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Glycerolipid Biosynthesis in *Saccharomyces cerevisiae*: *sn*-Glycerol-3-Phosphate and Dihydroxyacetone Phosphate Acyltransferase Activities

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Yeast acyl-coenzyme A:dihydroxyacetone-phosphate *O*-acyltransferase (DHAP acyltransferase; EC 2.3.1.42) was investigated to (i) determine whether its activity and that of acyl-coenzyme A:*sn*-glycerol-3-phosphate *O*-acyltransferase (glycerol-P acyltransferase; EC 2.3.1.15) represent dual catalytic functions of a single membranous enzyme, (ii) estimate the relative contributions of the glycerol-P and DHAP pathways for yeast glycerolipid synthesis, and (iii) evaluate the suitability of yeast for future genetic investigations of the eucaryotic glycerol-P and DHAP acyltransferase activities. The membranous DHAP acyltransferase activity showed an apparent K_m of 0.79 mM for DHAP, with a V_{max} of 5.3 nmol/min per mg, whereas the glycerol-P acyltransferase activity showed an apparent K_m of 0.05 mM for glycerol-P, with a V_{max} of 3.4 nmol/min per mg. Glycerol-P was a competitive inhibitor (K_i, 0.07 mM) of the DHAP acyltransferase activity, and DHAP was a competitive inhibitor (K_i, 0.91 mM) of the glycerol-P acyltransferase activity. The two acyltransferase activities exhibited marked similarities in their pH dependence, acyl-coenzyme A chain length preference and substrate concentration dependencies, thermolability, and patterns of inactivation by *N*-ethylmaleimide, trypsin, and detergents. Thus, the data strongly suggest that yeast glycerol-P and DHAP acyltransferase activities represent dual catalytic functions of a single membrane-bound enzyme. Furthermore, since no acyl-DHAP oxidoreductase activity could be detected in yeast membranes, the DHAP pathway for glycerolipid synthesis may not operate in yeast.

Glycerolipid biosynthesis in yeast (20, 33), as in mammals (22, 23, 36, 53), begins with the stepwise acylation of *sn*-glycerol 3-phosphate (glycerol-P) using long-chain fatty acyl-coenzyme A's (acyl-CoA's). Recent studies on the biosynthesis (12, 26, 49) and reduction (13, 16, 27, 49) of acyl-dihydroxyacetone phosphate (acyl-DHAP) in liver particulate fractions have led to the proposal of a separate, independent route (the DHAP pathway) that allows DHAP to serve as a precursor for the backbone of glycerolipid. Despite extensive work (1, 18, 35, 37, 38-40, 43), the relative contributions of the glycerol-P and DHAP pathways to phospholipid and triacylglycerol synthesis remain to be established in eucaryotic systems (53, 55).

The hepatic DHAP acyltransferase (12, 26, 49), acyl-DHAP oxidoreductase (25, 26, 49), and glycerol-P acyltransferase activities (46, 55) have been localized in both the mitochondrial and microsomal subcellular compartments. Our investigations have emphasized the microsomal activities because only microsomes contain the diacylglycerol acyltransferase, diacylglycerol choline-phosphotransferase, and diacylglycerol ethanolaminephosphotransferase activities necessary for triacylglycerol, phosphatidylcholine, and phosphatidylethanolamine syntheses (46). Since these are the most abundant cellular glycerolipids, the endoplasmic reticulum is believed to be the principal site of glycerolipid synthesis (46).

In previous studies of various mammalian tissues (45, 46), we presented evidence challenging the implicit assumption that the microsomal acylation of glycerol-P and DHAP occurs by separate enzymes. This work strongly suggested that a single microsomal enzyme catalyzes the acylation of both glycerol-P and DHAP. Calculations based on the determined kinetic parameters (45, 46) and previously reported glycerol-P and DHAP pools for fat cells (2) and liver (11) suggested that the glycerol-P pathway should be predominant in these tissues.

Conclusive evidence that a single enzyme catalyzes two reactions could be obtained either by purifying the enzyme to homogeneity or by genetic analysis of the structural gene in question.

The microsomal glycerol-P acyltransferase has thus far resisted all attempts to extensively purify it (36, 52, 58, 59). Moreover, the only mutants of the glycerol-P acyltransferase activity are in the procaryote *Escherichia coli* (3, 47). *E. coli* does not contain significant DHAP acyltransferase activity (54). In this paper, we report a biochemical investigation designed to evaluate whether *Saccharomyces cerevisiae* might be suitable for subsequent genetic investigation of eucaryotic glycerol-P and DHAP acyltransferase activities. This work extends our previous conclusion that a single enzyme catalyzes the acylation of both glycerol-P and DHAP in several mammalian tissues to a lower eucaryotic cell type.

