Enzymatic Reduction of Alkyl and Acyl Derivatives of Dihydroxyacetone Phosphate by Reduced Pyridine Nucleotides

(Received for publication, April 19, 1972)

Edward F. Labelle, Jr.* and Amiya K. Hajra
From the Neuroscience Laboratory, Mental Health Research Institute, and the Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan 48104

SUMMARY

The reductions of either acyl or alkyl derivatives of dihydroxyacetone phosphate by enzymes from ascites tumor cell microsomes with NADPH were studied. The reaction rate was measured by determining the formation of labeled lipid from [4-3H]NADPH. The products were characterized as either 1-acyl or 1-alkyl [2-3H]glycerol 3-phosphate formed from the corresponding keto derivatives. The enzymes were found to be distributed in both mitochondrial and microsomal fractions obtained from different organs, i.e. brain, liver, kidney, etc.

When [4-3H]NADH was substituted for [4-3H]NADPH, very little formation of labeled lipid from the dihydroxyacetone phosphate derivatives was observed, showing that the enzymes are specific for NADPH as the coenzyme. The same results were obtained when the reduction was studied with nonradioactive NADH or NADPH and 32P-labeled acyl or alkyl dihydroxyacetone phosphate.

Experiments with A-[4-3H]NADPH and B-[4-3H]NADPH proved that only the B-hydrogen from the nicotinamide ring was transferred to reduce the keto compounds. Some preliminary results indicated that one enzyme may be responsible for the reduction of both acyl and alkyl derivatives of dihydroxyacetone phosphate.

Acyl dihydroxyacetone phosphate is enzymatically reduced by NADPH to 1-acyl sn-glycerol 3-phosphate (1). Recently a similar reduction of alkyl dihydroxyacetone phosphate, an intermediate in glycerol ether biosynthesis, to 1-alkyl glycerol-3-P by NADPH in various systems has been described (2, 3). The reduced products, i.e. 1-alkyl or 1-acyl glycerol-3-P, are enzymatically acylated to form phosphatidic acid or its ether analog which is further metabolized to various glycerolipids containing an ether or ester bond (2, 3). Therefore, the reduction of alkyl or acyl derivatives of dihydroxyacetone phosphate seems to be an important biochemical reaction for the metabolism of glycerolipids.

In guinea pig liver mitochondria we have shown that NADH cannot replace NADPH for the enzymatic reduction of acyl-dihydroxyacetone phosphate. Because NADH is the cofactor for glycerophosphate dehydrogenase (EC 1.1.1.8), which is mainly responsible for the formation of glycerol-3-P, a precursor of phosphatidic acid in tissues (4, 5), and NADPH is the cofactor for the acyl dihydroxyacetone phosphate pathway, the 3H-labeled reduced pyridine nucleotides were used to compare the pathways leading to the formation of glycerolipid in different tissues (6). However, some recent studies by Rao et al. (7-9) show that such a comparison may not be entirely valid. These authors reported that NADH is more effective than NADPH in the formation of phosphatidate from dihydroxyacetone phosphate in rat liver. They proposed that acyl dihydroxyacetone phosphate reductase is probably nonspecific toward the two reduced pyridine nucleotides and is even more active toward NADH than NADPH. However, the direct reduction of acyl dihydroxyacetone phosphate by NADH (or NADPH) in rat liver was not studied.

To resolve this question, we examined the reduction of palmitoyl dihydroxyacetone phosphate and hexadecyl dihydroxyacetone phosphate in various systems by either NADH or NADPH. The reactions were studied mainly by measuring the formation of labeled lipid from 4-3H-labeled reduced pyridine nucleotides and unlabeled keto lipids. The results verify our earlier conclusion that NADPH, not NADH, acts as the coenzyme in the reduction of acyl or alkyl dihydroxyacetone phosphate. The distribution and properties of the reductase from different tissues and the stereospecificity of the enzymes towards 4H-labeled NADPH (A or B) were also studied.

MATERIALS AND METHODS

[1-3H]Ethanol, n-[1-3H]glucose, and H3PO4 were obtained from New England Nuclear (Cambridge, Mass.). Hexokinase, D-glucose 6-phosphate dehydrogenase, glutamic dehydrogenase, glycerol 3-phosphate dehydrogenase, lactate dehydrogenase, alkaline phosphatase, horse liver alcohol dehydrogenase, isocitrate dehydrogenase, reduced and oxidized forms of pyridine nucleotides were obtained from Boehringer (New York, N.Y.). The dimethyl ketal of dihydroxyacetone phosphate was obtained...
from Sigma (St. Louis, Mo.) and the free carbonyl compound was regenerated by hydrolysis with Dowex 50-H+ resin (10). Palmitoyl dihydroxyacetone phosphate and palmitoyl dihydroxyacetone were chemically synthesized by decomposing the palmitoyl diazocetone by H3PO4 (11). Hexadecyl dihydroxacetone phosphate was prepared by a similar method as outlined before (12).1

The mitochondrial and microsomal fractions from different organs (guinea pig liver and rat liver, brain, kidney, adipose tissue, heart, spleen, testes, lung) were prepared by differential centrifugation of a 10% homogonate in 0.25 M sucrose (13) containing Na2-EDTA (2 mM). The subcellular particles were washed twice with 0.25 M sucrose (no EDTA) and suspended in 0.25 M sucrose containing 2 to 8 ng of protein per ml. The preparations were kept frozen at -20° in small batches and thawed quickly before use.

Ehrlich ascites tumor cells (a hypotetraploid strain) were grown in the abdominal cavities of Swiss white mice and were harvested 8 days after inoculation. Cells were washed with 0.9% NaCl at 80 × g to remove any red blood cells (14) and then disrupted by sonication of a cell suspension of 0.25 M sucrose (1 ml of packed cell per 5 ml) with a Branson sonifier (Heat System Co., Great Neck, N.Y.) at position 4 at 0-2° for six 30-s periods. This homogenate was then centrifuged at 800 × g for 10 min and the supernatant was further centrifuged at 12,000 × g for 10 min. Microsomes were isolated from the second supernatant by centrifugation at 100,000 × g for 20 min. The microsomal residue was washed twice with 0.25 M sucrose, suspended in 0.25 M sucrose, and stored frozen. The enzyme studied here was optimally active even after several thawings.

