Biosynthesis of Glycerolipid Precursors in Rat Liver Peroxisomes and Their Transport and Conversion to Phosphatidate in the Endoplasmic Reticulum*

Arun K. Das, Shuichi Horie, and Amiya K. Hajra

From the Neuroscience Laboratory, Mental Health Research Institute and Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan 48104-1887

The transport of glycerolipid intermediates, viz. palmitoyl dihydroxyacetone phosphate (DHAP) and lysophosphatidate from peroxisomes and their conversion to phosphatidate in endoplasmic reticulum (microsomes) were studied in cell-free systems. The lipids were biosynthesized from [32P]DHAP, palmitoyl-CoA, and freshly made rat liver peroxisomes and microsomes in the presence or absence of Mg2+, NADPH, and bovine serum albumin (BSA). After incubation, the soluble fraction and the membranes were separated, and the distribution of radioactive lipids in these fractions were determined. The results showed that palmitoyl-DHAP and lysophosphatidate were recovered in the supernatant when BSA was present or when BSA was absent, but Mg2+ was removed after incubation by chelation with EDTA (or ATP). At low optimum palmitoyl-CoA concentration or when palmitoyl-CoA was generated in peroxisomes, and in the absence of BSA, the biosynthesized keto ether and ester lipids and lysophosphatidate were similarly present in the supernatant. Phosphatidate, however, was always localized in the membranes. Further fractionation showed that phosphatidate was associated with the microsomes. The critical micellar concentrations of palmitoyl-DHAP and 1-palmitoyl-<i>rac</i>-glycerol 3-phosphate, under the incubation conditions used, were determined to be 58 and 70 μM, respectively. These results suggest that at physiological concentrations the biosynthesized lysolipids are water soluble, and therefore, a carrier protein is unnecessary for their transport. These lipids freely diffuse from peroxisomes to endoplasmic reticulum where they are converted to membrane-bound phosphatidate.

In eukaryotes, enzymes catalyzing glycerolipid biosynthesis are present mainly in mitochondria and the endoplasmic reticulum (ER)* (Wilgram and Kennedy, 1963; Van den Bosch, 1974; Bell and Coleman, 1980; Hajra et al., 1986). The main site of cellular glycerolipid biosynthesis is believed to be the ER, from which the lipids are transported to different subcellular compartments (Kennedy, 1986; Bishop and Bell, 1988; Simoni, 1988). The enzyme composition of mitochondria suggests that only certain mitochondria-specific lipids (e.g. cardiolipin) are biosynthesized in these organelles (Kiyasu et al., 1963; Haldar et al., 1983). Glycerolipids and glycerol-ether lipids are also shown to be biosynthesized in animal peroxisomes (Hajra and Bishop, 1982). These organelles have been shown to contain the enzymes of the acyl dihydroxyacetone phosphate (acyl-DHAP) pathway, namely DHAP acyltransferase, alkyl-DHAP synthase, and acyl/alkyl-DHAP reductase (Hajra et al., 1979; Hajra and Bishop, 1982). DHAP acyltransferase, which catalyzes the biosynthesis of acyl-DHAP, has been shown by different workers, to be localized exclusively on the inner face of the peroxisomal membrane (Rock et al., 1977; Jones and Hajra, 1979; Bishop et al., 1982; Hardeman and Van den Bosch, 1988). Similarly, alkyl-DHAP synthase, which catalyzes the formation of ether bond, is localized inside the peroxisomal membrane (Bishop et al., 1982; Hardeman and Van den Bosch, 1988). However, acyl/alkyl-DHAP reductase, which catalyzes the formation of lysophosphatidate (and its ether analog) is localized on the outside (cytosolic side) of the peroxisomal membrane (Ghosh and Hajra, 1986a). This reductase is also present in the ER (Ghosh and Hajra, 1986a). A fourth lipid biosynthetic enzyme, acyl-CoA reductase (long chain alcohol-forming), has recently been localized to be present on the outside of peroxisomes (Burdett et al., 1991). Most other known glycerolipid-biosynthesizing enzymes are absent in peroxisomes (Hajra and Bishop, 1982; Ballas et al., 1984). From the composition and topography of peroxisomal enzymes, it seems that acyl-DHAP and alkyl-DHAP synthesized inside the peroxisomes are exported out and then reduced to 1-acyl and 1-alkyl-glycerol-3-P, respectively, by acyl/alkyl-DHAP reductase (and cytosolic NADPH) (Hajra et al., 1988; Hardeman and Van den Bosch, 1989). The lysophosphatidate, i.e. LPA, thus formed is transported to the ER where it is converted to phosphatidate, the precursor of all glycerolipids (Kennedy, 1986). Therefore, it is evident that only glycerolipid biosynthetic intermediates are synthesized in peroxisomes and are transported to the ER to form membrane glycerolipids and triglycerides.

