ENZYMIC SYNTHESIS OF ETHANOLAMINE PLASMALOGENS FROM 1-ALKYL-2-ACYL-*sn*-GLYCERO-3-(^{32}P)-PHOSPHORYLETHANOLAMINES BY MICROSOMES FROM RAT BRAIN

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1. Introduction

Several experiments [1–10] with intact cells have suggested that 1-alkyl-2-acyl-*sn*-glycero-3-phosphoryl-ethanolamines (alkylacyl-GPE) are the immediate precursors of 1-alkenyl-2-acyl-*sn*-glycero-3-phosphoryl-ethanolamines (ethanolamine plasmalogens). The first direct evidence for the existence of an enzyme catalyzing this conversion was reported in 1972 by Paltauf [11] for microsomes from hamster intestinal mucosa. While studying the enzymic synthesis by rat brain microsomes of alkylacyl-GPE [12, 13], we obtained indirect evidence for the further conversion of these products to ethanolamine plasmalogens. By incubations of alkylacyl-GPE with fresh rat brain microsomes, we have detected a dehydrogenase that catalyzes the last step in the biosynthesis of ethanolamine plasmalogens.

2. Methods

Alkylacyl-GPE labelled with ^{32}P were synthesized as described previously [12, 13] except that 12 mg of microsomal protein was present in a volume of 3.6 ml. Ethanolamine phosphoglycerides (EPG) were isolated by preparation thin-layer chromatography (TLC), alkenyl groups were removed by reaction with propanediol, diacyl compounds were removed by mild saponification, and the alkylacyl-GPE product was assayed according to Horrocks and Sun [14]. The alkylacyl-GPE was emulsified with Tween 20 in phosphate buffer. Incubations were carried out in tubes that were stoppered after flushing with nitrogen. After incubation, the conversion of alkylacyl-GPE to alkenylacyl-GPE was assayed by two-dimensional TLC [15]. Adult Fisher rats (180 g) were used as a source of brain microsomal and cytosol fractions [12].

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3. Results and discussion

As can be seen from table 1, 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylethanolamines labeled with ^{32}P are converted to the corresponding 1-alkenyl-2-acyl-*sn*-glycero-3-phosphorylethanolamines by a fresh cell-free system from the brain of mature rats. The reaction is stimulated by ATP and Mg^{2+} and almost completely inhibited by EDTA. The dehydrogenase enzyme is located in the microsomal fraction, but the enzyme has maximum activity in the presence of a heat-stable factor from the cytosol.

Paltauf [11], working with hamster intestinal mucosa, described an alkylacyl-GPE dehydrogenase with many similarities to fatty acid desaturases including requirements for oxygen and a reduced pyridine nucleotide. The calculated specific activity, 0.0035 nmoles/mg protein/hr, was more than 700-fold smaller than that of the brain enzyme. Blank et al. [16] reported a 0.93% conversion of 1-alkyl-2-acyl-*sn*-

Table 1
Alkylacyl-GPE dehydrogenase activity of fresh rat brain microsomes.

System	Net Conversion (%)	Specific activity (nmoles/mg protein/hr)
1 Complete*	4.24	1.66
2 Complete, 60 min incubation	5.20	1.02
3 Complete plus 2 mM $NADP^+$	3.86	1.52
4 Complete minus microsomes	2.00	0.79
5 Complete minus microsomes plus 2 mM $NADP^+$	0.54	0.21
6 Complete minus microsomes, ATP, Mg^{++}	−1.03	−0.40
7 Complete minus cytosol, ATP, Mg^{++}	1.93	0.76
8 Complete minus ATP, Mg^{++}	2.78	1.09
9 Complete minus Mg^{++}, plus 1 mM EDTA	0.14	0.06
10 Complete (5 mM ATP)	3.53	1.39
11 Complete (10 mM ATP)	5.06	1.99
12 Complete, boiled cytosol	6.38	2.50
13 Complete, Tris buffer	2.00	0.79

* Complete system: 12 nmoles of alkylacyl-GPE labeled with ^{32}P; 612 μg of microsomal protein and 160 μg of cytosol protein from rat brain; 10 mM ATP; 25 mM $MgCl_2$; 0.015% Tween 20; and 75 mM phosphate buffer, pH 7.6. Incubation was for 30 min at 39° in a final volume of 0.20 ml. The ethanolamine plasmalogens from the control (boiled cytosol, no microsomes) contained 3.83% of the added radioactivity.

glycerol-3-phosphate to alkenylacyl-GPE by a post-mitochondrial fraction of Ehrlich ascites cells in the presence of $NADP^+$, CDP-ethanolamine, ATP, and Mg^{2+}. In the rat brain system, $NADP^+$ was somewhat inhibitory, and NADPH inhibited the alkylacyl-GPE dehydrogenase almost completely. In the presence of air, alkylhydrolase (cleavage enzyme) activities were much higher and alkenylacyl-GPE formation was depressed. The alkylacyl-GPE dehydrogenase activity is comparable to the rate of formation of alkenylacyl-GPE in a system containing rat brain microsomes, 1-alkyl-2-acyl-*sn*-glycerols, CDP-ethanolamine, and Mg^{2+} [12, 13].

The sequence of reactions for the biosynthesis of ethanolamine plasmalogens is:

(a) 1-alkyl-2-acyl-*sn*-glycero-3-phosphates → 1-alkyl-2-acyl-*sn*-glycerols + Pi

(b) 1-alkyl-2-acyl-*sn*-glycerols + CDP-ethanolamine → 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylethanolamines + CMP

(c) 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylethanolamines ⟶ 1-alkenyl-2-acyl-*sn*-glycero-3-phosphorylethanolamines + 2 [H]

Reaction (a) has been demonstrated by Snyder et al. [17]. Radominska-Pyrek and Horrocks [12, 13] gave direct evidence for reaction (b) and indirect evidence for reaction (c). The present results provide direct evidence for reaction (c) at a rate consistent with the metabolic turnover of ethanolamine plasmalogens [18, 19], and are consistent with *in vivo* evidence implicating alkylacyl-GPE as the immediate precursor of alkenylacyl-GPE [1–10].

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