B-[4-3H]NADPH was prepared from [3H]glucose (6) and purified by the method of Pastore and Friedkin (15). The incubation mixture contained [3H]glucose (0.2 mM, 1 mCi), NADP+ (1 mm), crystalline hexokinase (0.4 mg), β-glucos 6-phosphate dehydrogenase (40 μg), ATP (10 mM), MgCl2 (5 mM), glycollglycine buffer (12.5 mM, pH 7.5) in a total volume of 2.0 ml. The mixture was incubated for 2 hours at 25° and the incubation mixture was diluted 20 times with water and put on a DEAE-cellulose column, 5 × 0.5 cm. NADPH was eluted from the column with glycollglycine buffer, NaCl gradient (15). The purity of the NADPH was checked by paper electrophoresis in 0.5 cm. NADPH was eluted from the column with glycollglycine buffer, NaCl gradient (15). The purity of the NADPH was checked by paper electrophoresis in 0.5 cm. NADPH was eluted from the column with glycollglycine buffer, NaCl gradient (15). The purity of the NADPH was checked by paper electrophoresis in 0.5 cm. NADPH was eluted from the column with glycollglycine buffer, NaCl gradient (15). The purity of the NADPH was checked by paper electrophoresis in 0.5 cm. NADPH was eluted from the column with glycollglycine buffer, NaCl gradient (15). The purity of the NADPH was checked by paper electrophoresis in 0.5 cm. NADPH was eluted from the column with glycollglycine buffer, NaCl gradient (15). The purity of the NADPH was checked by paper electrophoresis in 0.5 cm. NADPH was eluted from the column with glycollglycine buffer, NaCl gradient (15). The purity of the NADPH was checked by paper electrophoresis in 0.5 cm. NADPH was eluted from the column with glycollglycine buffer, NaCl gradient (15).

A-[4-3H]NADPH was prepared by reducing NADP+ with 1-[3H]ethanol and horse liver alcohol dehydrogenase. This enzyme transfers hydrogen to the A side at position 4 of the pyridine ring (16). Although NADP is the coenzyme of choice, horse liver alcohol dehydrogenase is known to utilize NADP+ also (17). The incubation mixture contained 1-[3H]ethanol (13 mM, 1 mCi), NADP+ (1 mM), horse liver alcohol dehydrogenase (0.1 mg), semicarbazide hydrochloride (7.5 mM)-glycine buffer (2.2 mM)-pyrophosphate buffer (7.5 mM) buffer at pH 8.8 in a total volume of 3.0 ml. The reduction of NADP+ in the reaction mixture was monitored by measuring the increase in absorbance at 340 nm. After 2 hours at room temperature (optical density at 340 nm - 1.02) the reaction mixture was diluted 20 fold with water and then transferred to a DEAE-cellulose column, 5 × 0.5 cm. The column was washed with 100 ml of 0.01 M glycylglycine buffer (pH 7.5) to remove the radioactive ethanol and the semicarbazide, etc., and the A-[4-3H]NADPH was eluted from the column with 10 ml of the same buffer containing NaCl (0.60 M) at pH 8.0. The purity of the [3H]NADPH was checked by high voltage paper electrophoresis as described above. About 75% of the radioactivity migrated with carrier NADPH. The yield of [3H]NADPH was about one-third of that expected from the absorbance change at 340 nm, possibly because of preferential utilization of H+ by the enzyme.

B-[4-3H]NADPH was prepared by dephosphorylation of B-[4-3H]NADPH with alkaline phosphatase (6, 18). The incubation was carried out as described before and the phosphatase activity after incubation was destroyed by heating at 100° for 2 min (6). The purity of NADH was also checked by high voltage paper electrophoresis. About 80% of radioactivity of the [3H]NADPH was found to migrate with standard NADH (12 cm from the origin). No radioactivity was found in the NADPH spot, but 10 to 15% of the radioactivity was present in a slow moving spot which was found to be inactive toward α-glycerophosphate dehydrogenase (see below).

The purities of the H labeled coenzymes were further confirmed by their activity toward different dehydrogenases. A sample of the B-[4-3H]NADPH was incubated for 10 min at 37° with α-ketoglutarate (23 mM), NH4Cl (46 mM), glutamic dehydrogenase (100 μg), and triethanolamine buffer (pH 7.0, 0.36 M) in a total volume of 0.11 ml. A control incubation was run without α-ketoglutarate. The radioactive product (glu- 

1 A. K. Hajra, manuscript in preparation.
µm), ATP (8 mM), CoA-SH (40 µM), MgCl₂ (4 mM), NaF (4 mM), Tris-Cl buffer (72 mM), dihydroxyacetone[32P]phosphate (62 µM, 10⁶ cpm), and ascites cell microsomes in a total volume of 1.2 ml. After 1 hour incubation the lipid was extracted from the incubation mixture (21) and the lipid extract was subjected to alkaline methanolysis as described previously (2). The alkaline-stable lipid was further purified by chromatography on a silicic acid column (2).

The [3H]-labeled alkyl ether lipid was dephosphorylated by acetylation (22, 23) then saponified. A mixture of [3H]-labeled lipid (1.5 × 10⁶ cpm) formed by enzymatic reduction of alkyl dihydroxyacetone phosphate with B-[3H]NADPH and synthetic 1-alkyl rac-glycerol-3-P (2 mg) was heated at 150°C for 20 hours with 2.0 ml of acetic anhydride-acetic acid (2:3) in a 16-mm screw-capped (Teflon-lined) test tube. After cooling the solution was evaporated under a nitrogen stream and the residue was saponified by heating at 100°C for 1 hour with 2 ml of 1 N KOH in 50% aqueous ethanol. After cooling, 2 ml of water were added and the mixture was extracted four times with 2 ml of diethylether. The combined ether extract was washed twice with 2 ml of water, dried over Na₂SO₄, and the ether removed by blowing a stream of nitrogen. The residue was dissolved in CHCl₃-methanol (2:1) and the radioactivity of an aliquot was determined. The recovery of [3H] was 98%.

The acetonate was prepared as described by Hanaoka et al. (23). A portion of the dephosphorylated [3H]-labeled lipid (2.5 × 10⁶ cpm) was added to 2.0 mg of chymio alcohol and the mixture was dissolved in 1.0 ml of acetonitrile and 5 µl of 70% HClO₄. After 30 min at room temperature, 5 ml of diethyl ether, 2 drops of concentrated NH₄OH (17%), and 5 ml of water were added to the solution, mixed well, and centrifuged. The ether layer was transferred to another tube and the aqueous layer was again extracted with 3 ml of ether. The combined ether layers were washed with 5 ml of water, dried over Na₂SO₄, and the acetonide recovered by removing ether by blowing nitrogen. The recovery of [3H] was 98%.

