diacylglycerols, 1,2-diacyl-*sn*-glycerols; diacyl-GPE, 1,2-diacyl-*sn*-glycero-3-phosphorylethanolamines; EPG, ethanolamine phosphoglycerides; TLC, thin-layer chromatography.

* Permanent address: Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland.

1–3% of the phospholipids from brains of man (27), rat (28–31), mouse (32), and chicken (33).

Diacyl-GPE is formed in brain tissue by the reaction catalyzed by CDP-ethanolamine:1,2-diacyl-*sn*-glycerol ethanolaminephosphotransferase (EC 2.7.8.1) in vitro (34–37) and in vivo (38, 39). When microsomes are incubated with labeled CDP-ethanolamine and Mg^{2+}, labeled EPG is formed due to the presence of endogenous diradylglycerols. An alkylacyl-GPE fraction with a very high specific radioactivity has been reported (34), but this result was due to a contaminant in the CDP-ethanolamine preparation (36). The formation of diacyl-GPE and alkenylacyl-GPE is stimulated many-fold by the appropriate exogenous diradylglycerols (34–37, 40), but saturated dialkylglycerols are not incorporated into dialkyl-GPE (34, 37). The purpose of the present investigation was to study the properties of the ethanolaminephosphotransferase from rat brain microsomes in the presence of endogenous diradylglycerols and exogenous alkylacylglycerols and diacylglycerols from natural sources in order to determine if alkylacylglycerols can be incorporated into alkylacyl-GPE.

METHODS

Rat brain microsomes

Brains were removed from Fisher rats, 14 wk of age, after ether anesthesia and decapitation. The brain tissue was dispersed in 10 vol of 0.32 M sucrose with a Potter-Elvehjem-type tissue grinder equipped with a Teflon pestle. The dispersion was centrifuged at 1000 *g* for 10 min. The supernate was centrifuged for 20 min at 18,000 *g*, and the resulting supernate was recentrifuged under the same conditions. A microsomal pellet was prepared from the latter supernate by centrifugation for 60 min at 105,000 *g* and was resuspended in fresh 0.32 M sucrose. After another centrifugation for 60 min at 105,000 *g*, the washed microsomal pellet was dispersed in 0.25 M Tris–HCl at pH 8.0 at a concentration equivalent to 500 mg (fresh wt) of brain/ml. All fractions were kept on ice between centrifugations, which were done in a refrigerated Spinco model L centrifuge with a 40 rotor. This dispersion was kept at −15°C up to 2 months with no loss of ethanolaminephosphotransferase activity. The protein content was determined (41) with bovine serum albumin as the standard.

Alkylacylglycerols

Chimera monstrosa (ratfish) liver oil (500 mg, Western Chemical Industries Ltd., Vancouver, Canada) was subjected to lipolysis in a reaction mixture (42, 43) that contained 0.9 M Tris–HCl at pH 8.0, 0.1 mM $CaCl_2$, 0.02% bile salts (Bacto-Oxgall, Difco Laboratories, Detroit, Mich.), and 80 mg of pancreatic lipase (steapsin, Nutritional Biochemicals Corp., Cleveland, Ohio) in a final volume of 10 ml. After incubation for 1 hr at 40°C, the reaction mixture was extracted with two 40-ml portions of diethyl ether. The extract was taken to dryness under N_2, dissolved in hexane, and separated by TLC on a 0.5-mm layer of silica gel HR (E. Merck A.G., Darmstadt, Germany) by development with 90:10 (v/v) toluene–acetone. The band containing 1-alkyl-2-acyl-*sn*-glycerols was eluted with chloroform. The eluate (about 60 mg) was emulsified in 0.1 M Tris–HCl at pH 7.6 containing 0.03% Tween 20 (Emulsion Engineering Inc., Elk Grove Village, Ill.). The 40 mM alkylacylglycerol emulsion was made by warming in a water bath, agitating with a Vortex mixer for 15 min, and sonicating (Blackstone model SS-2, Fisher Scientific Co., Pittsburgh, Pa.) for 1 min.

Diacylglycerols

Lecithin was separated from egg yolk lipids by chromatography on an alumina (M. Woelm, Eschwege, Germany) column. It was then treated with phospholipase C (*Clostridium welchii*, Calbiochem, Los Angeles, Calif.) according to Renkonen (44). The 1,2-diacyl-*sn*-glycerols were isolated by preparative TLC and emulsified as described above for alkylacylglycerols.

Gas–liquid chromatography

Methyl ester derivatives of the acyl groups of the diacylglycerols and alkylacylglycerols were prepared by alkaline methanolysis in the presence of silica gel (45). After purification by TLC, the methyl esters were separated by gas–liquid chromatography (45). Uncorrected peak areas measured with a Kent Chromolog 2 integrator (Kurt J. Lesker Co., Pittsburgh, Pa.) were used to calculate the composition of mixtures.

