THE GLYCEROL SOURCE FOR THE BIOSYNTHESIS OF ALKYL GLYCERYL ETHERS[1]

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SUMMARY

We have used a specific inhibitor to distinguish between glyceraldehyde-3-P and dihydroxyacetone-P as the glycerol source of alkyl glyceryl ethers. Only dihydroxyacetone-P reacted with fatty alcohols in the presence of the inhibitor and microsomes from preputial gland tumors to produce the *O*-alkyl linkage (CoA, ATP, and Mg^{++} are essential cofactors). The products formed have chromatographic properties consistent with the reaction sequence proposed by us in an earlier paper.

We recently reported (1,2) that the microsomal fraction from preputial gland tumors contains an enzyme system capable of converting long-chain fatty alcohols and DL-glyceraldehyde-3-P to *O*-alkylglycerols in the presence of ATP, CoA, Mg^{++}, and NADPH. This is the first cell-free system known to synthesize ether bonds in lipids. We later found that the washed microsomes contain a triose-P isomerase not removed by extensive washing, zonal centrifugation, or passage of the microsomes through Sephadex columns. The triose-P isomerase made it impossible to determine the true glycerol source in the initial step of the proposed reaction sequence (1).

High concentrations of phosphate ions will inhibit triose-P isomerase (3), but they also inhibit the biosynthesis of alkyl glyceryl ethers. Hartman (4) has just described the synthesis of halogenated derivatives of dihydroxyacetone-P that irreversibly inhibit triose-P isomerase by what is thought to be a specific

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modification of the active site. His report (4) prompted us to use the chlorine derivative of dihydroxyacetone-P (1-hydroxy-3-chloro-2-propanone phosphate) to pinpoint the glycerol source required in the initial step of the biosynthesis of alkyl glyceryl ethers. The findings we report in this communication show that the chlorine derivative of dihydroxyacetone-P could also be used to assess the role of dihydroxyacetone-P as an acyl acceptor (5,6).

METHODS

The source of preputial gland tumors (ESR-586) and cofactors, the preparation of microsomes, and the analytical methods used in these experiments were identical to those previously described (1,2). New materials used in these experiments were α-glycerophosphate dehydrogenase and triose-P isomerase (Sigma Chemical Co.). Triose-P isomerase, in 0.1 M phosphate buffer (pH 7.1), was analyzed (7) at 340 mμ in a Beckman DU spectrophotometer that was equipped with a Gilford Model 2000 recorder. The 1-hydroxy-3-chloro-2-propanone phosphate was a generous gift from Dr. Fred Hartman, Biology Division, Oak Ridge National Laboratory. The conditions of incubations are described in the table.

RESULTS AND DISCUSSION

The data in Figure 1 (B and C) demonstrate the presence of triose-P isomerase and the absence of glycerophosphate dehydrogenase (Fig. 1-D) in microsomal preparations of the preputial gland tumors washed as many as 3 times; Figure 1-A illustrates similar measurements made on preparations of commercial triose-P isomerase and glycerophosphate dehydrogenase. The slight slope in the initial O.D. curves in parts C and D of Figure 1 indicates that there is a slow oxidation of NADH by the microsomes unrelated to the reduction of dihydroxyacetone-P. Although glycerophosphate dehydrogenase was absent from the microsomes, we could not remove the isomerase by zonal centrifugation (8) or by passing the microsomes through Sephadex G-200 columns. Both DL-glyceraldehyde-3-P and dihydroxyacetone-P served equally well as the glycerol source in the biosynthesis of alkyl glyceryl ethers in the microsomal preparations (Table I). Other investigators (6) studying the acylation of dihydroxyacetone-P and

