BIOSYNTHESIS OF ALKYL LIPIDS: DISPLACEMENT OF THE ACYL MOIETY OF ACYLDIHYDROXYACETONE PHOSPHATE WITH FATTY ALCOHOL ANALOGS

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SUMMARY: The first step in the biosynthesis of ether-linked glycerolipids proceeds as follows: ROH + acyldihydroxyacetone-P ⟶ alkyldihydroxyacetone-P + RCOOH. Data obtained with a series of ^{3}H-labeled fatty alcohol analogs and [1-^{14}C]hexadecanol demonstrate that the microsomal enzyme from tumors that substitutes the alcohol group for the acyl group of acyldihydroxyacetone-P is not very selective. However, if hydroxyl groups are inserted at either the C-2 or C-16 position of hexadecanol, neither alkyldihydroxyacetone-P nor its dephosphorylated product is formed. The effect of modifying the terminal end of the alcohol was also apparent when iso and anteiso branched chain alcohols were used as substrates, i.e., the latter was incorporated into alkyldihydroxyacetone-P to a much greater extent.

INTRODUCTION

Utilization of long chain fatty alcohols (1) for the biosynthesis of the *O*-alkyl[2] moiety at the *sn*-1 position of glycerolipids is now firmly established (2-4). Key compounds formed by this pathway include alkyldihydroxyacetone-P (alkyl-DHAP)[3] (5-7), 1-alkyl-*sn*-glycero-3-P (6, 7), 1-alkyl-2-acyl-*sn*-glycero-3-P (6), the choline and ethanolamine phosphatides (8), 1-alkyl-2-acyl-*sn*-glycerols (8), and alkyldiacylglycerols (9). The fatty alcohols (1) react with acyl-DHAP (10, 11) or, alternately, alkylglycerols (12) can be phosphorylated to yield the complex alkyl glycerolipids; base groups are added via the cytidine pathway (8) or by exchange (13).

Hajra (10) found that alkyl-DHAP was formed by displacement of the acyl group of acyl-DHAP with a long chain fatty alcohol. This reaction has been confirmed in our laboratory (11), and Murooka *et al.* (14) have found

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[2]The term alkyl as used in this paper denotes unsubstituted, substituted, and branched chain *O*-alkyl moieties.

[3]Abbreviation: DHAP, dihydroxyacetone phosphate.

that the alkylation of homoserine by bacterial extracts also proceeds by an alkoxy displacement of an ester group. The entire alkyl chain (5, 6) and oxygen (15) of the alcohol is incorporated into the alkyl group of alkyl-DHAP, and Friedberg and coworkers (16, 17) have demonstrated that there is a tritium lost from the carbon of DHAP to which the acyl group is attached.

With few exceptions, the *O*-alkyl and *O*-alk-1-enyl moieties of glycerolipids consist of 16:0, 18:0, and 18:1 carbon chains, i.e., polyunsaturation is virtually absent (18). This unusual composition could be due to the specificity of the enzymes that are involved in the incorporation of the alcohol moiety into alkyl-DHAP or to the reductase (19-21) that converts fatty acids to fatty alcohols.

Microsomal enzymes from the preputial gland tumor are capable of incorporating $C_{10:0}$-$C_{18:0}$ fatty alcohols into alkyl-DHAP (5), and more recently we have demonstrated that a hydroxy-substituted alcohol (octadecane-1,12-diol) can also serve as a precursor of the *O*-alkyl moiety (22). Bandi *et al.* (23), who fed long chain alcohols to rats, reported that $\Delta 1$ and $\Delta 2$ unsaturated alcohols could also be incorporated into the *O*-alkyl moieties. The fact that various fatty alcohols can be incorporated into alkyl glycerolipids has suggested that the enzyme catalyzing the alcohol-acyl displacement reaction is not very selective. In the experiments described in this communication, we have examined the enzymic displacement of the acyl moiety of acyl-DHAP with several fatty alcohol analogs to determine which portions of the alcohol chain are critical for its participation as a substrate in formation of the *O*-alkyl linkage.

