THE ENZYMATIC EXCHANGE OF THE ACYL GROUP OF ACYL DIHYDROXYACETONE PHOSPHATE WITH FREE FATTY ACIDS

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SUMMARY: Ehrlich ascites cell microsomes catalyze the exchange of the acyl group of acyl dihydroxyacetone phosphate with free fatty acids. The reaction does not require ATP and CoA.

Acyl dihydroxyacetone phosphate (acyl DHAP[1]) has been shown to be a precursor of glycerolipids in a variety of systems (1,2). Acyl DHAP has also been found to be the precursor of glycerol ether lipids (1,2). It has been shown that acyl DHAP reacts with long chain alcohols to form 1-O-alkyl dihydroxyacetone-3-phosphate (alkyl DHAP[1]) (3,4). During the investigation of the formation of the ether bond it was noted that free fatty acid inhibited the formation of alkyl DHAP from acyl DHAP and fatty alcohol (3). Subsequently, it was found that this inhibition was due to the enzymatic exchange of the acyl moiety of acyl DHAP with free fatty acids. The details of the exchange reaction are described here.

MATERIALS AND METHODS

[1-^{14}C]fatty acids were obtained from either New England Nuclear (Boston, Mass.) or Amersham/Searle (Arlington Heights, Ill.). Triton X-100 (trademark, Rohm and Haas), Tris, CoA, 5,5' dithiobis(2-nitrobenzoic acid), ATP and hexokinase were obtained from Sigma (St. Louis, Mo.). The thin layer chromatography plates were from E. Merck obtained through Brinkmann (Westbury, N.Y.). The palmityl DHAP was chemically synthesized (5). The ascites cell microsomes were prepared as previously described (6) using a hypotetraploid strain of Ehrlich ascites cells which were a generous gift of Dr. H. N. Christensen.

[1] DHAP - dihydroxyacetone phosphate; G-3-P - glycerol-3-phosphate

Table 1. Requirements for the formation of ^{14}C-acyl DHAP from [1-^{14}C]palmitic acid and palmityl DHAP.

The reaction mixture consists of the following: [1-^{14}C]palmitic acid (20 nmoles, 2 X 10^6 cpm), palmityl DHAP (30 nmoles), Triton X-100 (50 µg), Tris-HCl (75 mM, pH 7.5), NaF (16.7 mM), and Ehrlich ascites cell microsomes (0.3 mg protein) in a total volume of 1.2 ml. The reaction mixture was incubated for 1/2 hour at 37°C. After the reaction, the lipids were extracted and separated by thin layer chromatography as described in the text. The radioactivity in the spots corresponding to standard palmityl DHAP was determined and is given below.

	Radioactivity in Palmityl DHAP
	CPM (X 10^4)
Whole system	6.0
" " - Enzyme	0.1
" " " + Boiled enzyme*	0.2
" " - Acyl DHAP	0.1
" " " " + 1-acyl-rac-glycerol-3-P (30 nmoles)	0.2
" " " " +DHAP (10 nmoles)	0.1

*The enzyme was heated at 100°C for 15 min. before use.

The exchange reaction was studied by determining the amount of radioactive acyl DHAP formed from ^{14}C-labeled fatty acids and nonradioactive acyl DHAP. The assay mixture was composed of the following: [1-^{14}C]palmitic acid (20 nmoles, 1.1 µCi), palmityl DHAP (30 nmoles), Triton-X-100 (50 µg), Tris-HCl (75 mM, pH 7.5), NaF (16.7 mM), and ascites cell microsomes (0.3 mg protein) in a total volume of 1.2 ml. The lipids (acyl DHAP, palmitic acid) and the detergent (Triton-X-100) were dried down together from chloroform solutions under a stream of nitrogen. The buffer and the sodium fluoride solution were then added and the lipids were dispersed by sonication in an ultrasonic bath. After addition of water, the mixture was sonicated again, the enzyme preparation added, and the tube mixed and incubated at 37°C in a shaking water bath. The reaction was stopped after the incubation period by addition of 4.5 ml of chloroform-methanol (1:2) and the lipids were extracted using an acidic Bligh and Dyer procedure (7). The upper layer was removed and the lower layer was dried under a stream of nitrogen. The lipids were then spotted on a thin layer chromatography plate