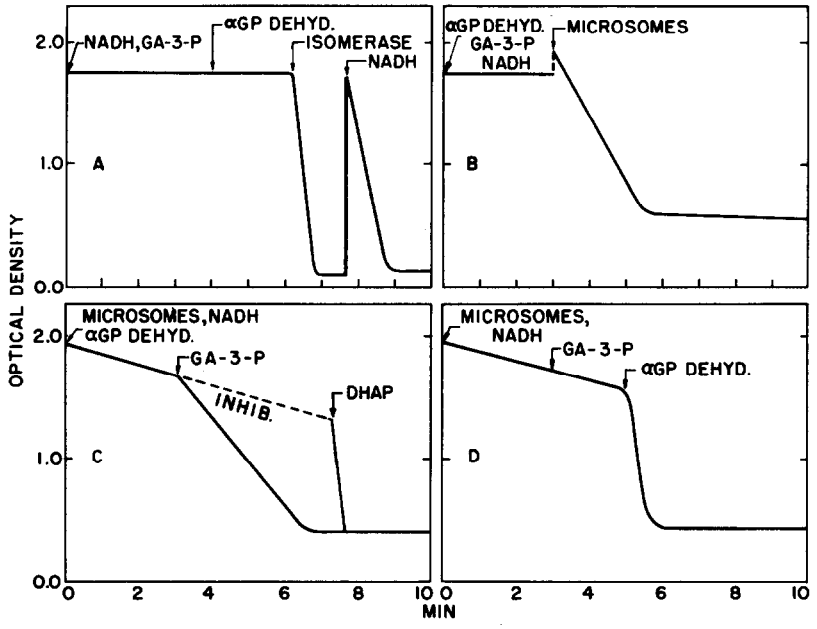

Figure 1. The presence of triose-P isomerase in microsomes from preputial gland tumors. The components added to the assay mixture (3 ml) were as follows: 5 μmoles DL-glyceraldehyde-3-P (GA-3-P); 5 μmoles dihydroxyacetone-P (DHAP); 1 μmole NADH; microsomes, washed 3 times, containing approximately 0.2 mg protein (similar results were obtained using microsomes washed 5 times); 300 μmoles potassium phosphate buffer, pH 7.1; 0.4 μmole 1-hydroxy-3-chloro-2-propanone phosphate (Inhib.); approximately 40 μg α-glycerophosphate dehydrogenase (αGP Dehyd.); and approximately 10 μg triose-P isomerase. Part A shows the control system for measuring triose-P isomerase with commercially available enzymes. Part B demonstrates the presence of a microsomal triose-P isomerase. Part C demonstrates that the microsomes oxidize NADH at a slow rate in the absence of glyceraldehyde-3-P and that the chlorine derivative of dihydroxyacetone-P completely inhibits the microsomal triose-P isomerase. Part D shows that αGP dehydrogenase was absent from the washed microsomes and suggests that the triose-P isomerase in microsomes is not simply contamination from the soluble fraction.

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glyceraldehyde-3-P have also found triose-P isomerase activity in microsomes that does not appear to be due to contamination of enzymes from the soluble fraction.

The halogenated dihydroxyacetone-P (0.4 μmoles per vial) completely inhibited the triose-P isomerase activity (Fig. 1-C) in the microsomal preparations as originally documented by Hartman for triose-P isomerase isolated from rabbit muscle (4). Our microsomal preparation containing added α-glycerophosphate dehydrogenase rapidly converted dihydroxyacetone-P to glycerophosphate in the presence of the inhibitor (Fig. 1-C), indicating that

Table I. The Effect of 1-Hydroxy-3-Chloro-2-Propanone Phosphate on the Biosynthesis of Alkyl Ether Bonds in Lipids from Dihydroxyacetone Phosphate and DL-Glyceraldehyde-3-Phosphate by Microsomes of Preputial Gland Tumors

System	Glycerol Source		Inhibitor*	Incorporation of 1-^{14}C-cetyl alcohol into *O*-alkylglycerols†	
	GA-3-P 5 μmoles	DHAP 5 μmoles	0.4 μmoles	% ^{14}C Exp. 1	% ^{14}C Exp. 2
1. Complete‡	+	-	-	23	30
2. Complete	-	+	-	24	30
3. Complete	+	-	+	3	3
4. Complete	-	+	+	25	28
5. Complete except microsomes were boiled 15 min	+	+	+	1	1

*Before adding other components of the complete system, each vial of microsomes was preincubated in the presence or absence of 1-hydroxy-3-chloro-2-propanone phosphate for 15 min at 37°C in 2 ml of 0.1 M phosphate buffer (pH 7.1). The complete system was then incubated for 1 hr at 37°C.