Periodate oxidation of the [3H]-labeled lipid was carried out with pyridine as the solvent (24). 4-[3H]-Labeled dephosphorylated lipid (1.2 × 10⁶ cpm) with carrier chymio alcohol (1.0 mg) was oxidized by stirring with sodium metaperiodate (10 mg) in pyridine (0.5 ml) at room temperature, with magnetic stirrer. After 4 hours the pyridine was removed under a gentle stream of nitrogen, the residue was dissolved in 3 ml of diethyl ether, and the ether layer was washed twice with 2 ml of boiled water. The extract was dried over anhydrous Na₂SO₄ and the ether was removed under nitrogen. The recovery of [3H] was 97%.

Chymio alcohol was oxidized by a mixture of periodate and permanganate as described by von Rudloff (25). A mixture of [3H]-labeled dephosphorylated lipid (1.5 × 10⁶ cpm) and chymio alcohol (2 mg) was stirred magnetically with 0.4 ml of oxidant (0.1 g of NaIO₄, 0.015 g of KMnO₄, and 5 ml of water), and 0.02 ml of 1 N H₂SO₄ in t-butyl alcohol (0.5 ml), overnight at room temperature. Four milliliters of 1 N KCl containing H₃PO₄ (21, 26) were added to the reaction mixture, and the oxidized lipid was extracted four times with 3 ml of hexane. The combined hexane extract was washed with water and an aliquot was used to determine the radioactivity. Only 2% of the [3H] (2.9 × 10⁶ cpm) was present in the lipid extract. On further extraction of the lower layer (MnO₄⁻, IO₃⁻ oxidation product) with diethyl ether no additional [3H] was recovered. When the hexane extract was checked on thin layer chromatography (hexane-ethyl ether-acetic acid 80:70:2) a spot corresponding to synthetic 2-O-hexadecyl glycolic acid (Rf = 0.75) was seen, showing that the chymio alcohol was oxidized to the expected product.

Incubations were carried out in 16-mm (inside diameter) test tubes. The lipid substrates, alkyl or acyl dihydroxyacetone phosphate, were emulsified in dilute buffer before adding to the incubation mixture. The detergent (Tween-20) and the lipids, both in chloroform solution, were dried down together under a stream of nitrogen and the residue was taken up in 0.05 M Tris buffer and dispersed by agitating in an ultrasonic water bath. After the addition of necessary cofactors, substrates and water, the reaction was started by the addition of microsomal or mitochondria fractions. The tubes were incubated at 37°C in a water bath and shaken continuously at 80 oscillations per min (Dutchoff Metabolic Shaking Bath). The reaction was stopped by adding 4.5 ml of chloroform-methanol (1:2) and the lipid was extracted by further addition of 1.5 ml of chloroform and 1.5 ml of 2 M KCl containing H₂PO₄ (21, 26). After mixing and centrifugation, the lower layer containing the lipid was transferred to another tube with a Pasteur pipette and the solvent was removed by blowing a gentle stream of nitrogen at 37°C. The lipid was further washed to remove any water-soluble material as described previously (23). High blank values were observed when the solution containing acid and [3H]-labeled NADPH or NADH was stored for a long time (18 hours), possibly because of slow breakdown of labeled pyridine nucleotides at low pH to form lipid-soluble materials. Generally the blank (no enzyme or boiled enzyme) values were less than 0.1% of the radioactivity used for incubation. These blank values were subtracted in the results presented.

Thin layer chromatography for phospholipids was performed on commercial silica gel plates (E. Merck, obtained through Brinkmann Instruments, N.J.), Solvents containing bisulfite (27) were used to separate the keto lipid from the acyl or alkyl glycerol-3-P. The radioactive spots on chromatograms containing either ³²P or ¹⁴C were located by radioautography (11). A Beckman model LS133 liquid scintillation counter was used for quantitative determination of radioactivity. Aliquots of radioactive lipids dried in counting vials were dissolved in a solvent containing tolune, ethanol, 2,5-diphenyloxazole (PPO), and 7,4-bis[2-4-methyl-5-phenyloxazolyl]benzene (diethyl POPOP) (950 ml, 50 ml, 5 g, 0.2 g), and the radioactivity was determined in the scintillation counter. Radioactive spots from thin layer chromatograms were cut out in a counting vial, the powder was dispersed in 0.5 ml of water by sonic oscillation, suspended in 10 ml of scintillation solvent containing toluene, BBS-3, 2,5-diphenyloxazole, and 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (1000 ml, 200 ml, 1 g, 0.25 g), and counted. The radioactivity in the [3H]-labeled spots on paper electrophoretograms was determined by combusting the [3H] in an oxidizer (Packard Tri-Carb model No. 300) and the resulting [3H]H₂O was counted in a dioxane-containing solvent (28).

Protein was determined by the method of Lowry et al. (29). The amount of synthetic alkyl or acyl dihydroxyacetone phosphate was determined by determining the total phosphate after digestion with magnesium nitrate (30). Other methods and materials were the same as described previously (11, 12, 20, 21).

1 Polyoxymethylene sorbitan mononolaurate.
2 The bisulfite solution should be freshly prepared and the commercial silica gel plates must be used to separate the keto and hydroxy lipids.
3 Bio-Solv 3; Beckman Instruments, Fullerton, Calif.
RESULTS

Requirements for Incorporation of \(^3\)H into Lipid—Table I shows the requirements for the formation of \(^3\)H-labeled lipid from [4-\(^3\)H]NADPH. The formation of labeled lipid was found to be completely dependent either on the addition of lipid substrates (acyl or alkyl dihydroxyacetone phosphate) or on the enzyme. Enzyme activity was largely lost by heating the microsomal preparation at 100° for 10 min. Fluoride was added to protect both the substrates and products from hydrolysis by lipid phosphomonoesterase (31). However, with the short term incubation in the presence of excess substrate, as employed here, fluoride did not affect the formation of \(^3\)H-lipid. Our other results show (see below) that long term incubation without fluoride increased the formation of labeled neutral lipid with a concomitant decrease of \(^3\)H in the phospholipid fraction, indicating that lipid phosphomonoesterase present in the enzyme preparation was more active when fluoride was not present. EDTA was included in the reaction mixture because the previous study showed Mg\(^{2+}\) and some other metal ions inhibited the reaction (1). Some stimulation of activity by EDTA was found with acyl dihydroxyacetone phosphate as the substrate but with alkyl dihydroxyacetone phosphate it had no effect (Table I). EDTA was routinely added to all incubation mixtures to prevent a possible inhibition by metal ions present either in the enzyme preparation or in the substrates.