The mechanism of intracellular transport of water-insoluble lipids is poorly understood. It is believed that such transport is mediated either by carrier proteins or by vesicles (Zilversmit, 1984; Simoni, 1988). However, the lipids synthesized in peroxisomes contain a single acyl (or alkyl) chain and are probably water soluble (high critical micellar concentrations. CMC) at physiological concentrations (<0.1 mM, see
Das and Hajra, 1984, 1989). Therefore, it is assumed that these lipids could freely diffuse from peroxisomes to ER without any special transport mechanism (Hajra and Bishop, 1982). We have previously provided preliminary evidence for such a mechanism by showing that in a cell-free system the acyl-DHAP and LPA biosynthesized in peroxisomes are present in the soluble fraction, whereas phosphatidate synthesized in the ER is present in membrane-bound fractions (Horie and Hajra, 1987; Hajra et al., 1988). However, the results reported by Hardeman and van den Bosch (1989) indicate that bovine serum albumin (BSA) present in the reaction mixture acts as a carrier protein which extracts acyl-DHAP or alkyl-DHAP from peroxisomes to the supernatant. In the absence of BSA, all of acyl/alkyl-DHAP remained associated with the peroxisomal membrane (Hardeman and Van den Bosch, 1989). A similar study by Haldar and Lipfert (1990) on the mitochondrial biosynthesis of LPA also indicates that BSA is necessary for the transport of LPA from mitochondria to the ER. Therefore, it seems that a putative carrier protein might be involved in the transport of lyso lipids from one subcellular compartment to another. In this paper we reexamine this problem regarding the formation of acyl-DHAP and LPA in peroxisomes and their in vitro transport to the ER where they are converted to phosphatidate. The roles of BSA and other cofactors, especially Mg^2+, in this transport process are also investigated.

EXPERIMENTAL PROCEDURES

Materials

[^32P]DHAP was prepared by the enzymatic phosphorylation of dihydroxyacetone with [γ-[^32P]ATP as described previously (Hajra and Burke, 1978). 1-Octyl-sn-glycerol-3-phosphate, phosphatidic acid (PA), ATP, NADPH, palmityl-CoA and cytochrome c were obtained from Sigma. Palmitoyl-DHAP was chemically synthesized as described previously (Hajra et al., 1983). 1-O-Palmitoyl-rac-glycerol 3-phosphate (LPA) was synthesized by reduction of 1-O-palmitoyl-DHAP with NaBH₄ as described before (Das and Hajra, 1984). Nycodenz (Nyegaard Co.) was purchased from Accurate Chemical & Scientific Research Corporation (Westbury, NY). Thin layer chromatographic (TLC) plates (E. Merck, Darmstadt) were from VWR Scientific (Chicago, IL). Adult Sprague-Dawley male rats (200–250 g) were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) and fed standard Purina Chow and water ad libitum. Other materials were used as described previously (Hajra et al., 1983; Das and Hajra, 1984).