Preparation of labeled CDP-ethanolamine

Carrier-free [^{32}P]orthophosphate (2 mCi, New England Nuclear, Boston, Mass.) was mixed with unlabeled orthophosphoric acid and reacted with ethanolamine to produce [^{32}P]phosphorylethanolamine, as described by Ansell and Chojnacki (46). After incubation of the purified [^{32}P]phosphorylethanolamine with CTP (Sigma Chemical Co., St. Louis, Mo.) and rat liver cytidylyltransferase (EC 2.7.7.14), the CMP-^{32}PE was separated by chromatography on Dowex 1-X8 ion exchange resin (formate form, Sigma) (47). The amount of CMP-^{32}PE was assayed by phosphorus determinations (48).

Assay of ethanolaminephosphotransferase activity

Assay mixtures were incubated in 15 × 125 mm test tubes with shaking in a water bath. Reactions were stopped by the addition of 3.0 ml of chloroform–methanol

2:1 (v/v) followed by a brief agitation. After the addition of 1.0 ml of 0.73 M NaCl and further agitation, the mixture was centrifuged for 10 min at low speed and the upper phase was discarded. The lower phase was washed with fresh upper phase (49), filtered, and taken to dryness in a stream of N_2. The lipids were then transferred with chloroform to a TLC plate with a 0.5-mm layer of silica gel G (E. Merck A.G.). Two-dimensional TLC with cleavage of the alkenyl groups with HCl between separations (Fig. 1) was used to separate the acyl-GPE derived from alkenylacyl-GPE from the mixture of alkylacyl-GPE and diacyl-GPE (50, 51). The latter mixture was resolved by solvent partition after saponification (27, 51). Samples containing silica gel or water were counted in 10 ml of XDC scintillation mixture (600 ml of 1,4-dioxane, 200 ml of xylene, 600 ml of 2-ethoxyethanol, 112 g of naphthalene, and 4 g of 2,5-*bis*-2-[5-*tert*-butylbenzoxazolyl]-thiophene), and lipid samples were counted in 0.4% 2,5-*bis*-2-[5-*tert*-butylbenzoxazolyl]-thiophene in toluene (24) in a Tri-Carb 3320 liquid scintillation spectrometer (Packard Instrument Co., Des Plaines, Ill.). Quenching was monitored with an external standard. Radioautograms were prepared with No-Screen medical X-ray film (Eastman Kodak Co., Rochester, N.Y.).

FIG. 1. A two-dimensional thin-layer radiochromatogram of rat brain microsomal phosphoglycerides after incubation with CMP-^{32}PE and alkylacylglycerols. The plate was developed vertically with chloroform–methanol–15 N ammonium hydroxide 65:25:4 (v/v/v), dried in a stream of air, exposed to HCl fumes for 5 min, dried again, and developed horizontally with chloroform–methanol–acetone–acetic acid–water 75:15:30:15:7.5 (by volume). The origin is at the lower right corner. Acid-stable ethanolamine phosphoglycerides are to the left of the acyl-GPE derived from alkenylacyl-GPE. The faint spot below the acyl-GPE is due to lyso ethanolamine phosphoglycerides.

RESULTS

Incorporation of radioactivity into ethanolamine phosphoglycerides

Without exogenous diradylglycerols, the washed rat brain microsomes incorporated a substantial amount of radioactivity into diacyl-GPE and alkenylacyl-GPE, but very little radioactivity was found in alkylacyl-GPE (Table 1). In the presence of 8 mM diacylglycerols, a 4.4-fold stimulation was found for diacyl-GPE, with very little change for alkenylacyl-GPE and diacyl-GPE. With 8 mM alkylacylglycerols, a 99-fold stimulation was found for alkylacyl-GPE, a 44% stimulation was found for alkenylacyl-GPE, and a 48% inhibition was found for diacyl-GPE in comparison with the values found without an exogenous lipid substrate. Similar effects on the labeling of ethanolamine phosphoglycerides were obtained in other experiments with some variation due to acyl group migration in the diradylglycerol substrates or to degree of emulsification. An incubation with 1-alkyl-3-acyl-*sn*-glycerols as the only exogenous substrate gave a 17% inhibition of the endogenous ethanolaminephosphotransferase activity. Only 70% of the endogenous activity was observed in the presence of 1-alkyl-2-acyl-*sn*-glycerols when the Tween 20 concentration was increased to 0.03%. The inclusion of egg yolk choline phosphoglycerides or bovine serum albumin in the emulsions did not increase the ethanolaminephosphotransferase activity.

