STUDIES ON THE BIOSYNTHESIS OF THE O-ALKYL BOND IN GLYCEROL ETHER LIPIDS

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The ether bond in glycerol ether lipids is biosynthesized via the reaction of 1-acyl dihydroxyacetone-3-phosphate (acyl DHAP) with long chain alcohol to form 1-O-alkyl dihydroxyacetone-3-phosphate (alkyl DHAP) (1-3). Alkyl DHAP, like its counterpart acyl DHAP, is then enzymatically reduced by NADPH to form 1-O-alkyl glycerol-3-phosphate (alkyl G-3-P) (4). Alkyl G-3-P is subsequently acylated to form the ether analog of phosphatidic acid (5), which is then converted to phosphorylcholine- and phosphorylethanolamine-containing glycerol ether lipids via the Kennedy pathway (6,7). A number of workers have shown that plasmalogens are biosynthesized via the direct dehydrogenation of different glycerol ether lipids (for a review, see Ref. 8). Therefore, it seems that the reaction catalyzed by the alkyl DHAP synthase, i.e. the reaction of acyl DHAP with long chain alcohol, is the primary reaction by which the ether bond is introduced into all glycerol ether lipids. Studies on some of the properties of this enzyme are described here.

MATERIALS AND METHODS

[3H] sodium borohydride and D-[U-14C] glucose were obtained from New England Nuclear (Cambridge, Mass.). L-glyceraldehyde was purchased from Tridom/Fluka (Hauppauge, N.Y.). NAD+, pyruvate, glycerol-3-phosphate dehydrogenase, aldolase, triose phosphate isomerase and lactate dehydrogenase were all obtained from Boehringer Mannheim (Indianapolis, Indiana). Glycerokinase was purchased from Sigma (St. Louis, Mo.). All other materials were as previously described (4,5).

1DHAP-dihydroxyacetone-3-phosphate; G-3-P sn-glycerol-3-phosphate
R,S-[1-3H]glycerol was prepared by the reduction of L-glycer-aldehyde with [3H]NaBH₄. Labeled [1-3H,U-14C]-sn-glycerol-3-phosphate (G-3-P) was prepared by the enzymatic phosphorylation of glycerol using glycerokinase and ATP. R,S-[1-3H,U-14C]DHAP was prepared by the enzymatic oxidation of labeled G-3-P using NAD⁺ in the presence of excess pyruvate and lactate dehydrogenase to convert NADH back to NAD⁺. R-[1-3H,U-14C]DHAP and S-[1-3H,U-14C]DRAP were prepared from the labeled DHAP by treatment with either aldolase or triose phosphate isomerase in water (9,10). Specifically labeled palmityl DHAP was prepared biosynthetically from the specifically labeled DRAP and palmityl CoA (11). Details of the preparation of the labeled substrates will be published elsewhere.

Assignment of the position of the hydrogen retained on the biosynthetic alkyl DRAP was performed by degrading the ether bond by HI treatment (12), then converting the liberated glycerol enzymatically to G-3-P. The G-3-P was then oxidized to DHAP as above. The resulting DHAP was then treated with aldolase or triose phosphate isomerase or both aldolase and triose phosphate isomerase. The known stereo-specificity of these reactions allows the assignment of the position of the hydrogen on C-1 to be made (9,10). Other methods were the same as described previously (4,5).

RESULTS

Properties of the Alkyl DHAP Synthase

The enzyme has been shown to be membrane bound and is generally found associated with either the microsomal or mitochondrial fractions in a variety of different tissues (however, see later) (1,13, 14). For the studies described in this report, the main source of enzyme used was Erhlich ascites tumor cell microsomes with the occasional use of guinea pig liver mitochondria and rat brain microsomes. In all these systems, long chain alcohols were shown to react with acyl DHAP to form alkyl DHAP. DHAP alone or acyl G-3-P cannot replace the acyl DHAP as the substrate (1). Free CoA and Mg⁺⁺ were found to inhibit the reaction. Mg⁺⁺ and ATP were thought originally to stimulate the reaction between alcohol and acyl DHAP (1). However, we have recently found that Mg⁺⁺ alone is inhibitory and ATP by itself does not stimulate the reaction but together, they appear stimulatory due to the apparent removal of free Mg⁺⁺ from the reaction mixture by ATP (13). A variety of long chain alcohols including polyunsaturates and diols have been found to react with acyl DHAP to form ether lipid (Table I). However, alcohols above C₂₀ or below C₁₀ do not react to any significant extent (Table I). Long chain fatty acids were found to inhibit the reaction catalysed by alkyl DHAP synthase (1). This inhibition