Methods

Isolation of Peroxisomes and Microsomes from Rat Liver—Subcellular fractionation of rat liver by differential centrifugation was done with minor modifications, as described previously (deDuve et al., 1955; Hajra et al., 1979). Briefly, the liver from adult rats was homogenized in ice-cold buffer containing 0.25 M sucrose, 10 mM TES, pH 7.5, 1 mM EDTA, 0.1% ethanol, 0.4 mM phenylmethylsulfonyl fluoride, and 0.2 mM leupeptin. The whole homogenate was subjected to differential centrifugation. The nuclear and mitochondrial pellets obtained at 600 × g for 10 min and 3,300 × g for 10 min, respectively, were discarded. The light mitochondrial fraction (L) sedimented at 25,000 × g for 10 min was re Suspended in the above homogenizing medium in a volume equivalent to one-fourth of the original liver weight, microsomes from the post-nuclear supernatant were sedimented at 100,000 × g for 60 min and suspended in the homogenizing medium in a volume corresponding to 1 ml/g of liver. These preparations of peroxisomes and microsomes were used fresh for the study of biosynthesis and transport of lipids. The remaining portions were stored at -20 °C for assaying marker enzymes (described below). The isolated peroxisomes and microsomes, when assayed for NADPH-cytochrome c reductase (microsomal marker enzyme, Williams and Kamin, 1962), were found to have activities of 4–9 nmol/min/mg protein for the peroxisomal fraction and 150 nmol/min/mg protein for the microsomal fraction. Calculations based on these marker enzyme activities indicate that the peroxisomes were contaminated by 3–5% of the total microsomal protein. The mitochondrial contamination was detected by its marker succinate-cytochrome c reductase, Schimdtman and Greenwald, 1968) in this isolated peroxisomes was about 2–5% (see also Ghosh and Hajra, 1986a). The purity of peroxisomes was calculated to be 90–95%. The specific activity of DHAP acyltransferase (DHAPAT) in peroxisomes was found to be 120.1 nmol/ min/mg protein under optimum conditions of assay (Jones and Hajra, 1979). The enrichment of DHAPAT from post nuclear supernatant to L-fraction was about 6- and from the L-fraction to peroxisomes was 7-fold. Between these two latter fractions, the specific activity of catalase, the marker enzyme for peroxisomes, was also found to be increased to 7-8-fold (260 ± 2100 ± 390 units/mg protein in L and peroxisomes, respectively). Specific activity of DHAPAT in isolated microsomes was 0.45 nmol/min/mg protein.

Biosynthesis of Lipids—Enzymatic syntheses of acyl-DHAP, LPA, and PA were studied using fresh liver peroxisomes and microsomes following a method described previously (Jones and Hajra, 1979). The incubation mixture in 2.4 ml contained Tris-HCl (75 mM, pH 7.5), MgCl₂ (3.3 mM), NaF (8.3 mM), palmityl-CoA (0.05 mg or 83 μM), liver peroxisomes (200 μg of protein), and [32P]DHAP (0.42 mM, 7000 cpm/nmol). Depending upon the experimental conditions (see under tables and figures), NADPH (4 μM), BSA (4 μg), and liver microsomes (200 μg of protein) were also present in the incubation mixtures. The reaction mixtures were incubated for 30 min at 37 °C. Lipids from the reaction mixtures were extracted by the method of Bligh and Dyer (1959) under acid conditions (Hajra, 1974). An aliquot of the CHCl₃ solution containing the products was used to determine the radioactivity and the radioactivity in the remaining was used to characterize the products by silica gel TLC using a solvent mixture of CHCl₃/methanol/acetic acid/5% Na-metabisulfite (100:40:12:4) (Hajra, 1968). The radioactive spots were localized by autoradiography and characterized with respect to the R₁ values of the known standards on the plates. In most experiments, three distinct and well separated [32P]-labeled lipids were found to be present on the radioautograms having R₁ values of 0.20, 0.35, and 0.72 corresponding to those of the standard samples of palmityl-DHAP, LPA, and PA, respectively. The radioactive spots were scraped off into liquid scintillation mixtures, mixed with 0.5 ml of H₂O and sonication, and the lipids were determined in a liquid scintillation counter by adding 2 ml of scintillation fluid (Universol, ICN Biomedicals, Inc., Irvine, CA). Separation of Peroxisomes and Microsomes after Incubation—A reaction mixture containing peroxisomes, microsomes, and other isolated subcellular organelles was immediately cooled on ice for 5 min after incubation, then added with an equal volume of cold sucrose-EDTA (0.25–0.01 M) solution whenever needed, and then subjected to centrifugation (+4 °C) at 8,000 rpm (7,720 × g) for 10 min in a Sorval RC2-B centrifuge using a SS-34 rotor to sediment peroxisomes. The supernatant was centrifuged at 40,000 rpm (104,000 × g) for 90 min using a Ti-50.2 Beckman rotor to sediment microsomes. Radioactive lipids from peroxisomal pellet, microsomal pellet, and from an aliquot of the final supernatant were isolated as described above. Radioactivity in total phospholipids and that of individual class, i.e. palmityl-DHAP, LPA, and PA present as membrane bound and in the supernatant (after centrifugation), were then determined by separating the lipid products on silica gel TLC plate followed by radioautography and then determining the [32P] counts as above. Other Methods—Protein was determined using BSA as standard by Lowry assay procedure (Lowry et al., 1951) after it was coprecipitated with deoxycholic acid (Bensadoun and Weinstein, 1978) to remove interfering Nycodenz from the sample. The amount of 1-[14C] hexadecyl DHAP formed in peroxisomes from long chain 1-[14C] hexadecanol was measured radio metrically employing a solvent partition method at high pH as described by Davis and Hajra (1981). The DHAP-DHAP product was isolated using an extraction solvent (CHCl₃/methanol/acetic acid/water, 100:40:12:4) which showed the presence of only one 14C-labeled spot (R₁ = 0.34) corresponding to the standard hexadecyl DHAP (Hajra et al., 1983). NADPH-cytochrome
RESULTS