Characterization of diradylglycerols

Small amounts of the 1,3 isomer were observed in the diacylglycerol and alkylacylglycerol preparations after TLC on silica gel HR. With silica gel G, the propor-

TABLE 1. Stimulation of incorporation of radioactivity from CMP-^{32}PE into ethanolamine phosphoglycerides by addition of diradylglycerols

Addition	Diacyl-GPE	Alkenyl-acyl-GPE	Alkylacyl-GPE
	nmoles/mg protein/hr		
None	22.1	6.2	0.2
8 mM Alkylacylglycerols	11.4	8.9	22.7
8 mM Diacylglycerols	96.2	6.5	0.3

Incubation mixture: 75 mM Tris–HCl, pH 8.0, 37 mM $MgCl_2$, 0.0060% Tween 20, 0.53 mM CMP-^{32}PE (8400 dpm), and 0.60 mg of microsomal protein from rat brain. Incubation was for 30 min at 39°C in a final volume of 0.40 ml. The enzymic activities can be converted to units of nmoles/g fresh tissue/hr by multiplication by 12.

TABLE 2. Acyl and alkyl group compositions of the diacylglycerols and alkylacylglycerols

Fraction	Diacylglycerols	Alkylacylglycerols	
	Acyl Groups	Acyl Groups	Alkyl Groups (Ref. 53)
	wt %	*wt %*	*mole %*
14:0[a]		0.6	3.2
16:0	31.6	19.2	16.8
16:1	2.1	2.8	11.5
18:0	12.2	1.3	2.7
18:1	31.8	76.1	63.2
18:2	18.1	0.1	
20:1		1.9	2.0
20:4	4.1		

The diacylglycerols were prepared from egg yolk choline phosphoglycerides and the alkylacylglycerols were prepared from *Chimera monstrosa* liver oil. The values are the averages of duplicate determinations. Qualitatively similar alkyl group compositions were obtained for trimethylsilyl ether derivatives of alkylglycerols from saponification of the alkylacylglycerols.

[a] Number of carbon atoms: number of double bonds.

tions of 1,2 and 1,3 isomers were nearly equal. Since completing this study, we have found that the inclusion of trimethyl borate in the developing solvent prevents acyl group migration on silica gel G (52).

The diacylglycerols used in these experiments contained an equal mixture of saturated and unsaturated acyl groups (Table 2). Nearly one-half of the latter contained two or more double bonds. In the alkylacylglycerols, about three-fourths of both the alkyl and acyl groups were monounsaturated.

Optimal conditions for biosynthesis of alkylacyl-GPE

Determinations of the amount of radioactivity incorporated into the diacyl-GPE and alkenylacyl-GPE as well as the alkylacyl-GPE were necessary because the reaction with endogenous diradylglycerols was significant, even in the presence of saturating concentrations of exogenous alkylacylglycerols. The increase in incorporation of radioactivity into the ethanolamine phosphoglycerides was proportional to the increase in microsomal protein added up to 1.5 mg/ml (Fig. 2). Between 1.5 and 2.4 mg/ml, the incorporation of radioactivity into alkylacyl-GPE was reduced, but the incorporation into alkenylacyl-GPE was increased. The microsomal protein concentration did not exceed 1.5 mg/ml in other experiments. The rate of incorporation of radioactivity into the diacyl-GPE and alkylacyl-GPE was somewhat higher during the first 15 min than during the following 45 min, with a further decrease in rate during the second hour (Fig. 3). These results are similar to those of Ansell and Metcalfe (36) and Porcellati, Biasion, and Pirotta (37) for exogenous diacylglycerols. The incubation time was limited to 30 min in other experiments.

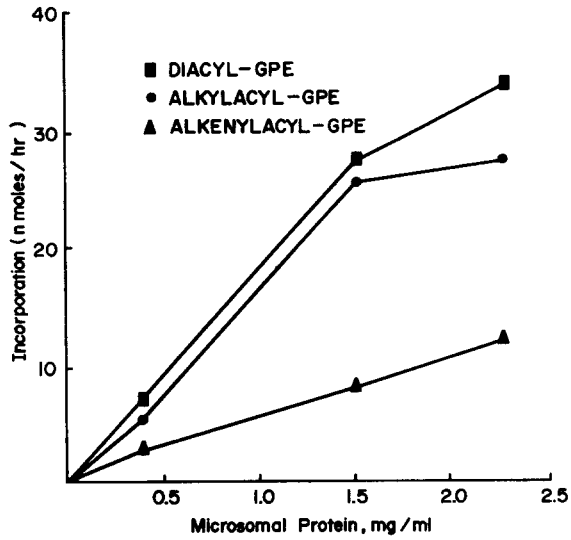

FIG. 2. Effect of protein concentration on the incorporation of radioactivity from CMP-^{32}PE into ethanolamine phosphoglycerides. Incubation mixture: 55 mM Tris-HCl (pH 8.0), 0.0065% Tween 20, 1.17 mM CMP-^{32}PE, 18 mM $MgCl_2$, 8.7 mM alkylacylglycerols, and different amounts of microsomal protein from rat brain. Incubation was for 30 min at 39°C in a final volume of 0.55 ml.