†*O*-Alkylglycerols were liberated from total lipid by $LiAlH_4$ reduction and verified by gas-liquid chromatography of the isopropylidene derivatives of the glyceryl ethers.

‡Each vial contained 30 μmoles ATP, 0.3 μmole CoA, 13 μmoles Mg^{++}, 1 mg microsomes (washed 3 times), and 100 mμmoles 1-^{14}C-cetyl alcohol in a final volume of 3 ml of 0.1 M phosphate buffer (pH 7.1).

the inhibitor did not affect α-glycerophosphate dehydrogenase activity. The data in Table I show that both dihydroxyacetone-P and DL-glyceraldehyde-3-P, with ^{14}C-cetyl alcohol, produced alkyl glyceryl ethers in the presence of microsomes, CoA, ATP, and Mg^{++}, but only the dihydroxyacetone-P served as the glycerol source in the presence of the inhibitor.

The products of the complete system formed in the presence or absence of the inhibitor had chromatographic properties (thin-layer and gas-liquid chromatography) identical to those described in our earlier report (1). These products have been partially characterized (9) by their chromatographic behavior before and after enzymic reduction with NADPH and by chromatography of derivatives obtained after the following chemical treatments: acidic and basic hydrolysis before and after $LiAlH_4$ or sodium borohydride reduction of the ketone groups,

acetolysis, periodate oxidation, or bisulfite addition. The data obtained support our earlier contention that the reaction sequence leading to the biosynthesis of glyceryl ethers involves the formation of ketone intermediates: *O*-alkyl dihydroxyacetone-P and *O*-alkyl dihydroxyacetone.

The possibility of dihydroxyacetone-P serving as a glycerol source was first suggested by us in two earlier reports (10,11) dealing with microsomal enzymes from normal cells. After private communication with us (12), Hajra (13) recently confirmed our earlier data (11) that showed dihydroxyacetone-P to be essentially equal to glyceraldehyde-3-P as an *O*-alkyl acceptor in the absence of any inhibitor. Our data with the inhibitor have clarified these earlier studies on the glycerol sources in the biosynthesis of *O*-alkyl bonds.

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REFERENCES

1. Snyder, F., Wykle, R. L., and Malone, B., *Biochem. Biophys. Res. Commun.*, 34, 315 (1969).
2. Snyder, F., Malone, B., and Wykle, R. L., *Biochem. Biophys. Res. Commun.*, 34, 40 (1969).
3. Oesper, P., and Meyerhof, O., *Arch. Biochem. Biophys.*, 27, 223 (1950).
4. Hartman, F. C., *Biochem. Biophys. Res. Commun.*, 33, 888 (1968).
5. Hajra, A. K., and Agranoff, B. W., *J. Biol. Chem.*, 243, 1617 (1968).
6. Rao, G. A., Sorrels, M. F., and Reiser, R., *Biochem. Biophys. Res. Commun.*, 31, 252 (1968).
7. Beisenherz, G., In: *Methods in Enzymology* (S. P. Colowick and N. O. Kaplan, eds.), Academic Press, New York, Vol. 1, p. 387 (1955).
8. Lee, T-C., Swartzendruber, D. C., and Snyder, F., *Biochem. Biophys. Res. Commun.*, 36, 748 (1969).
9. Wykle, R. L., and Snyder, F., *J. Am. Oil Chemists' Soc.*, 46, Abstract No. 109 (1969).
10. Snyder, F., Malone, B., and Blank, M. L., *Biochim. Biophys. Acta*, 187, 302 (1969).
11. Snyder, F., Malone, B., and Cumming, R. B., *Can. J. Biochem.*, in press (received July 4, 1969).
12. Fourth Annual Regional Lipid Meeting, Oak Ridge, Tennessee, May 8-9, 1969.
13. Hajra, A. K., *Biochem. Biophys. Res. Commun.*, 37, 486 (1969).