Characterization of Product—The properties of the \(^3\)H-labeled lipids enzymatically formed from [PH]NADPH and either acyl or alkyl dihydroxyacetone phosphate (Table I) resembled the corresponding acyl or alkyl glycerol-3-P derivatives. On thin layer chromatography in different solvent systems the \(^3\)H-labeled product migrated mainly with synthetic acyl or alkyl glycerol-3-phosphates. Fig. 1 shows the distribution of radioactivity of \(^3\)H-labeled lipid formed from [4-\(^3\)H]NADPH with alkyl dihydroxyacetone phosphate as the substrate. Two radioactive areas were located on the chromatogram. The major component, containing 87% of the radioactivity migrated the same distance as synthetic 1-alkyl glycerol-3-P (RF = 0.40). Similarly the \(^3\)H labeled product formed from [PH]NADPH and palmitoyl dihydroxyacetone phosphate migrated with synthetic 1-palmitoyl glycerol-3-P (RF = 0.40). The labeled lipids also migrated with the synthetic standards on thin layer chromatography in a different solvent system (chloroform-methanol-acetic acid-5% sodium bisulfite, 100:40:12:4). The RF for alkyl glycerol-3-P and the labeled lipid formed from alkyl dihydroxyacetone phosphate was 0.45, while it was 0.42 for acyl glycerol-3-P and the labeled lipid formed from acyl dihydroxyacetone phosphate. Similarly, the \(^3\)H-labeled product formed from acyl or alkyl dihydroxyacetone [\(^3\)P]phosphate and nonradioactive NADPH in the presence of enzyme migrated with the synthetic monoacyl or alkyl glycerophosphate (see Fig. 5).

The radioactive \(^3\)H-lipid which was present at the solvent front as shown in Fig. 1 was found to have the properties of a neutral lipid. On Silica Gel G plate developed with hexane-ether (3:7), this neutral lipid migrated with chymio alcohol. The amount of the \(^3\)H-labeled chymio alcohol formed was increased by prolonged incubation (30 min) of [PH]NADPH with alkyl dihydroxyacetone phosphate and enzyme. This lipid could be formed either by enzymatic reduction of the dephosphorylated (by lipid phosphomonoesterase present in the microsomal preparation) substrate, i.e. alkyl dihydroxyacetone or by dephosphorylation of 1 alkyl [2-\(^3\)H]glycerol 3-phosphate. That the

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Radioactivity in lipid from hexadecyl DHAP</th>
<th>Radioactivity in lipid from palmitoyl DHAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole system</td>
<td>26,200</td>
<td>21,000</td>
</tr>
<tr>
<td>Whole system — EDTA</td>
<td>27,000</td>
<td>17,200</td>
</tr>
<tr>
<td>Whole system — F(^-)</td>
<td>26,500</td>
<td>21,500</td>
</tr>
<tr>
<td>Whole system — F(^-) — EDTA</td>
<td>27,700</td>
<td>20,500</td>
</tr>
<tr>
<td>Whole system — lipid substrate</td>
<td>225</td>
<td>225</td>
</tr>
<tr>
<td>Whole system — enzyme, — lipid substrate</td>
<td>175</td>
<td>187</td>
</tr>
<tr>
<td>Whole system with boiled enzyme(^a)</td>
<td>83</td>
<td>83</td>
</tr>
<tr>
<td>Whole system with boiled enzyme(^a)</td>
<td>772</td>
<td>500</td>
</tr>
</tbody>
</table>

\(^a\) DHAP, dihydroxyacetone phosphate.

\(^b\) The microsomal suspension was heated at 100° for 5 min.

![Fig. 1. Distribution of radioactivity on a thin layer plate containing \(^3\)H-labeled lipid. Hexadecyl dihydroxyacetone phosphate, [PH]NADPH, and ascites microsomes were incubated for 5 min as described in Table I. The lipids were extracted and developed on a silica gel plate with chloroform-methanol-acetic acid-acetone-water (100:30:20:40:10). The plate was divided into bands and the bands were scraped out and counted (see "Materials and Methods"). The position of standard chemically synthesized 1-hexadecyl \(\Delta_5\)-glycerol 3-phosphate (ALK, GP) on the thin layer chromatography plate is also shown.](http://www.jbc.org/content-pdf/242/1/5825/F1.large.jpg)
latter hypothesis is probably correct is supported by the following findings. (a) When synthetic acyl or alkyl dihydroxyacetone were incubated with [4-3H]NADPH and ascites cell microsomes, only a small amount (1 to 3% of that from the corresponding dihydroxyacetone phosphate derivatives) of radioactive lipid was formed. (b) In a time course study it was observed that the increase in the radioactivity in the chimyl alcohol with the increasing time is accompanied by a corresponding decrease in the radioactivity of the 1-alkyl glycerol-3-P spot.

The identity of the 3H-labeled product formed from palmityl dihydroxyacetone phosphate was further confirmed by subjecting the 3H-labeled lipid to alkaline methanalysis (21, 32). After treatment with alkali all the 3H in the lipid was converted to water-soluble material. On high voltage paper electrophoresis at pH 1.5, 94% of this 3H-labeled compound migrated with glycerol 3-phosphate. The rest of the radioactivity (6%) which remained at the origin, was probably [2-3H]glycerol formed from labeled monoglyceride, which was enzymatically produced during incubation by dephosphorylation of 1-alkyl glycerol 3-P (see above). Similarly acyl dihydroxyacetone [32P]phosphate was enzymatically reduced by NADPH to form another 3P-lipid which was hydrolyzed to glycerol-3-32P by treatment with alkali. As mentioned above, on incubation with NADPH and ascites tumor cell microsomes, alkyl dihydroxyacetone [32P]phosphate was converted to a product which on thin layer chromatography migrated with 1-alkyl glycerol-3-P (Fig. 5), however, if long chain acyl-CoA’s (palmityl or oleoyl) were added to the incubation mixture the major 32P-lipid migrated with phosphatidic acid. This lipid was identified as 1-alkyl, 2-acyl glycerol-3-32P, because after hydrolysis by alkali (21) or snake venom phospholipase A (33) the resulting radioactive lipid migrated with alkyl glycerol-3-32P on thin layer chromatography. The structure of the enzymatically reduced product was confirmed by dephosphorylating the 3H-labeled lipid by acetylation (Fig. 2). After acetylation and saponification, the 3H-labeled lipid migrated with chimyl alcohol on thin layer chromatography developed either with hexane-diethyl ether (3:7) (Fig. 2) or with diethyl ether (RF = 0.2). The identification of the dephosphorylated lipid as 1-alkyl glycerol was confirmed by conversion to the acetonide. After treatment with acetone and HClO4 (see “Materials and Methods”), 97% of the 3H in the lipid migrated with authentic acetonide of chimyl alcohol (Fig. 2). The rest of the radioactivity was present in the original alkyl ether of glycerol. After oxidation by IO3-, the major product migrated to the same position as 2-O-alkyl glycoaldehyde (Fig. 2). However, on oxidation with a mixture of periodate and permanganate, only 2% of 3H was present in the lipid extract. When the resulting lipid was examined by thin layer chromatography (hexane-ether-acetic acid 90:70:2), no radioactivity was present in the 2-O-alkyl glyceraldehyde spot (RF = 0.75). The retention of 3H in the lipid after periodate oxidation but the complete absence of 3H in the periodate-permanganate-oxidized product, proved that 3H was present in the enzymatically reduced product exclusively in position 2 of the 1-alkyl glycerol derivative.