As found previously for diacylglycerols and alkenylacylglycerols (36, 37), high concentrations of alkylacylglycerols may inhibit the ethanolaminephosphotranferase activity (Fig. 4). A Lineweaver-Burk double reciprocal plot was not linear, as was also the case for the incorporation of CMP-^{32}PE into alkylacyl-GPE as a function of CMP-^{32}PE concentrations (Fig. 5). For both substrates, the saturating concentrations and K_m values

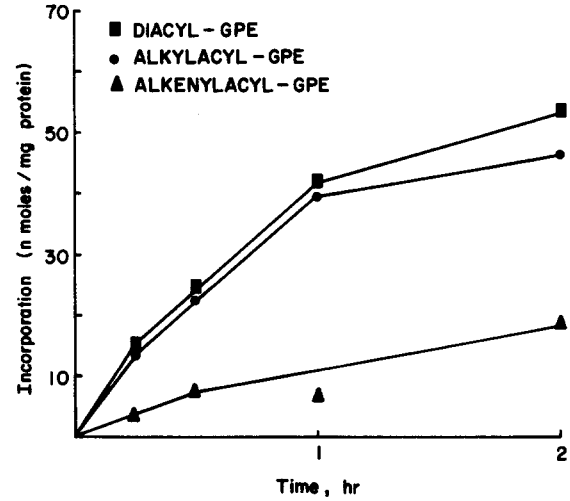

FIG. 3. Effect of time of incubation on the incorporation of radioactivity from CMP-^{32}PE into ethanolamine phosphoglycerides. Incubation mixture: 73 mM Tris-HCl (pH 8.0), 0.0058% Tween 20, 1.04 mM CMP-^{32}PE, 24 mM $MgCl_2$, 7.8 mM alkylacylglycerols, and 0.60 mg of microsomal protein from rat brain. Incubation was for different periods of time at 39°C in a final volume of 0.41 ml.

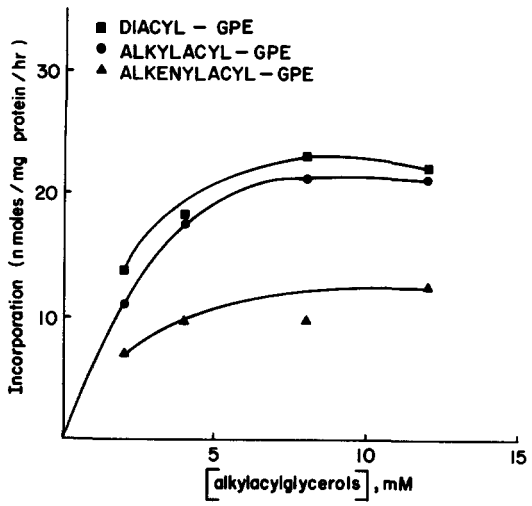

FIG. 4. Effect of concentration of alkylacylglycerols on the incorporation of radioactivity from CMP-^{32}PE into ethanolamine phosphoglycerides. Incubation mixture: 75 mM Tris–HCl, 0.0060% Tween 20, 1.07 mM CMP-^{32}PE, 25 mM $MgCl_2$, different concentrations of alkylacylglycerols, and 0.60 mg of microsomal protein from rat brain. Incubation was for 30 min at 39°C in a final volume of 0.40 ml.

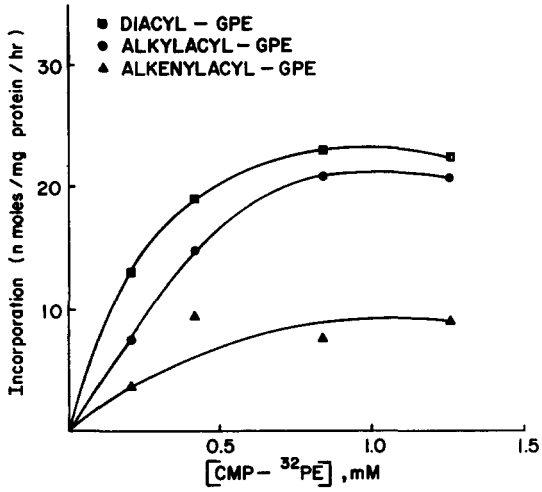

FIG. 5. Effect of CMP-^{32}PE concentration on the incorporation of radioactivity from CMP-^{32}PE into ethanolamine phosphoglycerides. Incubation mixture: 73 mM Tris–HCl, 0.0058% Tween 20, different concentrations of CMP-^{32}PE, 24 mM $MgCl_2$, 7.8 mM alkylacylglycerols, and 0.60 mg of microsomal protein from rat brain. Incubation was for 30 min at 39°C in a final volume of 0.41 ml.

