ALTERNATE TRIOSE PHOSPHATE PATHWAYS FOR GLYCERIDE BIOSYNTHESIS IN RAT LIVER*

G. Ananda Rao, M. F. Sorrels and Raymond Reiser

Department of Biochemistry and Biophysics
Texas A&M University
College Station, Texas 77843

Received March 18, 1968

The biosynthesis of glycerides in liver has been generally considered to occur by the glycerol 3-phosphate pathway (Weiss and Kennedy, 1956). Acylation of glycerol 3-phosphate occurs concomitantly with *de novo* synthesis of fatty acids (Howard and Lowenstein, 1965), through the mediation of thioesters of 4'-phosphopantetheine compounds such as acyl carrier protein (Goldfine *et al.*, 1967). On the other hand, glycerol 3-phosphate may be acylated with preformed fatty acids through the mediation of thioesters of CoASH (Johnston *et al.*, 1967; Smith *et al.*, 1967; Kuhn and Lynen, 1965). However, several observations suggested to us that the glycerol 3-phosphate pathway might not be the only mechanism for glyceride synthesis. Previous studies have shown that palmitoxy hydroxy acetone is converted to triglyceride by intestinal mucosa (Reiser and Williams, 1953) and that acyl dihydroxyacetone phosphate is present in liver mitochondria (Hajra and Agranoff, 1967). Furthermore, attempts to utilize the glycerol 3-phosphate pathway to produce glycerides with natural fatty acid distribution have been unsuccessful (Lands and Hart, 1964; Husbands and Reiser, 1966; Stoffel *et al.*, 1966). The following *in vitro* studies were carried out to compare the three trioses as direct acyl acceptors for glyceride biosynthesis.

* This work was supported by a grant from the National Institutes of Health (AM 06011).

Rats maintained for two weeks on a fat-free diet were killed by decapitation. The livers were removed quickly, washed with ice-cold 0.25 M sucrose and homogenized in three volumes of 0.25 M sucrose at 0°C. The homogenate was centrifuged at 1,000 xg for 15 minutes and the supernatant was again centrifuged at 11,000 xg for 20 minutes at 0°C. The mitochondria-free supernatant was subjected to Sephadex G-25 gel filtration to remove triose phosphates before use as the enzyme. The reaction mixtures contained enzyme equivalent to 0.5 ml of the homogenate, acetate-1-^{14}C (10^6 cpm; 20 mC per mmole), 20 µmoles of potassium citrate, 10 µmoles of ATP, 30 µmoles of GSH, 0.5 µmole of CoASH, 5 µmoles of NADPH, 10 µmoles of $MgCl_2$, 5 µmoles of $MnCl_2$, and 150 µmoles of potassium phosphate (pH 7.4) in a total volume of 3 ml. Graded levels of L-glycerol 3-phosphate, DL-glyceraldehyde 3-phosphate or dihydroxyacetone phosphate were included in the incubations except in the control flasks. After incubation for one hour at 37°C, the lipid was extracted and the degree of labeled acetate incorporation was measured. The results are given in Table I.

TABLE I

Incorporation of Acetate-1-^{14}C into Lipids in the Presence of Triose Phosphates by the Mitochondria-free Supernatant

Acyl acceptor	L-glycerol 3-phosphate	DL-glyceraldehyde 3-phosphate	Dihydroxyacetone phosphate
µmoles	cpm x 10^{-3}	cpm x 10^{-3}	cpm x 10^{-3}
none	140	140	140
0.5	210	210	180
1.0	250	290	160
2.0	300	340	170
4.0	380	330	170
6.0	380	340	140

Both glycerol 3-phosphate and DL-glyceraldehyde 3-phosphate stimulated the incorporation of acetate into lipids, but dihydroxyacetone phosphate did not. The extracted lipids were fractionated by thin-layer chromatography and the

radioactivities of the fractions compared (Johnston *et al.*, 1967). The glyceride fractions contained the major portion of ^{14}C activity, thus demonstrating the rapid acylation of triose phosphates during the synthesis of fatty acids. Since dihydroxyacetone phosphate did not stimulate lipid synthesis, the formation of glycerol 3-phosphate from glyceraldehyde 3-phosphate prior to acylation can be excluded. This route can also be excluded because inorganic phosphate present during incubation is inhibitory to triose phosphate isomerase (Beisenherz, 1955). These studies suggest direct participation of glycerol 3-phosphate and glyceraldehyde 3-phosphate in the acylation of nascent fatty acids. Similar results were obtained with enzyme preparations from livers of rats maintained on a stock ration.