Comparison of [4-3H]NADH and [3H]NADPH Incorporation—When [4-3H]NADH is substituted for [4-3H]NADPH as the co-factor for the reduction of either hexadecyl dihydroxyacetone phosphate or palmityl dihydroxyacetone phosphate by ascites cell microsomes, very little [3H]-labeled lipid was formed. Fig. 3 shows such a comparison of NADPH versus NADH as the co-factor with the increasing concentration of enzyme. The activity of the enzyme with [4-3H]NADPH was found to be fairly linear for up to 0.2 mg of protein. But with [3H]NADH as the cofactor the enzyme activity was found to be negligible. However, at higher concentrations of NADH a small formation of labeled lipid from alkyl or acyl dihydroxyacetone phosphate was seen (Fig. 4). The formation of labeled lipid from NADH was shown to be linear with time (Fig. 4); but the enzyme activity with NADH was found to be only 6 to 7% of that for NADPH, although the concentration of NADH was 4 times that of NADPH (100 versus 40 μM).

The possibility that the added NADH was rapidly oxidized by enzyme (or enzymes) present in the crude particulate fraction and hence was not available for the reduction of acyl or alkyl dihydroxyacetone phosphate, was checked by determining the amount of NADPH present at the end of incubation. After incubation of hexadecyl dihydroxyacetone phosphate, [4-3H]-
NADH with ascites cell microsomes for 15 min, as described in Fig. 1, glycero-phosphate dehydrogenase (10 µg) and dihydroxyacetone phosphate (3 µmoles) were added and the reaction mixture was incubated further for 15 min. An aliquot of the mixture was subjected to paper electrophoresis (see "Materials and Methods") and the radioactivity in the glycerol-3-P spot was determined. More than 50% of the 3H originally present in [3H]NADH was recovered in the glycerophosphate spot, showing that the major portion of the added 1-{4-[3H]NADH was still available at the end of incubation.

The low activity of the reductase for the reduction of acyl and alkyl dihydroxyacetone phosphate by NADH compared to that of NADPH was also verified by using nonradioactive reduced pyridine nucleotides and acyl or alkyl dihydroxyacetone [3P]-phosphate derivatives. The radioactive precursors were incubated with NADH or NADPH and ascites cell microsomes and the resulting reduced lipid was separated from the keto lipid by thin layer chromatography using a developing solvent containing bisulfite. Fig. 5 shows the radioautogram of the thin layer chromatography plate after separation of the lipids from each other. It is seen that a radioactive lipid was formed when NADPH was present whose migration rate corresponded to acyl or alkyl glycerol-3-P derivative. Only faint spots of corresponding lipids were seen when NADH was used as the cofactor (Fig. 5). With hexadecyl dihydroxyacetone [3P]phosphate and NADPH as the substrate 22% of 3P was present in the 1-alkyl glycerol-3-P spot and with acyl dihydroxyacetone [3P]phosphate as the substrate, 66% of the radioactivity was recovered in the reduced lipid. The corresponding figures for NADH were 0.07 and 0.08%, respectively.

The reductase activity was found to be widely distributed in both mitochondrial and microsomal fractions of different organs (Table II). Although the optimum assay conditions in each organelle was not determined, it is seen that in every case the enzyme was more active with NADPH than with NADH. However, when the concentration of the coenzyme was increased (from 0.08 to 0.8 mM) an increase in activity with NADH was...
noticed with the particulate fraction from rat liver. The activity with NADPH remained practically unchanged.

Optimum Conditions for Incorporation of $^3$H into Lipid from Labeled NADPH—The formation of labeled lipid from $[4-3^H]$NADPH by ascites cell microsomes was found to be linear with time and with increasing amount of protein up to 0.15 mg, as shown in Figs. 3 and 4. Fig. 6 shows the increase in the reaction rate with changing NADPH concentration with either hexadecyl dihydroxyacetone phosphate or palmitoyl dihydroxyacetone phosphate as the substrate. The enzyme was saturated at a relatively low concentration of NADPH (40 to 50 $\mu$M). The double reciprocal plots are also shown from which the $V_{max}$ and $K_m$ values were calculated. The $K_m$ for NADPH was 10 $\mu$M and $V_{max}$ with hexadecyl dihydroxyacetone phosphate was 27 nmoles per min per mg of protein and with palmitoyl dihydroxyacetone phosphate it was 23 nmoles per min per mg. The differences between the alkyl and acyl derivatives may not be significant and some variations in relative activities were observed.

Fig. 7 shows the effect of increasing the concentration of the lipid substrates on the activity of the enzyme. In this particular experiment, more activity was observed with acyl dihydroxyacetone phosphate than with alkyl dihydroxyacetone phosphate.