METHODS

Preparations of substrates

The [1-^{3}H]fatty alcohols were prepared by reduction (24) of the corresponding acid with LiAl$^3H_4$ obtained from New England Nuclear Corporation (Boston, Mass.). After extraction of the labeled alcohols from the reaction

mixture, their radiopurity was checked by thin-layer chromatography on Silica Gel G; the diol lipid preparations were chromatographed in hexane: diethyl ether:methanol:acetic acid (70:30:5:1, v/v) or in chloroform:methanol: acetic acid (98:2:4, v/v), whereas the monohydroxy lipids were chromatographed in only the former solvent system. Specific activities of the alcohols used as substrates in the incubations are listed in Table 1. Acyl-DHAP was synthesized according to the procedure we described earlier (25).

Incubations

For most incubations, the enzyme source was from preputial gland tumors (ESR-586) that had been transplanted subcutaneously in C57BL/6 mice (Cumberland View Farms, Clinton, Tenn.). Microsomes were separated in the usual fashion and washed three times in sucrose buffer (5). They were stored in the freezer until used. For one series, we used microsomes from Ehrlich ascites cells [grown in Swiss albino mice (HA/ICR)] that had been washed once in the sucrose buffer. This microsomal preparation was not frozen, but was used immediately after washing.

In addition to the enzyme source, the complete system for all the incubations contained 50 nmoles of labeled fatty alcohol, 100 nmoles of hexadecanoyl-DHAP, 10 mM ATP, 4 mM $MgCl_2$, and 42 mM NaF in 3 ml 0.1 M phosphate buffer (pH 7.1). Each labeled alcohol was added to the incubation vials in approximately 10 µl of 95% ethanol (5). The quantity of microsomes added to each vial was 0.75 mg protein for the preputial gland tumor samples and 4.2 mg protein for the Ehrlich ascites cell samples. Identical portions of the same microsomal preparation were used for all measurements. All samples were incubated in duplicate for 1 hr at 37°C. At the end of the incubation, lipids were extracted by the Bligh and Dyer method (26).

Analytical procedures

To determine the quantity of alkyl lipids synthesized from each alcohol, samples were chromatographed on Silica Gel HR for separating alkyl-DHAP and

on Silica Gel G for separating alkyldihydroxyacetone. When diol substrates were used, chromatography was done in chloroform:methanol:ammonium hydroxide (65:35:5, v/v), diethyl ether:acetic acid (200:1, v/v), and chloroform: methanol:acetic acid (98:2:4, v/v) systems. When other alcohols were used as substrates, the products were chromatographed in hexane:diethyl ether: methanol:acetic acid (70:30:5:1, v/v) and chloroform:methanol:acetic acid (98:2:1, v/v). In addition, all lipid extracts were reduced with Vitride[4] (27) and the products analyzed in a diethyl ether:acetic acid (200:1, v/v) system. If alkylglycerols were detected, they were purified in the same chromatographic system so that their isopropylidene derivative (28) could be prepared. When diols were used as substrate, we also subjected an aliquot of the lipid extract to acid hydrolysis (7% HCl in MeOH at 100°C for 10 min) since the *O*-alkyl moiety of the ketone intermediates is cleaved under these conditions (9). Protein was determined by the method of Lowry *et al.* (29).

RESULTS

Table 1 shows the relative quantities of total alkyl glycerolipids (alkyl-DHAP and alkyldihydroxyacetone) formed from each of the alcohols used as substrates. No reaction was detectable with the 1,16- or 1,2-diols, since only the isopropylidene derivative of the hexadecane-1,2-diol and a small amount of unreacted diol were isolated after the lipid extracts had been subjected to Vitride reduction and then acetone-$HClO_4$ treatment.