(Table 3) are quite similar to the values found with other radylacylglycerols. Saturating concentrations of alkylacylglycerols and CMP-^{32}PE were used in all other experiments except those shown in Table 1 in which the CMP-^{32}PE concentration was about two times the K_m value. No incorporation of radioactivity into ethanolamine phosphoglycerides was found in the absence of divalent cations. The remainder of the incubation conditions were identical to those specified by Ansell and Metcalfe (36).

TABLE 3. Michaelis constants for CDPE:1-radyl-2-acyl-*sn*-glycerol ethanolaminephosphotransferase (EC 2.7.8.1) from the microsomal fraction of brain tissue

Reference	Animal	Lipid Acceptor	K_m Values	
			CDPE	Diradylglycerol
			M	
36	Rat	Diacylglycerols	2.6×10^{-4}	1.5×10^{-3}
37	Chicken	Diacylglycerols	2.1×10^{-4}	2.1×10^{-3}
36	Rat	Alkenylacylglycerols	2.2×10^{-4}	1.7×10^{-3}
37	Chicken	Alkenylacylglycerols		2.4×10^{-3}
36	Rat	Alkylacylglycerols	2.8×10^{-4}	1.9×10^{-3}

The results reported in Figs. 2–5 were obtained with alkylacylglycerol emulsions that had been prepared several days before incubation. With aged emulsions, the incorporation of radioactivity into diacyl-GPE was always greater than the incorporation of radioactivity into alkylacyl-GPE. In another experiment with a fresh alkylacylglycerol emulsion, the ratio of radioactivity recovered in the diacyl-GPE to the radioactivity recovered in the alkylacyl-GPE was 1.11 with 2 mM alkylacylglycerols, 0.87 with 4 mM alkylacylglycerols, and 0.51 with 8 mM alkylacylglycerols. These results confirm the inhibition of diacyl-GPE formation by fresh alkylacylglycerol emulsions (Table 1).

DISCUSSION

An enzyme is present in the microsomal fraction from rat brains that catalyzes the reaction:

$$\text{CDPE} + \text{alkylacylglycerol} \rightarrow \text{alkylacyl-GPE} + \text{CMP}$$

This enzyme has K_m values and a divalent cation requirement in common with the enzyme described by Porcellati et al. (37) in chicken brain and by Ansell and Metcalfe (36) in rat brain. These similarities and the inhibition of diacyl-GPE formation by alkylacylglycerols indicate that the same enzyme is responsible for the transfer of phosphorylethanolamine from CDP-ethanolamine to any of the three 1-radyl-2-acyl-*sn*-glycerols.

A further reaction

$$\text{alkylacyl-GPE} \rightarrow \text{alkenylacyl-GPE}$$

is suggested by the lack of inhibition of alkenylacyl-GPE formation in the presence of alkylacylglycerols and by the discrepancy in the degree of stimulation of alkylacyl-GPE and diacyl-GPE by exogenous substrates. If the radioactivity found in the alkenylacyl-GPE is included with the radioactivity found in the alkylacyl-GPE, then the degree of stimulation shown in Table 1 is 4.35 for diacylglycerols and 4.93 for alkylacylglycerols. An oxidation of alkylacyl-GPE to alkenylacyl-GPE would also explain the changes in distribution of radioactivity in the EPG during the second hour of incubation (Fig. 3),

the nonlinearity of alkylacyl-GPE formation with protein concentration (Fig. 2), and the small amount of radioactivity found in alkylacyl-GPE after incubations of microsomes with labeled CDPE in the absence of exogenous diradylglycerols. After 30 min of incubation, Porcellati et al. (37) could not detect radioactivity in the alkylacyl-GPE, and we found only 0.8% of the EPG radioactivity in the alkylacyl-GPE. The distribution of radioactivity in the EPG after these incubations should be proportional to the content of the corresponding endogenous diradylglycerols in the microsomes if each type of EPG is formed from the corresponding diradylglycerol. The diacylglycerols from mouse brain have been characterized (54). No chemical or metabolic evidence for the existence of alkenylacylglycerols in mammals have been reported (1), but labeled compounds with the properties of alkylacylglycerols have been detected in mouse brain at short times after intracerebral injections of [^{14}C]hexadecanol (26).