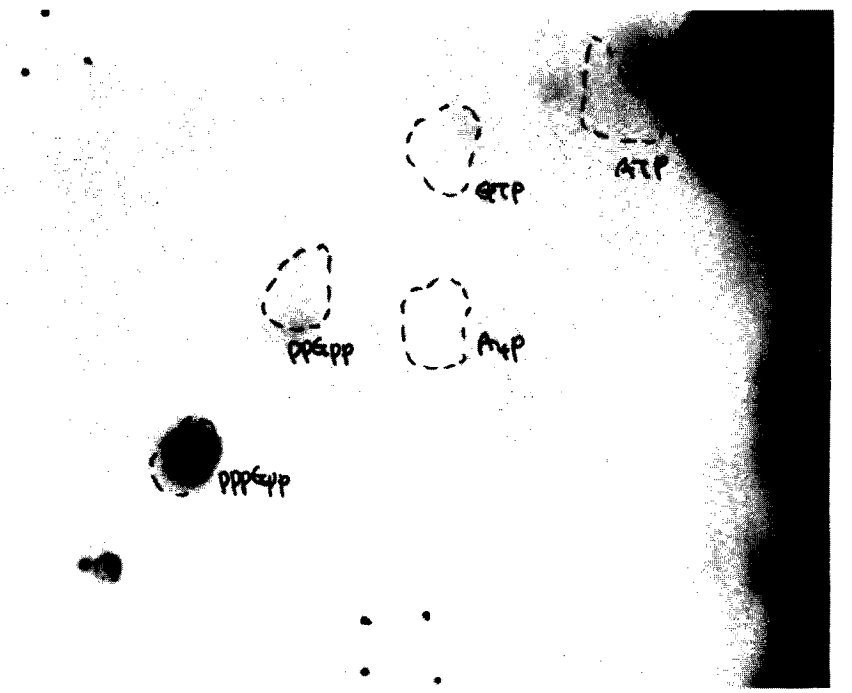


FIGURE 2. Conversion of yeast ppGpp to pppGpp by glyceraldehyde-3-P dehydrogenase and 3-P-glycerate phosphokinase. ppGpp was purified by preparative thin-layer chromatography from yeast culture which has been shifted from room temperature to 38° for six minutes. Experimental details were described in MATERIALS AND METHODS. Dotted circles represent the positions where marker nucleotides migrated. Radioactivity in pppGpp and ppGpp spots are 806 and 136 cpm respectively. The chromatogram was exposed to Kodak nonscreen X-ray films for six days.

Alkaline hydrolysis of yeast ppGpp: To 60 µl sample containing radioactive ppGpp, 6 µl each of 0.1 M EDTA and 2 N KOH were added. After overnight incubation at 37°, approximately 8 µl 2 M formic acid was added to the reaction mixture. Nucleotides were then separated by thin-layer chromatography.

RESULTS

FIGURE 1 shows a radioautogram of ^{32}P labelled compounds extracted from yeast cells after heat shock and resolved by two dimensional thin-layer chromatography. A compound which comigrates exactly with marker ppGpp is evident. This compound was quantitatively adsorbed by activated charcoal,

Table 3. Comparison of acyl group exchange reaction activity of different fatty acids with palmityl DHAP.

The reaction mixture is the same as in Table 1 except that a variety of labeled fatty acids were substituted for palmitic acid. The exchange reaction rate with palmitic acid was .06 nmole/min/mg protein.

Fatty acid used	Relative acyl exchange activity
Palmitic acid	100
Stearic acid	40
Oleic acid	88
Linoleic acid	111
Linolenic acid	134
Arachidonic acid	70

its co-migration with authentic acyl DHAP in several different thin layer chromatography systems and also by its conversion to 1-acyl-rac-G-3-P upon treatment with $NaBH_4$ (8). When [1-^{14}C]palmitic acid was replaced by [1-^{14}C]linolenic acid in the reaction mixture (see later, Table 3), the product was shown to migrate with linolenyl DHAP on argentation thin layer chromatography (9).