Effect of BSA on the Biosynthesis and Localization of Lipids—Studies were carried out to investigate the role of BSA in the biosynthesis of glycerolipid intermediates. Table 1 shows the formation of acyl-DHAP, LPA, and PA in peroxisomes or microsomes or in combinations of them under different experimental conditions, such as in the presence or absence of NADPH and of BSA. As shown, palmitoyl-DHAP is formed mainly in peroxisomes. Most of this keto lipid was reduced (95–97%) to palmitoyl glycerol-3-P (i.e. LPA) in this organelle when NADPH was present in the incubation mixture. When the incubation mixture contained microsomes in addition to peroxisomes and NADPH, PA was the major product. Since the peroxisomes and microsomes used were not completely free from each other (see “Experimental Procedures”), a small fraction of the total lysop-PA formed in the presence of NADPH was converted to PA when peroxisomal fraction was used (Table I). Similarly, a small amount of palmitoyl-DHAP was formed when microsomes were used and was ultimately transformed into PA in the presence of NADPH (Table I). The effect of BSA on peroxisomal and microsomal lipid biosynthesis revealed that although BSA stimulated the formation of the lipids, it was not required if Mg²⁺ was present in the incubation mixture. However, BSA stimulated overall lipid production by 2.5–3 times (Table I). In a separate experiment, the effect of Mg²⁺ on peroxisomal DHAPAT in the absence of BSA was investigated. The specific activities of the enzyme were 3.4 nmol/min/mg protein and 0.26 nmol/min/mg protein in the presence or absence of Mg²⁺, respectively.

The effects of BSA on the localization (membrane-bound or soluble) of acyl-DHAP, LPA, and PA formed are shown in Fig. 1. Peroxisomes and microsomes were sedimented together by centrifuging the mixture at 100,000 × g for 90 min at 4 °C after the end of incubation and the type and amount of lipids in the membrane pellet and the supernatant were determined. As shown in Fig. 1A, palmitoyl-DHAP and LPA produced in peroxisomes in the presence of BSA were mostly recovered in the supernatant. In contrast, when BSA was absent but Mg²⁺ was present, these lipids remained mainly in the membrane fraction (Fig. 1B). However, since the incubation mixture contained Mg²⁺, therefore, it is probable that these lysolipids which should be water soluble at these low concentrations (high CMC, see “Appendix”) probably formed insoluble salts with divalent Mg²⁺ and remained with the membrane fraction in the absence of BSA. To verify this, Mg²⁺ in the mixture (no BSA) was chelated by adding EDTA at the end of incubation, and the distribution of radioactive lipids were determined. The results showed that under such conditions, both acyl-DHAP and LPA were present in the supernatant even though BSA was absent in the incubation mixture (Fig. 1C). The results also showed that when PA was formed in the presence of microsomes (and NADPH), it remained bound to the membrane fraction regardless of whether BSA or EDTA was present or absent in the system (Fig. 1, A–C).