Saturated dialkylglycerols are not incorporated into dialkyl-GPE (34, 37). These observations could be due to difficulties in emulsifying saturated substrates or to a requirement for a 2-acyl group on the diradylglycerol for binding to the enzyme. Dialkyl-GPE or other EPG with two ether bonds are probably not present in brain tissue (1). In order to reflect the apparent substrate specificity, the enzyme should be designated CDP-ethanolamine:1-radyl-2-acyl-*sn*-glycerol ethanolaminephosphotransferase.

The rate observed for the formation of the diacyl-GPE was 36% of the highest rate reported by Ansell and Metcalfe (36). The difference is due to the age of the rats (36) and to differences in CDPE concentrations in the incubation mixture. Similarly, a higher rate of formation of alkylacyl-GPE could probably be obtained with microsomes from younger animals with higher CDPE concentrations. In rat brain, the activity of the enzyme system involved in the formation of alkyl groups is highest at 5 days of age (55).

In order to confirm the metabolic sequence of alkylacylglycerols, alkylacyl-GPE, alkenylacyl-GPE that was proposed on the basis of in vivo studies (1, 25), it is necessary to demonstrate that each reaction can occur in vitro. Snyder, Blank, and Malone (10) produced alkylacylglycerols and alkylacyl-GPE by incubating alkylacyl-GP with microsomes from mouse preputial gland tumors, CDPE, and Mg^{2+}. The alkylacyl-GPE were formed at a rate of 0.19 nmole/mg protein/hr. In this investigation, we have demonstrated that the ethanolaminephosphotransferase from rat brain will catalyze the incorporation of alkylacylglycerols into alkylacyl-GPE at a rate of 22.7 nmoles/mg protein/hr. This reaction, which has been postulated to occur in the metabolic pathway for the biosynthesis of ethanolamine plasmalogens (1, 2), has been assayed in vitro for the first time with alkylacylglycerols as an added substrate. The dehydrogenation of alkylacyl-GPE to alkenylacyl-GPE has been demonstrated in incubation mixtures containing ^{32}P-labeled alkylacyl-GPE and rat brain microsomes (56).

This investigation was supported in part by PHS research grant NS-08291 of the National Institute of Neurological Diseases and Stroke, U.S. Public Health Service.

Manuscript received 14 January 1972; accepted 19 April 1972.

REFERENCES

1. Horrocks, L. A. 1972. Content, composition and metabolism of mammalian and avian lipids that contain ether groups. *In* Ether Lipids: Chemistry and Biology. F. Snyder, editor. Academic Press, New York. In press.
2. Snyder, F. 1972. The enzymic pathways of ether-linked lipids and their precursors. *In* Ether Lipids: Chemistry and Biology. F. Snyder, editor. Academic Press, New York. In press.
3. Hajra, A. K. 1969. Biosynthesis of alkyl-ether containing lipid from dihydroxyacetone phosphate. *Biochem. Biophys. Res. Commun.* **37:** 486–492.
4. Wykle, R. L., and F. Snyder. 1969. The glycerol source for the biosynthesis of alkyl glyceryl ethers. *Biochem. Biophys. Res. Commun.* **37:** 658–662.
5. Snyder, F., B. Malone, and R. B. Cumming. 1970. Synthesis of glyceryl ethers by microsomal enzymes derived from fibroblasts (L-M cells) grown in suspension cultures. *Can. J. Biochem.* **48:** 212–215.
6. Snyder, F., B. Malone, and M. L. Blank. 1970. Enzymic synthesis of *O*-alkyl bonds in glycerolipids. *J. Biol. Chem.* **245:** 1790–1799.
7. Snyder, F., M. L. Blank, B. Malone, and R. L. Wykle. 1970. Identification of *O*-alkyldihydroxyacetone phosphate, *O*-alkyldihydroxyacetone, and diacyl glyceryl ethers after enzymic synthesis. *J. Biol. Chem.* **245:** 1800–1805.
8. Hajra, A. K. 1970. Acyl dihydroxyacetone phosphate: precursor of alkyl ethers. *Biochem. Biophys. Res. Commun.* **39:** 1037–1044.
9. Wykle, R. L., and F. Snyder. 1970. Biosynthesis of an *O*-alkyl analogue of phosphatidic acid and *O*-alkylglycerols via *O*-alkyl ketone intermediates by microsomal enzymes of Ehrlich ascites tumor. *J. Biol. Chem.* **245:** 3047–3058.
10. Snyder, F., M. L. Blank, and B. Malone. 1970. Requirement of cytidine derivatives in the biosynthesis of *O*-alkyl phospholipids. *J. Biol. Chem.* **245:** 4016–4018.
11. Wykle, R. L., M. L. Blank, and F. Snyder. 1970. The biosynthesis of plasmalogens in a cell-free system. *FEBS Lett.* **12:** 57–60.
12. Snyder, F., M. L. Blank, and R. L. Wykle. 1971. The enzymic synthesis of ethanolamine plasmalogens. *J. Biol. Chem.* **246:** 3639–3645.
13. Blank, M. L., R. L. Wykle, and F. Snyder. 1971. Enzymic synthesis of ethanolamine plasmalogens from an *O*-alkylglycerolipid. *FEBS Lett.* **18:** 92–94.
14. Keenan, R. W., J. B. Brown, and B. H. Marks. 1961. Plasmalogen and ester phospholipid biosynthesis in dog-heart-lung preparations. *Biochim. Biophys. Acta.* **51:** 226–229.