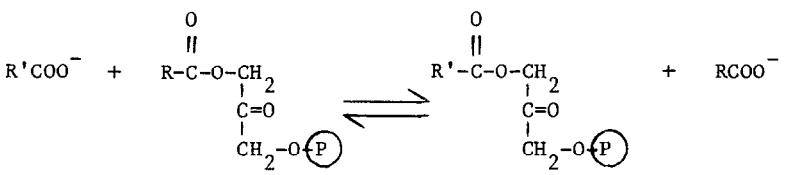
These results indicate that the acyl group of acyl DHAP exchanges with ^{14}C-fatty acid to form radioactive acyl DHAP. Acyl CoA does not seem to be involved in this reaction, as is seen by the nonconversion of DHAP to acyl DHAP in this system despite the presence of acyl CoA:DHAP acyltransferase in Ehrlich ascites cell microsomes (1). To confirm this, we checked the effect of ATP and CoA on this system. The addition of ATP + Mg^{++} did stimulate the reaction compared to that of Mg^{++} alone (Table 2). However, Mg^{++} was found to inhibit the reaction strongly (Table 2) and the ATP stimulation was the result of removal of Mg^{++} by ATP from the reaction mixture. Compared to the control, ATP plus Mg^{++} did not stimulate the reaction (Table 2) and the removal of any endogenous ATP by hexokinase and glucose did not affect the reaction (Table 2). Similarly, addition of 5,5'dithiobis(2-nitrobenzoic acid) to remove any endogenous CoA (10)

did not result in inhibition of the exchange reaction. Direct addition of CoA resulted in inhibition of the reaction rather than stimulation (Table 2). Long chain fatty alcohols inhibited the reaction upon addition to the reaction mixture (Table 2).

Palmitic acid can be replaced by other long chain fatty acids (Table 3). Polyunsaturated fatty acids gave higher rates of reaction compared to the corresponding saturated fatty acids, possibly due to their higher solubility in the reaction mixture.

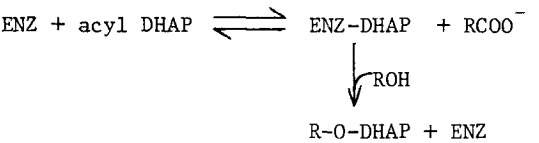
DISCUSSION

The above results show that the ascites cell microsomes catalyze the following reaction:



We found that guinea pig liver mitochondria and rat brain microsomes also catalyze the exchange reaction. The physiological significance of this reaction is not clear. The properties of the reaction are quite different from the acyl CoA:DHAP acyltransferase reaction. For example, neither Mg^{++}, ATP nor CoA are needed for the exchange reaction and the specificity towards the fatty acid utilized is different, i.e. the acyl transferase will not accept polyunsaturated acyl CoA's (1). On the other hand, the exchange reaction has some similarities with the reaction which forms alkyl DHAP (3). For example, we recently found that Mg^{++} also inhibits the conversion of acyl DHAP to alkyl DHAP (Davis & Hajra, unpublished work) and that the stimulation of ether bond synthesis by ATP (3) is due to the binding of Mg^{++} in the reaction mixture by the added ATP. The addition of CoA inhibits both reactions (3). The inhibition of the reactions by CoA was probably due to the reaction of the substrate, acyl DHAP, with CoA to form long chain fatty acyl CoA's (11). Like the utilization of a wide range of long chain alcohols including polyunsaturated alcohols for the synthesis of alkyl DHAP (3,12),

the exchange reaction also utilizes a wide variety of long chain fatty acids (Table 3). Additionally, hexadecanol inhibits the exchange reaction (Table 2) and fatty acid, as previously described, inhibits the formation of alkyl DHAP (3). On the basis of these similarities, it may be assumed that this exchange reaction is also catalyzed by the enzyme responsible for the synthesis of alkyl DHAP. A mechanism consistent with these results can be depicted as below:



This mechanism would explain the reversible exchange of the acyl group of acyl DHAP with free fatty acid, the inhibition of the ether bond synthesis by fatty acid, and the inhibition of the exchange reaction by long chain fatty alcohol. Further aspects of these reactions are under study in this laboratory.

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