THE BIOSYNTHESIS OF ETHANOLAMINE PLASMALOGENS BY A POSTMITOCHONDRIAL FRACTION FROM RAT BRAIN[1]

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SUMMARY: Ethanolamine plasmalogens were synthesized from 1-^{14}C-alkyl-*sn*-glycero-3-phosphorylethanolamine by mitochondrial supernatants of rat brain. The data indicate that the desaturation of the *O*-alkyl moiety to the *O*-alk-1-enyl moiety occurs on intact 1-alkyl-2-acyl-*sn*-glycero-3-phosphoryl-ethanolamine.

INTRODUCTION

The conversion of *O*-alkyl moieties to *O*-alk-1-enyl groups in glycerolipids has been shown both *in vivo* (1-8) and with cell-free systems (9-13). The cofactors involved in this reaction are ATP, Mg^{++}, and NADPH or NADH (11-13); the reaction is inhibited by cyanide but not by carbon monoxide and has a requirement for molecular oxygen (12, 13). These studies indicated that the biosynthesis of the *O*-alk-1-enyl bond is catalyzed by a mixed-function oxidase system that is similar to that involved in the desaturation of fatty acids (14).

Experimental data at this time suggest that the immediate precursor of the 1-alk-1-enyl-2-acyl-*sn*-glycero-3-phosphorylethanolamine (alk-1-enylacyl-GPE) is either 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylethanolamine (alkylacyl-GPE) (7, 11, 15) or 1-alkyl-*sn*-glycero-3-phosphorylethanolamine (alkyl-GPE) (6). Our report describes ethanolamine plasmalogen biosynthesis by a cell-free system from rat brain that is similar to that previously described for tumors (10, 13).

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MATERIALS AND METHODS

Whole brains from 12 to 14-day-old male and female rats were homogenized at 0° in 0.25 M sucrose (1.5 ml/g wet tissue) using 4-5 strokes of a Potter-Elvehjem homogenizer attached to a Lourdes homogenizing motor set at 50. The postmitochondrial supernatant was prepared from the homogenate as previously described (10). Incubation conditions, cofactor concentrations (ATP, CoA, NADPH, Mg^{++}), and extraction of the lipids were also the same as described earlier (10, 11, 13). The D-glucose-6-P and glucose-6-P dehydrogenase were purchased from Sigma Chemical Co., and other cofactors were purchased from P-L. Biochemicals, Inc.

The substrate used in these experiments, ^{14}C-alkyl-GPE, was prepared from Ehrlich ascites cell lipids after intraperitoneal injection of 1-^{14}C-hexadecanol (58 μCi/μmole/mouse) into mice bearing the tumor cells. The ascites cells were recovered 18-24 hours after injection of the labeled hexadecanol and the lipids extracted (16). The phospholipids were isolated on a short silicic acid column (17), spread as a thin film in a pear-shaped flask, and exposed to HCl gas for 2 hours to hydrolyze the *O*-alk-1-enyl moieties (18). The ^{14}C-alkyl-acyl-GPE was then isolated by preparative thin layer chromatography (TLC) using a solvent system of chloroform-methanol-NH_4OH (65:35:5, v/v); this fraction was further purified by TLC in chloroform-methanol-glacial acetic acid-water (50:25:8:2, v/v). ^{14}C-Alkyl-GPE was obtained by removing the acyl groups from the 2-position of the ^{14}C-labeled alkylacyl-GPE with phospholipase A. Analysis of the ^{14}C-alkyl-GPE preparation by two different treatments, BF_3-methanol (19) and $NaAlH_2(OCH_2CH_2OCH_3)_2$ reduction (10), and subsequent TLC showed that the substrate contained 91% of the ^{14}C in the *O*-alkyl moiety and 8% in the *O*-acyl groups at the 1-position of the glycerolipid; the plasmalogens had previously been eliminated by exposing the ethanolamine phospholipid fraction to HCl.

The ethanolamine plasmalogens were isolated by separating the lipids by TLC using chloroform-methanol-NH_4OH (65:35:5, v/v) in one direction; after exposing to HCl fumes, the plate was developed in a second direction (after