These results show that when Mg²⁺ in the incubation mixture was removed by EDTA at the end of incubations, acyl-DHAP and LPA became soluble and did not sediment with the membrane fraction. Intracellular Mg²⁺ is present mostly in the chelated form with cellular ATP (Lehniger, 1982). When ATP was added to the incubation mixture (no BSA), most (75%) of acyl-DHAP and LPA were present in the supernatant (data not shown, but see Table II) similar to the case when EDTA was added.

Localization of Membrane-bound PA—The results in Fig. 1, A–C, showed that the PA once formed was recovered with the membrane pellet which consisted of both peroxisomes and microsomes. To determine whether or not PA synthesized in microsomes remained exclusively bound with this organelle, peroxisomes and microsomes were separated from each other after the end of incubation. When such an experiment was performed following conventional differential centrifugation, i.e. 25,000 × g for 10 min to sediment peroxisomes and 100,000 × g for 60 min to sediment microsomes, it was observed that a large (50–60%) fraction of microsomes (as determined by the marker enzyme NADPH-cytochrome c reductase) sedimented with peroxisomes. Since the incubation mixture for PA biosynthesis contained Mg²⁺, the microsomes probably aggregated in the presence of this divalent cation (Schenkman and Cinti, 1978) and sedimented at lower centrifugal force. Optimum conditions were developed to separate microsomes (ER vesicles) from peroxisomes after binding the Mg²⁺ with EDTA at the end of the reaction. The best separation was

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**Table 1**

Biosynthesis of palmitoyl-DHAP, LPA, and PA in peroxisomes and microsomes under various experimental conditions

| Reaction conditions | −BSA | +BSA |
|---------------------|-----|-----|
|                     | Palmitoyl-DHAP | LPA | PA | Palmitoyl-DHAP | LPA | PA |
| −BSA                | nmol |     |    | nmol |     |    |
| PX                  | 3.2 ± 0.68 | 0.0 | 0.0 | 9.9 ± 0.62 | 0.0 | 0.0 |
| PX + NADPH          | 0.4 ± 0.13 | 3.0 ± 0.52 | 0.9 ± 0.22 | 0.6 ± 0.15 | 9.8 ± 0.88 | 1.6 ± 0.14 |
| PX + MS             | 3.2 ± 0.56 | 0.0 | 0.0 | 10.1 ± 0.51 | 0.2 ± 0.10 | 0.0 |
| PX + MS + NADPH     | 0.3 ± 0.20 | 0.4 ± 0.15 | 3.9 ± 0.70 | 0.4 ± 0.08 | 2.3 ± 0.11 | 9.6 ± 0.64 |
| MS                  | 0.35 | 0.0 | 0.0 | 0.73 | 0.0 | 0.0 |
| MS + NADPH          | 0.02 | 0.02 | 0.31 | 0.09 | 0.04 | 0.47 |

*Average ± S.D. (n = 4).

*Average of two experiments (range ± 6% of the average).


Table II

Distributions of acyl-DHAP and alkyl-DHAP biosynthesized in peroxisomes using the acyl-CoA-generating system

The incubation mixture for the biosynthesis of acyl-DHAP contained Tris-HCl (75 mM, pH 7.5), NaF (8.3 mM), MgCl₂ (8.3 mM), ATP (8.4 mM), reduced glutathione (4.2 mM), CoA (50 μM), ammonium palmitate (20 μM), liver peroxisomes (200 μg of protein), and [3H]DHAP (0.42 mM, 7000 cpm/nmol) in a total volume of 1.2 ml. For biosynthesis of alkyl-DHAP, the same mixture as above was used except that the pH of the buffer was 8.0, DHAP was non-radioactive, and [3H]hexadecanol (33.3 μM, 60,000 cpm/nmol) was included. The incubations (37 °C for 30 min) were carried out either in the absence or presence of BSA (2 mg). At the end of incubation the mixtures were cooled in ice and centrifuged at 25,000 g for 10 min to sediment the peroxisomes. The amount of acyl-[3H]DHAP present in the pellet and supernatant was determined by acid precipitation and chemical analysis. The lipids (acyl-DHAP and alkyl-DHAP) biosynthesized in peroxisomes using acyl-CoA as the precursor were determined by solvent partition as described in the text. The percentages of radioactive lipid product present in each fraction are shown. The average total amount of acyl-DHAP and alkyl-DHAP formed was 0.97 and 0.18 nmol, respectively.