We found that 14-methylpentadecanol was incorporated into the alkyl ether linkages to about the same degree as is n-hexadecanol, whereas hexadec-9c-enol and 12-methyltetradecanol were incorporated to a much greater extent than n-hexadecanol. The relative reactivity of the two branched chain fatty alcohols in displacing the acyl moiety of acyl-DHAP is of the same order for the microsomal preparations from both tumors. Substituting the methyl group adjacent to the terminal carbon atom (iso type)

[4]Trade name for $NaAlH_2(OCH_2CH_2OCH_3)_2$ (Eastman Organic Chem., Rochester, N.Y.).

Table 1

Replacement of acyl moiety of acyl-DHAP with fatty alcohols catalyzed by microsomes from preputial gland tumor[a]

Labeled fatty alcohol used as substrate	Specific activity μCi/μmole	μCi incubated per vial	Microsomal source	Hexadecanoyl-DHAP	Products formed: Alkyl-DHAP	Products formed: Alkyl-DHA
1. [1-^{14}C]Hexadecanol	6.46	0.323	PGT[b]	+	9.5, 12.7	4.5, 6.0
				−	0.1, 0.3	0, 0
2. [1-^{14}C]Hexadec-9c-enol	5.68	0.284	PGT	+	13.8, 10.5	46.1, 36.9
				−	0.5, 0.5	0, 0
3. [1-^{3}H]-12-methyl-tetradecanol	6.88	0.344	PGT	+	16.9, 17.3	41.5, 41.0
				−	0.2	0, 0
4. [1-^{3}H]-14-methyl-pentadecanol	5.08	0.254	PGT	+	10.9, 11.1	9.5, 10.2
				−	0.3, 1.1	0, 0
5. [1-^{3}H]Hexadecane-1,16-diol	5.68	0.284	PGT	+	None	None
				−	None	None
6. [1-^{3}H]Hexadecane-1,2-diol	9.14	0.457	PGT	+	None	None
				−	None	None
7. [1-^{14}C]Hexadecanol	6.46	0.323	EAC[b]	+	12.7, 14.5	4.0, 3.6
				−	0.9, 0.1	0, 0
8. [1-^{3}H]-12-methyl-hexadecanol	6.88	0.344	EAC	+	18.6, 17.8	24.2, 29.7
				−	0.3, 0.3	0, 0
9. [1-^{3}H]-14-methyl-hexadecanol	5.08	0.254	EAC	+	9.1, 4.7	3.5, 2.0
				−	0.5, 0.2	0, 0

[a] See text (Incubations) for description of incubation system.

[b] PGT indicates preputial gland tumor, and EAC indicates Ehrlich ascites cell tumor.

appears to greatly hinder its reactivity as compared to when the methyl group is substituted in an anteiso position. In these experiments, we observed differences in the relative amounts of alkyl-DHAP and alkyldihydroxyacetone formed. The two most reactive alcohols, when incubated with microsomes from the preputial gland tumors, formed mostly alkyldihydroxyacetone, whereas alkyl-DHAP predominated when hexadecanol and 14-methylpentadecanol were the substrates. The same trend was observed with microsomes from Ehrlich ascites cells but less pronounced than with microsomes from the preputial gland tumors. These data imply that the phosphatase that dephosphorylates alkyl-DHAP is influenced by the nature of the groupings substituted on the *O*-alkyl moiety.

Our data demonstrate that the insertion of a double bond, substitution of a hydroxy group near the center of the alcohol chain (22), or substitution of methyl groups in the iso or anteiso position do not hinder and, in some instances, can even enhance the incorporation of the alcohol moiety into alkyl-DHAP. Thus, these experiments show that the enzyme that catalyzes the formation of alkyl-DHAP from fatty alcohols and acyl-DHAP is not highly selective, although it cannot utilize alcohols that have functional groups (1,2- or 1,16-diols) at either end of the aliphatic chain. It would appear that the type of ether-linked chains occurring in nature results from the specificity of acyl-CoA reductase which forms the fatty alcohol.

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