### Table II

Enzymatic activity for reduction of palmitoyl and hexadecyl derivatives of dihydroxyacetone phosphate in particulate fractions from different tissues

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>Lipid substrate</th>
<th>Concentration of pyridine nucleotide</th>
<th>Radioactivity in lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>[$^3$H] NADPH</td>
<td>From [$^3$H] NADPH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[nM]</td>
<td>[nM]</td>
</tr>
<tr>
<td>Ascites microsomes</td>
<td>Hexadecyl-DHAP</td>
<td>40</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Palmitoyl-DHAP</td>
<td>40</td>
<td>0.4</td>
</tr>
<tr>
<td>Guinea pig liver mito-</td>
<td>Hexadecyl-DHAP</td>
<td>40</td>
<td>0.00</td>
</tr>
<tr>
<td>ochondria</td>
<td></td>
<td></td>
<td>4.4</td>
</tr>
<tr>
<td>Rat brain mitochondria</td>
<td>Hexadecyl-DHAP</td>
<td>40</td>
<td>0.00</td>
</tr>
<tr>
<td>Rat brain microsomes</td>
<td>Hexadecyl-DHAP</td>
<td>40</td>
<td>0.00</td>
</tr>
<tr>
<td>Rat liver mitochondria</td>
<td>Hexadecyl-DHAP</td>
<td>40</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Palmitoyl-DHAP</td>
<td>40</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Polymaloyl-DHAP</td>
<td>500</td>
<td>0.1</td>
</tr>
<tr>
<td>Rat liver microsomes</td>
<td>Hexadecyl-DHAP</td>
<td>40</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Palmitoyl-DHAP</td>
<td>80</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>Polymaloyl-DHAP</td>
<td>800</td>
<td>0.72</td>
</tr>
<tr>
<td>Rat kidney mitochondria</td>
<td>Hexadecyl-DHAP</td>
<td>40</td>
<td>0.99</td>
</tr>
<tr>
<td>Rat kidney microsomes</td>
<td>Hexadecyl-DHAP</td>
<td>40</td>
<td>0.66</td>
</tr>
<tr>
<td>Rat heart mitochondria</td>
<td>Hexadecyl-DHAP</td>
<td>40</td>
<td>0.74</td>
</tr>
<tr>
<td>Rat heart microsomes</td>
<td>Hexadecyl-DHAP</td>
<td>40</td>
<td>1.86</td>
</tr>
<tr>
<td>Rat adipose tissue</td>
<td>Hexadecyl-DHAP</td>
<td>40</td>
<td>3.42</td>
</tr>
<tr>
<td>mitochondria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat adipose tissue</td>
<td>Hexadecyl-DHAP</td>
<td>40</td>
<td>6.55</td>
</tr>
<tr>
<td>microsomes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat spleen mitochondria</td>
<td>Hexadecyl-DHAP</td>
<td>40</td>
<td>1.38</td>
</tr>
<tr>
<td>Rat lung mitochondria</td>
<td>Hexadecyl-DHAP</td>
<td>40</td>
<td>1.21</td>
</tr>
<tr>
<td>Rat testes mitochondria</td>
<td>Hexadecyl-DHAP</td>
<td>40</td>
<td>0.96</td>
</tr>
</tbody>
</table>

a DHAP, dihydroxyacetone phosphate.
These differences are probably due to the variations in the emulsions prepared and are unavoidable when the enzyme, substrate, and product are all insoluble and occur in separate phases.

The reductase has a very broad pH optimum between pH 6.5 and 7.5 with either acyl or alkyl dihydroxyacetone phosphate.

Table III gives the optimum activity of the enzyme from different sources. The $K_m$ and $V_{max}$ values were determined by changing the concentration of $^3$H-labeled NADPH, keeping the concentration of acyl or alkyl dihydroxyacetone phosphate constant. Whereas high activity was found in both ascites cell microsomes and guinea pig liver mitochondria, the affinity of the enzyme for NADPH was higher in tumor cells ($lower K_m$) than in liver. This probably explains the relatively high activity of the acyl dihydroxyacetone phosphate pathway (6) and also the increased concentration of either lipid in tumor cells (34, 35). Although the activity of the enzyme in brain is fairly low, it has high affinity for the cofactor, which probably leads to the increased amount of alkyl and alkenyl ethers in brain (36).

Specificity of Reductase toward A- or B-[4-$^3$H]NADPH—Oxido-reductase employing pyridine nucleotides as the coenzymes show absolute specificity in utilizing (or transferring) hydrogen toward either A or B side at position 4 of the nicotinamide ring (16). As shown above and also in Table IV, $^3$H-labeled reduced lipids were enzymatically formed by reducing either hexadecyl or palmitoyl dihydroxyacetone phosphate with B-[4-$^3$H]NADPH. However, under identical conditions, no $^3$H was incorporated into lipid from A-[4-$^3$H]NADPH (Table IV) showing that the reductase was specific for the B-NADPH. To show that the A-[4-$^3$H]NADPH was active toward the enzyme (donating 'HI, the coenzyme was continuously regenerated in the incubation mixture either by adding isocitric dehydrogenase and isocitrate or by adding glucose 6-phosphate dehydrogenase and glucose 6-phosphate to the reaction mixture. In the former case, because isocitric dehydrogenase is an A-specific enzyme (16) the reduction of [4-$^3$H]NADP+ (enzymatically formed by oxidation of A-[4-$^3$H]NADPH) with nonradioactive isocitrate would produce B-[4-$^3$H]NADPH. Whereas with glucose 6-phosphate dehydrogenase (B-specific) and glucose 6-phosphate, the A-[4-$^3$H]NADPH would be regenerated back. Thus it is seen (Table IV, Part B), that when isocitric dehydrogenase and isocitrate were present in the reaction mixture, $^3$H-labeled lipid was enzymatically formed from A-[4-$^3$H]NADPH and hexadecyl dihydroxyacetone phosphate. The incorporation of $^3$H was low, due to the presence of NADP+ in the A-[4-$^3$H]NADPH preparation, which after reduction lowered the specific activity of NADPH. Very little radioactivity was incorporated into the lipid when glucose 6-phosphate dehydrogenase and glucose 6-phosphate were used for regeneration of NADPH, proving that the enzymes responsible for the reduction of acyl or alkyl dihydroxyacetone phosphate specifically transfer hydrogen from the B side of the nicotinamide ring.

**Competition between Two Lipid Substrates**—Throughout this study it was apparent that the activities of the enzymes for the reduction of either acyl or alkyl dihydroxyacetone phosphate are remarkably similar in different tissues and under different experimental conditions. These results naturally point toward the possibility that the same enzyme is responsible for the reduction of these two similar substrates. At the present stage, with the crude particle bound enzyme, a detailed study cannot be made to prove the above hypothesis. However, a preliminary study of competition between these two substrates for the enzyme showed that possibly the same enzyme was involved in the reduction of both the substrates. The reduction of constant amount of alkyl dihydroxyacetone [32P]phosphate by NADPH by ascites microsomes with the increasing amount of nonradioactive acyl dihydroxyacetone phosphate was studied (Fig. 8). As the concentration of acyl dihydroxyacetone phosphate is increased, the reduction of alkyl dihydroxyacetone phosphate is inhibited, suggesting a competition between two substrates for the common reductase.