| Lipids       | Pellet | Supernatant |
|--------------|--------|-------------|
|              | -BSA   | +BSA        | -BSA   | +BSA        |
| Acyl-DHAP    |        |             |        |             |
| Expt. 1      | 9.1    | 10.0        | 90.8   | 90.0        |
| Expt. 2      | 11.7   | 12.4        | 88.2   | 87.6        |
| Alkyl-DHAP   |        |             |        |             |
| Expt. 1      | 11.0   | 11.5        | 88.9   | 88.4        |
| Expt. 2      | 9.3    | 10.6        | 90.7   | 89.4        |

FIG. 2. Subcellular localization of biosynthetic PA. The reaction mixture contained Tris-HCl (75 mM, pH 7.5), NaF (8.3 mM), MgCl₂ (8.3 mM), palmitoyl-CoA (80 μM), NADPH (200 μM), BSA (4 mg), [3H]DHAP (0.42 mM, 7000 cpm/nmol), liver peroxisomes (200 μg of protein), and microsomes (200 μg of protein) in a total volume of 2.4 ml. The mixtures were incubated at 37 °C for 30 min, cooled on ice, and equal volumes of ice-cold sucrose-EDTA (0.25-0.01 M) were added to each tube. After mixing, the mixtures were centrifuged at 7,720 × g for 10 min to sediment peroxisomal fractions (solid bar, ■), and the resulting supernatants were centrifuged at 100,000 × g for 90 min to separate microsomes (hatched bar, □) from the soluble fraction (dotted bar, □). The NADPH-cytochrome c reductase activity and the amount of each radioactive lipid present were determined in the aliquots of each fraction. The results presented above were the values corrected for the microsomal contamination of the peroxisomal fractions (see text for details). The values are the average of two experiments (range ± 6% of the average values).
Palmitoyl-DHAP and LPA were present in the supernatant. This low palmitoyl-CoA concentration was employed to study the biosynthesis and transport of the lipids in the absence of BSA and Mg$^{2+}$.

Fig. 4 shows the results of the biosynthesis and transport of palmitoyl-DHAP, LPA, and localization of PA synthesized in a mixture of peroxisomes and microsomes at low (7 µM) palmitoyl-CoA concentration and in the absence of BSA and Mg$^{2+}$. After incubation, the peroxisomes and microsomes in the reaction mixture were separated from each other by differential centrifugation without the addition of EDTA, and the distribution of the radioactive lipids in these fractions and in the supernatant was determined. Under these conditions palmitoyl-DHAP and LPA were present in the supernatant fraction, whereas PA remained associated with the ER vesicles (Fig. 4).

**Distribution of Acyl-DHAP and Alkyl-DHAP Biosynthesized in Peroxisomes Using an Acyl-CoA-generating System**—Acyl-CoA ligase is present in the peroxisomes (Mannaerts et al., 1982) and theacyl-CoAs generated in peroxisomes are probably physiologically utilized to synthesize acyl-DHAP. Alkyl-DHAP is also biosynthesized in peroxisomes (Hajra and Bishop, 1982), and this ether lipid should be transported out of peroxisomes to ER to form membrane ether lipids. To study the formation and export of these lipids from peroxisomes under putative physiological conditions, a fatty acyl-CoA-generating system instead of acyl-CoA was used in the presence of DHAP and hexadecanol. The results are shown in Table II. As seen in this table most (>85%) of the biosynthesized acyl-DHAP and alkyl-DHAP are localized in the soluble fraction and are not associated with peroxisomes. A carrier protein, such as BSA, is not necessary for such export of these keto lipids from peroxisomes where they are biosynthesized (Table II). The results also show that Mg$^{2+}$ does not form insoluble salts with these keto lipids if it remains chelated with excess ATP in the incubation mixtures (Table II).

