ENZYMIC SYNTHESIS OF ETHANOLAMINE PLASMALOGENS FROM AN *O*-ALKYL GLYCEROLIPID

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1. Introduction

In vivo studies directed toward the elucidation of plasmalogen biosynthesis have indicated that *O*-alkyl lipids can serve as precursors of *O*-alk-1-enyl lipids [1–7]. Our work with an enzymic system from neoplastic cells that synthesizes plasmalogens [8, 9] also supported this concept. (a) The substrate (hexadecanol and dihydroxyacetone-P) and cofactor requirements (CoA, ATP, and Mg^{2+}) were identical for both types of ethers, except for the need of $NADP^+$ (or NAD^+) in plasmalogen synthesis, and (b) the $^3H/^{14}C$ isotope ratios of the *O*-alkylglycerols and *O*-alk-1-enyl-glycerols derived from the products synthesized from 9,10-^{3}H-hexadecanol and uniformly ^{14}C-labeled dihydroxyacetone-P were identical. However, it was not possible for us completely to rule out other reactions that could account for the synthesis of the *O*-alk-1-enyl moiety, since a suitable substrate containing the *O*-alkyl bond was not available in the earlier studies.

We have now prepared and tested 1-(9,10-^{3}H-alkyl)-2-acyl-(U)^{14}C-glycerol-3-P as a potential precursor of plasmalogens in the enzymic system. Using this precursor, the CoA requirement was not essential, whereas $NADP^+$ was still required for the conversion of the substrate to ethanolamine plasmalogens. The data demonstrate that the *O*-alkyl moiety is transformed to an *O*-alk-1-enyl moiety without cleavage of the ether bond and that the $NADP^+$ requirement is twofold: (a) It not only serves to generate NADPH* which was necessary for reduction of the ketone group of *O*-alkyldihydroxyacetone-P as shown in our earlier work [8–12] but, as shown here, (b) it is also involved as a cofactor in the formation of the *O*-alk-1-enyl bond.

* Unpublished observation.

2. Methods

Unless otherwise stated, the methodology, materials, and enzyme source used were identical to those previously described in detail [8–12]. The 1-(9,10-^{3}H-alkyl)-2-acyl-(U)^{14}C-glycerol-3-P was prepared from (U)^{14}C-D-fructose-1,6-diphosphate (190 mCi/mmole) and 9,10-^{3}H-hexadecanol (400 mCi/mmole) and isolated as before [12]; the preparation contained no radioactivity in *O*-alk-1-enyl lipids as determined by radioassay of dimethylacetals and *O*-alk-1-enylglycerols. 1-(1-^{14}C-alkyl)-2-acylglycerol-3-P was prepared in the same manner from 1-^{14}C-hexadecanol (46 mCi/mmole) and unlabeled dihydroxyacetone-P. The legend of table 1 lists the components, concentrations, and conditions used for each incubation.

3. Results and discussion

The data in table 1 demonstrate that 1-(1-^{14}C-alkyl)-2-acylglycerol-3-P serves as an effective precursor of ethanolamine plasmalogens (1-alk-1-enyl-2-acylglyceryl-3-phosphorylethanolamine) when this precursor is incubated with a postmitochondrial fraction of Ehrlich ascites cells, $NADP^+$, CDP-ethanolamine, ATP, and Mg^{2+}; coenzyme A was not essential. Plasmalogens were not synthesized from the alkyl analog of phosphatidic acid in the absence of $NADP^+$.

When the doubly labeled alkyl analog of phos-

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Table 1
The biosynthesis of ethanolamine plasmalogens from 1-(1-^{14}C-alkyl)2-acylglycerol-3-P by a postmitochondrial fraction of Ehrlich ascites cells.

System	^{14}C in total ethanolamine phospholipid fraction (%)	^{14}C in ethanolamine plasmalogens (%)
1 Complete	10.1	9.24
2 Complete minus $NADP^+$	7.28	0.71
3 Complete minus CoA	8.56	6.95

[a] The complete system contained the ^{14}C-substrate, ATP (10 mM), CoA (100 μM), Mg^{2+} (4 mM), CDP-ethanolamine (1 mM), $NADP^+$ (2 mM), and postmitochondrial fraction (30 mg protein) in a final volume of 3 ml Tris buffer (0.1 M, pH 7.1). The 1-(1-^{14}C-alkyl)2-acylglycerol-3-P (145,600 dpm per vial) was added to the system in 10 μl of 95% ethanol. Incubations were carried out at 37° for 90 min.

phatidic acid was used as a substrate, the ^{3}H and ^{14}C were incorporated into alkylacylglycerylphosphoryl-ethanolamine and alk-1-enylacylglycerylphosphoryl-ethanolamine in essentially the same ratios present in the precursor (table 2). The dimethylacetals of the aldehydes liberated from the *O*-alk-1-enyl moiety contained only ^{3}H, as expected from the location of ^{14}C and ^{3}H in the ether-linked precursor. Additional evidence that indicated the *O*-alkyl linkage was not cleaved is that $>98.5\%$ of the ^{3}H was still associated with either the *O*-alkyl or *O*-alk-1-enyl glycerolipids after a 90-min incubation (table 2), i.e., only 1.5% of the activity could be isolated as fatty alcohols (derived from acyl moieties) after reduction with $NaAlH_2(OCH_2CH_2OCH_3)_2$. The lack of *O*-alkyl cleavage enzymes in Ehrlich ascites cells has previously been established [13].

Our findings indicate that the *O*-alkyl moiety can indeed be transformed to an *O*-alk-1-enyl linkage, presumably via a dehydrogenation mechanism involving $NADP^+$ as a cofactor. The nature of this mechanism remains unknown, although *in vivo* experiments [2] have suggested that a rather complex substitution reaction(s) might be responsible. The experiments reported in this communication do not answer the question of which *O*-alkyl glycerolipid serves as the direct precursor of plasmalogens, but stimulation of ethanolamine plasmalogen synthesis by CDP-ethanol-

Table 2
Incorporation of 1-(9,10-^{3}H-alkyl)-2-acyl-(U)^{14}C-glycerol-3-P into ether-linked ethanolamine phospholipids[a].

Sample	^{3}H/^{14}C ratio
1 Substrate (alkylacylglycerol-P)	24
2 Alkylglycerols from ethanolamine phospholipids after reduction	23
3 Alk-1-enylglycerols[b] from ethanolamine phospholipids after reduction	22

[a] The complete incubation system was identical to that described in the legend of table 1 except that the 1-(9,10-^{3}H-alkyl)-2-acyl-(U)^{14}C-glycerol-3-P (1.6×10^6 dpm ^{3}H per vial), serving as substrate, was added in 10 μl diethyl ether: 95% ethanol, 2:1 (v/v). Under these conditions, 8% (based on ^{3}H assay) of the substrate was incorporated into the ethanolamine phospholipids and 11.6% of this activity was in the plasmalogen fraction; the alkyl ethanolamine phospholipids contained 86.8% of the ^{3}H. The products from five vials were pooled to obtain the data for samples (2) and (3). Reduction of the samples was accomplished with $NaAlH_2(OCH_2CH_2OCH_3)_2$.

[b] The dimethylacetals of the aldehydes liberated by methanolic HCl contained only ^{3}H.

amine [14] suggests that the transformation occurs directly on alkylacylglycerylphosphorylethanolamine.

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