In order to confirm the direct acylation of the triose phosphates, 5 μmoles of glycerol 3-phosphate and 10 μmoles each of DL-glyceraldehyde 3-phosphate and dihydroxyacetone phosphate were incubated respectively under conditions which would exclude their interconversions. Reaction mixtures were prepared containing in addition to the triose phosphate, either mitochondria or microsomes equivalent to 1 ml of the homogenate, 1 μmole of potassium palmitate-1-^{14}C, 30 μmoles of ATP , 30 μmoles of GSH, 0.5 μmoles of CoASH, 10 μmoles of $MgCl_2$, 25 μmoles of KF and 150 μmoles of potassium phosphate (pH 7.4) in a total volume of 2.5 ml. Incubations were carried out at 37°C for 30 minutes. The results are presented in Table II.

The microsomal fraction utilized all three triose phosphates for lipid synthesis, glycerol 3-phosphate being the most active. With mitochondria, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate were the most active. Most of the incorporated ^{14}C activity was found to be associated with a polar lipid as indicated by thin-layer chromatography. In the case of glycerol 3-phosphate, the lipid was found to be phosphatidic acid (Johnston *et al.*, 1967; Smith *et al.*, 1967). Presumably, corresponding monoacyl derivatives would be formed from glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Significant incorporation of palmitate-1-^{14}C into glycerides was observed in

TABLE II

Incorporation of Palmitate-1-^{14}C in Presence of Triose Phosphates

Acyl acceptor	mμmoles of palmitate-1-^{14}C incorporated into glycerides in presence of	
	Microsomes	Mitochondria
None	150	230
Glycerol 3-phosphate	880	280
Glyceraldehyde 3-phosphate	350	330
Dihydroxyacetone phosphate	330	420

control flasks containing no acyl acceptors. Apparently, in spite of careful washing procedures, some acyl acceptors remain bound to the particulate fractions. These studies demonstrate that the triose phosphates other than L-glycerol 3-phosphate are utilized for acylation and that specificities toward this process depend on the subcellular particles used.

The hexose monophosphate shunt may be a source of glyceraldehyde 3-phosphate for direct acylation. This could be related to the role of the shunt as a source of NADPH for fatty acid synthesis, since fatty acid synthesis and acylation may be interlocking phenomena. The participation of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate in glyceride biosynthesis indicates that the process is more complex than has been realized. The differences between the primary hydroxyl group in dihydroxyacetone phosphate and the secondary hydroxyl group in glyceraldehyde 3-phosphate may provide the specificity for the first acylation by either a saturated or an unsaturated fatty acid and hence be responsible for the acyl distribution in the final glyceride product.

SUMMARY Incorporation of acetate-1-^{14}C derived fatty acids into glycerides by enzyme preparations from rat liver is shown to be enhanced by L-glycerol 3-phosphate or glyceraldehyde 3-phosphate but not by dihydroxyacetone phosphate. All three triose phosphates stimulate the esterification

of palmitate-1-^{14}C in the presence of either microsomes or mitochondria. These observations show that other mechanisms than the glycerol 3-phosphate pathway should be considered for glyceride biosynthesis.

REFERENCES

Beisenherz, G., in Colowick, S. P., and Kaplan, N. O. (editors), Methods in Enzymology 1, 387 (1965).

Goldfine, H., Ailhaud, G. P., and Vagelos, P. R., J. Biol. Chem. 242, 4466 (1967).

Hajra, A. K., and Agranoff, B. W., J. Biol. Chem. 242, 1074 (1967).

Howard, C. F., and Lowenstein, J. M., J. Biol. Chem. 240, 4170 (1965).

Husbands, D. R., and Reiser, R., Fed. Proc. 25, 405 (1966).

Johnston, J. M., Rao, G. A., Lowe, P. A., and Schwarz, B. E., Lipids 2, 14 (1967).

Kuhn, N. J., and Lynen, F., Biochem. J. 94, 240 (1965).

Lands, W. E. M., and Hart, P., J. Lipid Res. 5, 81 (1964).

Reiser, R., and Williams, M. C., J. Biol. Chem. 202, 815 (1953).

Smith, M. E., Sedgwick, B., Brindley, D. N., and Hübscher, G., Europ. J. Biochem. 3, 70 (1967).

Stoffel, W., Schiefer, H. G., and Wolf, G. D., Hoppe-Seylers Zeitschrift f. Physiol. Chemie. 347, 102 (1966).

Weiss, S. B., and Kennedy, E. P., J. Am. Chem. Soc. 78, 3550 (1956).