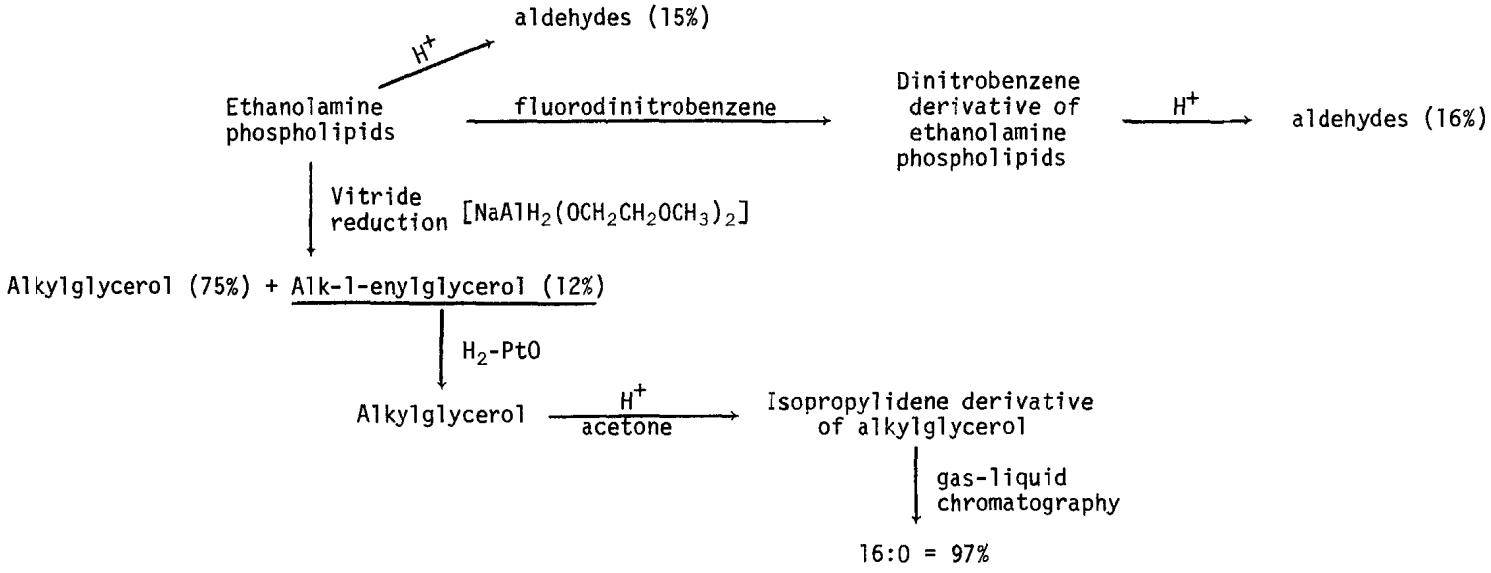

Figure 1. Identification of ^{14}C-ethanolamine plasmalogens. All analytical procedures used were the same as those described earlier (10). The percentages of ^{14}C in the indicated products from each procedure are shown.

90° rotation) in hexane-diethyl ether (80:20, v/v) (20, 21). The layer was then exposed to iodine vapors and the aldehyde areas, as well as other zones along the plate, were scraped for radioassay in a liquid scintillation spectrometer (22). A pooled sample of the ethanolamine phospholipids was purified by preparative TLC, and the ^{14}C-alk-1-enylacyl-GPE was identified by the procedures (Fig. 1) previously described (10).

RESULTS AND DISCUSSION

Two labeled phospholipid classes were synthesized from ^{14}C-alkyl-GPE by the rat brain preparations. In typical incubations (Table 1), 25% of the substrate was acylated to form alkylacyl-GPE and alk-1-enylacyl-GPE, while 35% remained unreacted and contained no *O*-alk-1-enyl groups. Addition of 100 nmoles of linoleic acid to the basic incubation system did not increase the extent to which alkyl-GPE was acylated. Similar incubations with a tumor

Table I

Biosynthesis of Ethanolamine Plasmalogens by Postmitochondrial Supernatants from Rat Brain

Additions to incubation mixture[b]	Percent of ^{14}C in alk-1-enyl moieties of ethanolamine phospholipid fraction[a]		
	Experiment 1	Experiment 2	Experiment 3
1. None	4.2; 4.5	6.8; 5.1	----
2. NADPH[c]	16.9[d]	24.3; 25.9	20.8; 19.3
3. $NADP^+$ (2 mM)	13.2; 13.4	----	----

[a]Approximately 25% of ^{14}C-substrate was incorporated into the total ethanolamine phospholipid fraction in all incubations.

[b]The incubation mixture contained the ^{14}C-substrate, ATP (10 mM), CoA (0.1 mM), Mg^{++} (4 mM), and postmitochondrial fraction (≈15 mg protein) in a final volume of 3 ml Tris buffer (0.1 M, pH 7.1). The 1-[1-^{14}C]alkyl-*sn*-glycero-3-phosphorylethanolamine (≈2 μCi/μmole) was added in 10 μl of chloroform-methanol (2:1, v/v); experiments 1 and 2 contained 6 nmoles and experiment 3 contained 30 nmoles of the substrate. Incubations were carried out at 37° for 90 min.