15. Keenan, R. W. 1960. Studies on phospholipid biosynthesis with special reference to the plasmalogens. Ph.D. Dissertation, The Ohio State University. University Microfilms 60-6381.
16. Schmid, H. H. O., and T. Takahashi. 1970. Reductive and oxidative biosynthesis of plasmalogens in myelinating brain. *J. Lipid Res.* **11:** 412–419.
17. Bell, O. E., Jr., M. L. Blank, and F. Snyder. 1971. The incorporation of ^{18}O and ^{14}C from long-chain alcohols into the alkyl and alk-1-enyl ethers of phospholipids of developing rat brain. *Biochim. Biophys. Acta.* **231:** 579–583.
18. Stoffel, W., and D. LeKim. 1971. Studies on the biosynthesis of plasmalogens. Precursors in the biosynthesis of plasmalogens: on the stereospecificity of the biochemical dehydrogenation of the 1-*O*-alkyl glyceryl to the 1-*O*-alk-1′-enyl glyceryl ether bond. *Hoppe-Seyler's Z. Physiol. Chem.* **352:** 501–511.
19. Paltauf, F. 1971. Biosynthesis of plasmalogens from alkyl- and alkyl-acyl-glycerophosphoryl ethanolamine in rat brain. *FEBS Lett.* **17:** 118–120.
20. Bickerstaffe, R., and J. F. Mead. 1968. Metabolism of chimyl alcohol and phosphatidyl ethanolamine in the rat brain. *Lipids.* **3:** 317–320.
21. Joffe, S. 1969. Interrelationships among the ethanolamine phosphatides in myelinating rat brain. *J. Neurochem.* **16:** 715–723.
22. Debuch, H., H. Friedemann, and J. Müller. 1970. Über die Bildung der Plasmalogene zur Zeit der Myelinisierung bei der Ratte. III. Einbau von ^{14}C-markiertem *O*-(1,2-Diacyl-bzw. *O*-(1-alkyl-2-acyl-*sn*-glycerin-3-phosphoryl)-äthanolamin in Plasmalogen. *Hoppe-Seyler's Z. Physiol. Chem.* **351:** 613–621.
23. Debuch, H., J. Müller, and H. Fürniss. 1971. Über die Bildung der Plasmalogene zur Zeit der Myelinisierung bei der Ratte. IV. Einbau von ^{14}C-markiertem *O*-(1-alkyl-*sn*-glycerin-3-phosphoryl) äthanolamin—ein direkter Vorläufer der Plasmalogene. *Hoppe-Seyler's Z. Physiol. Chem.* **352:** 984–990.
24. Horrocks, L. A. 1969. Metabolism of the ethanolamine phosphoglycerides of mouse brain myelin and microsomes. *J. Neurochem.* **16:** 13–18.
25. Horrocks, L. A., and G. B. Ansell. 1967. The incorporation of ethanolamine into ether-containing lipids in rat brain. *Lipids.* **2:** 329–333.
26. Horrocks, L. A. 1971. The biosynthesis of plasmalogens from hexadecanol. Third Meeting of the International Society for Neurochemistry. Akademiái Kiadó, Budapest. 312.
27. Panganamala, R. V., L. A. Horrocks, J. C. Geer, and D. G. Cornwell. 1971. Positions of double bonds in the monounsaturated alk-1-enyl groups from the plasmalogens of human heart and brain. *Chem. Phys. Lipids.* **6:** 97–102.
28. Wells, M. A., and J. C. Dittmer. 1967. A comprehensive study of the postnatal changes in the concentration of the lipids of developing rat brain. *Biochemistry.* **6:** 3169–3175.
29. Ansell, G. B., and S. Spanner. 1961. The probable occurrence of long-chain ether analogues of phosphatidylethanolamine in brain tissue. *Biochem. J.* **81:** 36P–37P.
30. Ansell, G. B., and S. Spanner. 1963. The occurrence of a long-chain ether analogue of phosphatidylethanolamine in brain tissue. *Biochem. J.* **88:** 56–64.
31. Horrocks, L. A., and G. B. Ansell. 1967. Studies on the phospholipids of rat brain which contain glyceryl ethers. *Biochim. Biophys. Acta.* **137:** 90–97.
32. Sun, G. Y., and L. A. Horrocks. 1969. The metabolism of palmitic acid in the phospholipids, neutral glycerides and galactolipids of mouse brain. *J. Neurochem.* **16:** 181–189.
33. Porcellati, G., M. G. Biasion, and G. Arienti. 1970. The incorporation of phosphorylethanolamine into the phospholipids of brain microsomes *in vitro*. *Lipids.* **5:** 725–733.