**DISCUSSION**

Results from this cell-free system show that acyl-DHAP synthesized inside the peroxisomes is exported across the membrane and is enzymatically reduced to acyl glycerol-3-P which is transported to the ER where it is acylated to form PA. Apparently, acyl-DHAP and LPA at low concentrations in the incubation medium (1-16 µM, Table I) are present as water-soluble monomers which could freely diffuse in and out of peroxisomes to the soluble fraction. We have determined the CMCs of palmitoyl-DHAP and LPA in the absence of BSA and MgCl$_2$. The reaction mixture in 2.4 ml contained Tris-HCl (75 mm, pH 7.5), NaF (8.3 mm), [32P]DHAP (0.42 mm, 7000 cpm/nmol), palmitoyl-CoA (7 µM), and either (i) peroxisomes (68 µg of protein); or (ii) a mixture of peroxisomes (68 µg of protein), microsomes (68 µg of protein) and NADPH (200 µM). Incubations (37°C for 30 min), separation of peroxisomes (solid bar), microsomes (hatched bar), and the amount of radioactivity present in palmitoyl-DHAP (left), LPA (middle), and PA (right) in all the fractions were determined as described in the legend for Fig. 2 and text. Data represent the average of two experiments (range ± 4-7%).

**Fig. 4. Biosynthesis and subcellular localization of palmitoyl-DHAP (palmit DHAP), LPA, and PA in absence of BSA and MgCl$_2$.**

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indicated that at pH 7.0 and low ionic strength the CMC of palmitoyl-CoA is about 40-50 μM (Smith and Powell, 1986; Constantines and Stein, 1985), whereas it decreases substantially (8-10 μM) at high ionic strength. Under the conditions of the incubations we used (75 mM Tris-HCl, pH 7.5, 8.3 mM NaF), the CMC of palmitoyl-CoA was determined to be 41 μM (see “Appendix”). Therefore, the finding that optimum DHAPAT activity is at about 10 μM of palmitoyl-CoA in the absence of BSA and Mg²⁺ (Fig. 3) indicates that palmitoyl-CoA monomers, not the micelles, inhibit DHAP acyltransferase. However, as pointed out by Tanford (1980), micelles start to form long before the CMC is reached, and therefore, it is still possible that this acyl transferase is inhibited by palmitoyl-CoA micelles which are present in the incubation mixture at low concentrations of this amphiatic substrate. Mg²⁺ also changes the CMC of acyl-CoAs by forming salt (Constantines and Stein, 1986), and the observed stimulation of DHAPAT activity by Mg²⁺ in the absence of BSA (Table I and Fig. 1) may also be due to the suppression of palmitoyl-CoA micelle formation in the incubation mixture.

Hardeman and van den Bosch (1989) have also reported that under the conditions described in Fig. 4 is not stimulated by the addition of rat liver cytosol. They presented preliminary evidence that a soluble 14-kDa protein is the stimulatory factor. We found, however, that peroxisomal biosynthesis of acyl-DHAP under the conditions described in Fig. 4 is not stimulated by the addition of rat liver cytosol.² It is, of course, possible that a specific liver cytosolic protein, such as fatty acid-binding protein (Glatz and van der Vusse, 1980), may bind acyl-DHAP and LPA thus facilitating their transport between intercellular compartments. However, results presented here demonstrate that such a carrier protein is not essential for the transport of acyl-DHAP and LPA from peroxisomes to microsomes. Almost complete conversion of these peroxisomal lipids to microsomal PA occurs under conditions where these lipids are present as soluble form in a large volume of incubation mixture (no BSA, no Mg²⁺, Fig. 4) or bound to a soluble protein, i.e. BSA (Fig. 1A) or even when present as insoluble salt with Mg²⁺ in the absence of BSA (Fig. 1B). Morphological studies showed that peroxisomes and endoplasmic reticulum are closely associated in cells (Novikoff and Novikoff, 1982). Therefore, it seems plausible to assume that in vivo, with peroxisomes and ER being concentrated in a much smaller cellular volume, such transport takes place without the involvement of a specific carrier protein.