### Table III

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Substrate</th>
<th>$K_m$ $(NADPH)$</th>
<th>$V_{max}$ (mmoles/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ascites microsomes</strong></td>
<td>Hexadecyl-DHAPa</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Palmitoyl-DHAP</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td><strong>Guinea pig liver mitochondria</strong></td>
<td>Hexadecyl-DHAP</td>
<td>50</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Palmitoyl-DHAP</td>
<td>50</td>
<td>33</td>
</tr>
<tr>
<td><strong>Rat brain microsomes</strong></td>
<td>Hexadecyl-DHAP</td>
<td>5</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>Palmitoyl-DHAP</td>
<td>7</td>
<td>3.1</td>
</tr>
</tbody>
</table>

* DHAP, dihydroxyacetone phosphate.

### Table IV

**Incorporation of tritium into lipid from either A- or B-[4-$^3$H]NADPH**

The incubation conditions were the same as described in Table I, using ascites cell microsomes as the enzyme source. Either B-[4-$^3$H]NADPH (8 µM, 104 cpm) or A-[4-$^3$H]NADPH (8 µM, 104 cpm) were used. In Part B, in addition to the necessary substrates and cofactors as above, either isocitrate (50 mM), isocitric dehydrogenase (50 µg) and MgCl₂ (1 mM), or glucose 6-phosphate (50 mM) and glucose 6-phosphate dehydrogenase (10 µg) were present in the reaction mixture. The incubation time was 15 min for Part A and 60 min for Part B. The radioactivity in lipid extract was determined as described in the text.

<table>
<thead>
<tr>
<th>A. Lipid substrate</th>
<th>[4-$^3$H]NADPH used</th>
<th>Radioactivity in lipid</th>
<th>cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexadecyl-DHAPa</td>
<td>B</td>
<td></td>
<td>5480</td>
</tr>
<tr>
<td>Hexadecyl-DHAP</td>
<td>A</td>
<td></td>
<td>150</td>
</tr>
<tr>
<td>Palmitoyl-DHAP</td>
<td>R</td>
<td></td>
<td>4944</td>
</tr>
<tr>
<td>Palmitoyl-DHAP a</td>
<td>A</td>
<td></td>
<td>36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Lipid substrate</th>
<th>[4-$^3$H]NADPH used</th>
<th>Regeneration system</th>
<th>Radioactivity in lipid</th>
<th>cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexadecyl-DHAP</td>
<td>A</td>
<td>Isocitrate-isocitric dehydrogenase</td>
<td>1705</td>
<td></td>
</tr>
<tr>
<td>Hexadecyl-DHAP a</td>
<td>A</td>
<td>Glucose 6-phosphate-glucone 6-phosphate dehydrogenase</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

* DHAP, dihydroxyacetone phosphate.
as assayed against glutamic dehydrogenase (71%), the incor-

dissent of the enzymes and substrates were used for these assays,

differences. Assuming the purity of [3H]NADPH was the same 

that the decreased activity was due to an isotope effect (3H versus 

optical method.

Therefore, a radioactive assay with 3H-labeled pyridine nucleo-

tides was devised. This assay gave very low blank values and 

long time (10 to 15 min) and interfered with the optical assay.

The 3H-labeled pyridine nucleotides were found to be 70% 


tergent caused an increased transmission of light, possibly due 

as crude microsomal and mitochondrial fractions were employed 

liver microsomal or mitochondrial protein), it is more than the 

argine dehydrogenase (EC 1.1.1.8) in the microsomal preparation. Dihy-

sphate in a total volume of 1.2 ml. The mixture was incubated 

at 37° for 15 min and the total lipid extract was put on thin layer 

silica gel plate to separate the alkyl [32P]glycerol S-phosphate 

[32P]DHAP (60 nmoles, 42,000 cpm), ascites cell microsomes (0.125 

mg), and varying amounts of palmitoyl dihydroxyacetone phos-

phate by acyl dihydroxyacetone phosphate. The incu-

bation mixture contained phosphate buffer (16.6 mm, pH 7.4), 

NaH2EDTA (0.33 mm), NaF (5.0 mm), NADPH (40 μm), alkyl 

[32P]DHAP (60 nmoles, 42,000 cpm), ascites cell microsomes (0.125 

mg), and varying amounts of palmitoyl dihydroxyacetone phos-

phate in a total volume of 1.2 ml. The mixture was incubated 

at 37° for 15 min and the total lipid extract was put on thin layer 

silica gel plate to separate the alkyl [32P]glycerol S-phosphate 

(ALKYL[32P](G-3-P) from alkyl dihydroxyacetone [32P]phosphate 

(DHAP) as described in the text. The radioactivity in alkyl 

[32P]glycerol 3-phosphate is reported here.

**Discussion**

The usual optical assay used most commonly with pyridine 
nucleotide dependent oxidoreductases was not suitable for our 

studies because of the low activity of the enzyme. Moreover, 

ad heterogeneous populations were employed as the source for the enzyme, the addition of substrates and 

detergent caused an increased transmission of light, possibly due to 

the lysis of membranes by the detergent and substrates (which 

are also detergents). This change in optical density lasted for a 

long time (10 to 15 min) and interfered with the optical assay. 

Therefore, a radioactive assay with [3H]-labeled pyridine nucleo-

tides was devised. This assay gave very low blank values and 

increased the assay sensitivity more than 100-fold over the usual 

optical method.

The [3H]-labeled pyridine nucleotides were found to be 70% 

enzymatically active when tested with either glutamic dehydro-

enase or glycerol 3-phosphate dehydrogenase. It is possible 

that the decreased activity was due to an isotope effect (3H versus 

H) in the enzymatic reduction; however, because an enormous 

excess of the enzymes and substrates were used for these assays, 

it is unlikely that the isotope effect would be responsible for such 

differences. Assuming the purity of [3H]NADPH was the same 

as assayed against glutamic dehydrogenase (71%), the incor-

poration of [3H] from [4-3H]NADPH into lipid from acyl or alkyl 

dihydroxyacetone phosphate, was found to be 90% of that cal-

culated from the oxidation of NADPH (decrease in absorbance 

at 340 nm) showing a negligible isotopic effect for the enzymes 

studied here.