34. McMurray, W. C. 1964. Metabolism of phosphatides in developing rat brain. II. Labelling of plasmalogens and other alkali-stable lipids from radioactive cytosine nucleotides. *J. Neurochem.* **11:** 315–326.
35. Ansell, G. B. 1968. The metabolism of brain phospholipids. *Sci. Basis Med. Annu. Rev.* 383–400.
36. Ansell, G. B., and R. F. Metcalfe. 1971. Studies on the CDP-ethanolamine-1,2-diglyceride ethanolaminephosphotransferase of rat brain. *J. Neurochem.* **18:** 647–665.
37. Porcellati, G., M. G. Biasion, and M. Pirotta. 1970. The labeling of brain ethanolamine phosphoglycerides from cytidine diphosphate ethanolamine *in vitro*. *Lipids.* **5:** 734–742.
38. Ansell, G. B., and S. Spanner. 1967. The metabolism of labelled ethanolamine in the brain of the rat *in vivo*. *J. Neurochem.* **14:** 873–885.
39. Sun, G. Y., and L. A. Horrocks. 1971. The incorporation of [^{14}C]-palmitic acid into the lipids of mouse brain *in vivo*. *J. Neurochem.* **18:** 1963–1969.
40. Kiyasu, J. Y., and E. P. Kennedy. 1960. The enzymatic synthesis of plasmalogens. *J. Biol. Chem.* **235:** 2590–2594.
41. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
42. Mattson, F. H., and R. A. Volpenhein. 1961. The use of pancreatic lipase for determining the distribution of fatty acids in partial and complete glycerides. *J. Lipid Res.* **2:** 58–62.
43. Snyder, F., and C. Piantadosi. 1968. Deacylation of isomeric diacyl [1-^{14}C] alkoxyglycerols by pancreatic lipase. *Biochim. Biophys. Acta.* **152:** 794–797.
44. Renkonen, O. 1966. Individual molecular species of phospholipids. III. Molecular species of ox-brain lecithins. *Biochim. Biophys. Acta.* **125:** 288–309.
45. Sun, G. Y., and L. A. Horrocks. 1968. The fatty acid and aldehyde composition of the major phospholipids of mouse brain. *Lipids.* **3:** 79–83.
46. Ansell, G. B., and T. Chojnacki. 1966. The incorporation of the phosphate esters of *N*-substituted aminoethanols into the phospholipids of brain and liver. *Biochem. J.* **98:** 303–310.
47. Chojnacki, T., and R. F. Metcalfe. 1966. Enzymatic synthesis of labelled cytidine-5′-diphosphate ethanolamine. *Nature.* **210:** 947–948.
48. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
49. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
50. Horrocks, L. A. 1968. The alk-1-enyl group content of mammalian myelin phosphoglycerides by quantitative two-dimensional thin-layer chromatography. *J. Lipid Res.* **9:** 469–472.
51. Horrocks, L. A., and G. Y. Sun. 1972. Ethanolamine plasmalogens. *In* Research Methods in Neurochemistry. R. Rodnight and N. Marks, editors. Plenum Press, New York. 223–231.
52. Pollack, J. D., D. S. Clark, and N. L. Somerson. 1971. Four-directional-development thin-layer chromatography of lipids using trimethyl borate. *J. Lipid Res.* **12:** 563–569.

53. Guyer, K. E., W. A. Hoffman, L. A. Horrocks, and D. G. Cornwell. 1963. Studies on the composition of glyceryl ethers and their preparation from diacyl glyceryl ethers in liver oils. *J. Lipid Res.* **4:** 385–391.
54. Sun, G. Y. 1970. Composition of acyl groups in the neutral glycerides from mouse brain. *J. Neurochem.* **17:** 445–446.
55. Snyder, F., M. Hibbs, and B. Malone. 1971. Enzymic synthesis of *O*-alkyl glycerolipids in brain and liver of rats during fetal and postnatal development. *Biochim. Biophys. Acta.* **231:** 409–411.
56. Horrocks, L. A., and A. Radominska-Pyrek. 1972. Enzymic synthesis of ethanolamine plasmalogens from 1-alkyl-2-acyl-*sn*-glycero-3-(^{32}P)-phosphorylethanolamines by microsomes from rat brain. *FEBS Lett.* **22:** 190–192.