REFERENCES

Ballas, L. M., Lazarow, P. B., and Bell, R. M. (1984) Biochim. Biophys. Acta 796, 287-300
Bell, R. M., and Coleman, R. A. (1980) Annu. Rev. Biochem. 49, 459-487
Bensadoun, A., and Weinstein, D. (1976) Anal. Biochem. 70, 241-250
Bishop, J. E., Salem, M., and Hajra, A. K. (1982) Ann. N. Y. Acad. Sci. 386, 411-413
Bishop, W. R., and Bell, R. M. (1988) Annu. Rev. Cell Biol. 4, 579-610
Bligh, E. G., and Dyer, W. J. (1959) Canad. J. Biochem. 37, 911-917
Brandes, R., Olley, J., and Shapiro, B. (1963) Biochem. J. 86, 244-247
Brydels, K., Larkins, L., Das, A. K., and Hajra, A. K. (1991) J. Biol. Chem. 266, 12201-12206
Constantines, P. F., and Stein, J. M. (1985) J. Biol. Chem. 260, 7573-7580
Das, A. K., and Hajra, A. K. (1983) Biochim. Biophys. Acta 796, 178-189
Das, A. K., and Hajra, A. K. (1989) Lippida 26, 329-333
Davis, P. A., and Hajra, A. K. (1981) Arch. Biochem. Biophys. 211, 20-29
Declercq, P. E., Hangman, H. P., Van Veldhoven, P. P., Debeer, L.

² A. K. Das and A. K. Hajra, unpublished observations.
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J., Van Golde, L. M. G., and Mannaerts, G. P. (1984) J. Biol. Chem. 259, 9064–9075
De Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R., and Appelmans, F. (1955) Biochem. J. 60, 604–617
Ghosh, M. K., and Hajra, A. K. (1986a) Arch. Biochem. Biophys. 245, 523–530
Ghosh, M. K., and Hajra, A. K. (1986b) Anal. Biochem. 159, 169–174
Glatz, J. F. C., and Van der Vusse, G. J. (1989) Mol. Cell. Biochem. 88, 37–44
Hajra, A. K. (1986) Biochem. Biophys. Res. Commun. 33, 929–935
Hajra, A. K. (1974) Lipids 9, 502–505
Hajra, A. K. and Bishop, J. E. (1990) Anal. Biochem. 193, 265–275
Mannaerts, G. P., van Veldhoven, P., van Broekhoven, A., Vandelbroek, G., and Debeer, L. J. (1982) Biochem. J. 204, 17–23
Novikoff, A. B., and Novikoff, P. M. (1982) Ann. N. Y. Acad. Sci. 386, 138–152
Peters, T. J., Muller, M., and de Duve, C. (1972) J. Exp. Med. 136, 1117–1139
Powell, G. L., Grothusen, J. R., Zimmerman, J. K., Evans, C. A., and Fish, W. W. (1981) J. Biol. Chem. 256, 12740–12747
Rabert, U., Volki, A., and Debach, H. (1966) Happe Seyler's Z Physiol. Chem. 367, 215–222
Rock, C. O., Fitzgerald, V., and Snyder, F. (1977) J. Biol. Chem. 252, 6363–6366
Schenkman, J. B., and Cinti, D. L. (1978) Methods Enzymol. 52, 83–89
Schwartzman, C., and Greenawalt, J. W. (1968) J. Cell Biol. 38, 158–175
Simoni, R. D. (1988) Prog. Clin. Biol. Res. 282, 29–41
Smith, R. H., and Powell, G. J. (1986) Arch. Biochem. Biophys. 244, 281–289
Tanford, C. (1980) in The Hydrophobic Effects, 2nd ed., pp. 63–70, Wiley Interscience, New York
Tippett, P. S., and Neet, K. E. (1982) J. Biol. Chem. 257, 12839–12844
Vancura, A., Carroll, M. A., and Haldar, D. (1991) Biochem. Biophys. Res. Commun. 175, 338–343
Van den Bosch, H. (1974) Annu. Rev. Biochem. 43, 243–277
Wilgram, G. F., and Kennedy, E. P. (1963) J. Biol. Chem. 238, 2615–2619
Williams, C. H., and Kamin, H. (1962) J. Biol. Chem. 237, 587–592
Woldegiorgis, G., Spennetta, T., Corkey, B. E., Wilkinson, J. R., and Shrago, E. (1985) Anal. Biochem. 150, 8–12
Zahler, W. L., and Cleland, W. W. (1969) Biochem. Biophys. Acta 176, 699–703
Zahler, W. L., Barden, R. E., and Cleland, W. W. (1968) Biochim. Biophys. Acta 164, 1–11
Zilversmit, D. B. (1964) J. Lipid Res. 25, 1563–1569