The results show that NADPH, not NADH, serves as the coenzyme for the reduction of both acyl and alkyl dihydroxy-

acetone phosphate. Although the activity of the enzyme (or enzymes) varies from one fraction to another, the activity is at 

least ten to fifty times more with NADPH than with NADH. Some activity with NADH was found, especially at the high 

concentration of the coenzyme. However, it is well known that 

the dehydrogenases are not absolutely specific for a particular 

pyridine nucleotide and will accept many analogs of pyridine 
nucleotides as the coenzyme (37). We found that glyceroephr-

hosphate dehydrogenase which has been shown to be specific only 

for NADH (38), will reduce dihydroxyacetone phosphate to 

glycerol-3-P when NADPH is used as the coenzyme at high 

concentration (10 mM).5 The low activity of NADH in reducing 

[32P]phosphate derivatives (Fig. 5) shows that no incorporation of 3H from B-[4-3H]NADH into lipid can 

be explained either by an "isotope effect" (enzyme cannot uti-

lize 3H) or by a different stereospecificity of the enzyme with 

NADH as the cofactor, i.e. only A-[4-3H]NADH is enzymatically 

active, etc. Therefore by every available criterion the reduc-

tase studied here is fairly specific for NADPH.

This conclusion about the specificity of the reductase toward 

NADPH does not explain the results obtained by Puleo et al. 

(7-9). Because these workers used high concentration of NADH 

(7, 8), some reduction of acyl dihydroxyacetone phosphate would 

take place but the rate still would be much lower than that of 

NADPH as the coenzyme. However, these results could be 

explained by the possible presence of glyceroephsphate dehydro-


genase (EC 1.1.1.8) in the microsomal preparation. Dihy-

droxyacetone phosphate would be reduced by NADH by this 

enzyme to glycerol-3-P which would be enzymatically acylated 

to form phosphatidic acid (4). Boxer and Shonk (39) reported 

the presence of glyceroephsphate dehydrogenase in rat liver and 

kidney mitochondria which could not be removed by repeated 

washing. Puleo et al. reported the absence of this enzyme from 

the rat liver microsomal preparation (7). However, the optical 

assay employed by these workers may not be sensitive enough to 

estimate a low level of the dehydrogenase. We found dihydroxy-

acetone-[32P]phosphate is converted to glycerol-3-[32P] by NADH 

(9, 38), but not by NADPH) when incubated with mitochondrial and 

microsomal fractions from rat liver and brain, guinea pig liver, 

ascites cell, etc. (40). Like triose phosphate isomerase (2, 39, 

41), the activity of this enzyme could not be removed by repeated 

washing to the particles with 0.25 M sucrose. The bound en-

zyme activity varied from 0.2 to 0.57; of the total NADH-de-

pendent glycerophosphate dehydrogenase in the cell homogenate. 

Although this activity of the bound enzyme is low (2 to 4 nmoles 

of dihydroxyacetone phosphate reduced per min per mg of rat 

liver microsomal or mitochondrial protein), it is more than the 

acyl-CoA:glycerol-3-P acyltransferase activity present in these 

fractions. Therefore, in the presence of NADH, dihydroxy-

acetone phosphate, acyl-CoA, and other cofactors, phosphatidic 

acid would form by the well known acylation (4, 5) of glycero-

phosphate. Most of the results obtained by Reiser et al., i.e. 

low enzyme activity in mitochondria, formation of phosphatidic 

acid in the presence of NADH, etc., may possibly be due to the 

presence of bound glycerolphosphate dehydrogenase in the microsomal fraction.

Like other pyridine nucleotide-dependent oxidoreductases, the enzymes responsible for the reduction of the dihydroxyacetone phosphate derivatives can utilize hydrogen only from one side at position 4 of the nicotinamide ring of NADPH. In this case the hydrogen (or H\textsuperscript{+}) from the B side (16) of the ring was transferred. It is interesting to note that the same stereospecificity exists between these enzymes and glycerol 3-phosphate dehydrogenase with the production of the same optical isomers of the glycerol derivatives.

The enzyme (or enzymes) responsible for the reduction of the acyl or alkyl derivatives of dihydroxyacetone phosphate was found to be widely distributed in different organs (Table II). The enzyme was always found to be membrane-bound and was present in mitochondrial and microsomal fractions. Compared to the soluble dehydrogenases the activity of this enzyme is low. However, because of the insolubility of the enzymes, substrates, and products in the reaction mixture, and also due to the presence of detergents, the true activity of the reductase present in membrane is probably not measured by the present assay method. In any event, the rates of reduction of the dihydroxyacetone phosphate derivatives were much higher than the enzymatic rate of acylation or alkylation of dihydroxyacetone phosphate (12, 20), showing that the reduction of these keto derivatives are not rate limiting for the biosynthesis of glycolipids.

By several criteria it was seen that the glycerol portions of the reduced lipids have the expected \(\alpha\) or \(\beta\)-glycerol 3-phosphate configuration. The alkyl and acyl glycerophosphate could be further acylated enzymatically\(^4\) and the resulting compound could be hydrolyzed by snake venom phospholipase A to lysophosphatidate. Van Deenen and DeHaas showed that phospholipase A would only hydrolyze the ester bond at carbon 2 of glycerol having only the \(\alpha\)-configuration (42). Also the enzymatically reduced lipid from acyl dihydroxyacetone phosphate could be hydrolyzed to glycerophosphate which can act as a substrate for glycerophosphate dehydrogenase (1). This dehydrogenase is also known to act only on \(\alpha\)-glycerol 3-phosphate (43). Attempts are now being made to prepare a larger amount of these enzymatically reduced lipids for a direct determination of configuration by determining the optical activity.

As discussed previously, the results probably indicate that a single enzyme catalyzes the reduction of both acyl and alkyl derivatives of dihydroxyacetone phosphate. The similarities in the distribution and the activity of the enzyme and especially the inhibition of the reduction of alkyl dihydroxyacetone phosphate by acyl dihydroxyacetone phosphate indicate that one enzyme is involved. It is possible, however, that there are more than one enzyme having wide specificity for different substrates. No definite conclusion could be reached until the enzyme (or enzymes) is purified and the properties are studied. We recently found that 60 to 80\% of the enzyme activity present in the ascites cell microsomes remained in the 100,000 \(\times\) g supernatant after centrifugation by treatment of the membrane preparation with dilute solution of different detergents. Work is under progress in our laboratory to determine the properties and if possible, purify this “solubilized” reductase.

\(^4\) Acyl-CoA:1-acyl glycerol-3-P acyltransferase was also found to be stereo-specific in the transfer of acyl group. The \(\alpha\) isomer, i.e. \(\alpha\)-3-acyl glycerol-1-P, did not serve as a substrate for this enzyme. A. K. Hajra, unpublished experiments.

**REFERENCES**

Enzymatic Reduction of Alkyl and Acyl Derivatives of Dihydroxyacetone Phosphate by Reduced Pyridine Nucleotides
Edward F. LaBelle, Jr. and Amiya K. Hajra


Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/247/18/5825.full.html#ref-list-1