[c]NADPH generating system consisting of D-glucose-6-P (6 mM), glucose-glucose-6-P dehydrogenase (5 units) and $NADP^+$ (2 mM) added to the incubation mixture.

[d]Four pooled samples.

preparation resulted in a greater degree of acylation of the ^{14}C-alkyl-GPE (13) than was found in this study. The amount of ^{14}C in the *O*-alk-1-enyl moieties of the ethanolamine phospholipids was also higher when tumor preparations were used.

From 15 to 25% of the label in the ethanolamine phospholipid fraction from rat brain was found in *O*-alk-1-enyl moieties. The ^{14}C-ethanolamine plasmalogens from the brain were identified by the procedures shown in Fig. 1; these data offer proof that the product formed from the incubation of ^{14}C-alkyl-GPE with the postmitochondrial supernatant fraction from young rat brain and the necessary cofactors was indeed alk-1-enylacyl-GPE. Although activity (≈20%) was found in a fraction which migrated with phosphatidic acid in basic and acidic solvent systems and a trace in the total choline phospholipids, none of it was present in the *O*-alk-1-enyl moieties of these two fractions.

Approximately 10-20% of the carbon-14 was found in the neutral lipid fraction. After the neutral lipids were treated with BF_3-methanol (19), 4.5% of the label in the products was found in dimethylacetals. No further studies of the neutral lipid fraction were undertaken.

The effects of $NADP^+$ and NADPH on the formation of ^{14}C-alk-1-enylacyl-GPE by brain preparations are shown in Table 1. The requirement for $NADP^+$/NADPH has previously been shown using postmitochondrial fractions from tumors (13). Reduction of $NADP^+$ occurred rapidly in the tumor system in the absence of a NADPH generating system. We assume that this also may occur in the brain preparations. Since ^{14}C-labeled *O*-alk-1-enyl moieties were found only in the ethanolamine phospholipid fraction and none was found in the lyso form, it appears that the actual substrate for plasmalogen synthesis in the brain is alkylacyl-GPE; this is further substantiated by our more detailed studies obtained with the tumor preparations (13). The system described here should be useful for exploring the biochemical mechanisms responsible for plasmalogen synthesis in nervous tissues.

REFERENCES

1. Thompson, G. A., Jr. *Biochim. Biophys. Acta 152*, 409 (1968).
2. Wood, R., and Healy, K. *Biochem. Biophys. Res. Commun. 38*, 205 (1970).
3. Blank, M. L., Wykle, R. L., Piantadosi, C., and Snyder, F. *Biochim. Biophys. Acta 210*, 442 (1970).
4. Bell, O. E., Jr., Blank, M. L., and Snyder, F. *Biochim. Biophys. Acta 231*, 579 (1971).
5. Stoffel, W., and LeKim, D. *Z. Physiol. Chem. 352*, 501 (1971).
6. Debuch, H., Müller, J., and Fürniss, H. *Z. Physiol. Chem. 352*, 984 (1971).
7. Paltauf, F. *FEBS Letters 17*, 118 (1971).
8. Paltauf, F. *Biochim. Biophys. Acta 239*, 38 (1971).
9. Wykle, R. L., Blank, M. L., and Snyder, F. *FEBS Letters 12*, 57 (1970).
10. Snyder, F., Blank, M. L., and Wykle, R. L. *J. Biol. Chem. 246*, 3639 (1971).
11. Blank, M. L., Wykle, R. L., and Snyder, F. *FEBS Letters 18*, 92 (1971).
12. Paltauf, F. *FEBS Letters 20*, 79 (1972).
13. Snyder, F., Wykle, R. L., Blank, M. L., Lumb, R. H., Malone, B., and Piantadosi, C. *Federation Proc. 31*, 454 (1972).
14. Oshino, N., Imai, Y., and Sato, R. *Biochim. Biophys. Acta 128*, 13 (1966).
15. Wykle, R. L., Blank, M. L., and Snyder, F. *Federation Proc. 30*, 1243 (1971).
16. Bligh, E. G., and Dyer, W. J. *Can. J. Biochem. Physiol. 37*, 911 (1959).
17. Borgström, B. *Acta Physiol. Scand. 25*, 101 (1952).
18. Ferrell, W. J., Radloff, D. M., and Radloff, J. F. *Anal. Biochem. 37*, 227 (1970).
19. Morrison, W. R., and Smith, L. M. *J. Lipid Res. 5*, 600 (1964).
20. Schmid, H. H. O., and Mangold, H. K. *Biochim. Biophys. Acta 125*, 182 (1966).
21. Viswanathan, C. V., Phillips, F., and Lundberg, W. O. *J. Chromatog. 35*, 66 (1968).
22. Snyder, F., and Smith, D. *Separation Sci. 1*, 709 (1966).