MATERIALS AND METHODS

Materials. [2-^{3}H]glycerol (200 mCi/mmol) was a product of New England Nuclear Corp., Boston, Mass. Proteose peptone and yeast extract were from Difco Laboratories, Detroit, Mich. GasPak 150 anaerobic culture systems were obtained from the BBL division of Becton, Dickinson, and Co., Cockeysville, Md. All other chemicals and enzymes were the highest grade commercially available and have been described previously (45, 46).

Synthesis of radioactively labeled substrates. [2-^{3}H]glycerol-P was synthesized by the glycerol kinase-dependent phosphorylation of [2-^{3}H]glycerol according to the procedure of Chang and Kennedy (7), except that a step gradient from 0 to 2 N formic acid was used to elute the product from the ion-exchange column. Chromatography on Whatman no. 1 paper in methyl cellosolve–methyl ethyl ketone–3 N NH_4OH (7:2:3, vol/vol/vol) confirmed that the product contained no unreacted [^{3}H]glycerol or other radioactive impurity. [^{32}P]DHAP was prepared by the glycerol kinase-dependent phosphorylation of dihydroxyacetone with [γ-^{32}P]ATP as described previously (44), except that purification of the [^{32}P]ATP from the Glynn-Chappell (10) reaction mixture was omitted with some preparations. Chromatography on Whatman no. 1 paper in *t*-butyl alcohol–water–picric acid (40 ml:10 ml:2 g) (15) confirmed that the purified, lyophilized [^{32}P]DHAP product contained less than 5% $^{32}P_i$ and no other radioactive impurity. The quantities of [2-^{3}H]glycerol-P and [^{32}P]DHAP synthesized were estimated spectrophotometrically by using glycerol-P dehydrogenase (41).

Palmitoyl-[^{32}P]DHAP was synthesized enzymatically from [^{32}P]DHAP and palmitoyl-CoA by using microsomes from isolated fat cells under conditions described previously (45), scaled up 10- to 20-fold. The combined chloroform phase, containing lipid extracted from reaction mixtures, was evaporated to 1 ml and then mixed with 2 ml of methanol and 0.8 ml of 1 M KCl containing 0.2 M tris(hydroxymethyl)aminomethane (Tris) base (48). The single phase of this basic Bligh and Dyer extraction (5) was immediately broken by the addition of 1 ml of chloroform and 1 ml of KCl-Tris solution, followed by brief centrifugation. Under these conditions, the monoacyl radioactive product partitioned mainly into the upper phase, while the residual diacyl microsomal phospholipids remained in the lower phase (48). The upper aqueous methanolic phase was brought to pH 1 with about 50 μl of concentrated HCl and extracted with 3 ml of chloroform to recover the purified radioactive product, which migrated similar to an authentic sample of acyl-DHAP on silica gel HR in chloroform–methanol–acetic acid–5% sodium metabisulfite in water (100:40:12:4, by volume) (26). The final chloroform phase was evaporated to dryness, and the palmitoyl-[^{32}P]DHAP (20 nmol) was dispersed at 4°C in 16 mM potassium phosphate buffer (pH 7.4, 1 ml) by five 3-s bursts from a Branson Sonifier, with 30-s cooling periods between bursts.

Enzyme assays. Glycerol-P and DHAP acyltransferase activites were assessed by monitoring the incorporation of [2-^{3}H]glycerol-P and [^{32}P]DHAP, respectively, into chloroform-extractable material under conditions similar to those described previously (45, 46). Assays were performed in screw-capped culture tubes (13 by 100 mm) at 23°C in a final volume of 100 μl. The standard incubation mixture contained 75 mM Tris-hydrochloride (pH 7.4), 4 mM $MgCl_2$, 2 mg of bovine serum albumin per ml, 8 mM NaF, 1 mM dithiothreitol, 80 μM palmitoyl-CoA, and either 0.3 mM [2-^{3}H]glycerol-P (50 mCi/mmol) or 1.0 mM [^{32}P]DHAP (10 to 100 mCi/mmol). Reactions were initiated by adding 0 to 25 μg of membrane protein and were terminated after 10 min by the addition of 3 ml of chloroform-methanol (1:2, vol/vol) and 0.7 ml of 1% perchloric acid. The quantity of [^{3}H]glycerol-P or [^{32}P]DHAP converted to lipid was determined as described previously (45, 46), except that no carrier lipids were added to the extraction and the chloroform phase was only washed twice with 2-ml portions of 1% perchloric acid. Both the glycerol-P and DHAP acyltransferase activities were proportional to the amount of added protein up to at least 40 μg and to an incubation time of up to 15 min. More than 95% of the labeled phospholipid product was extracted and recovered under the conditions used. Thin-layer chromatography on silica gel HR, using the sodium metabisulfite system (26), showed that 80% of the ^{3}H-labeled material present in the final chloroform phase of glycerol-P acyltransferase assays migrated similar to phosphatidic acid, while the remainder migrated similar to lysophosphatidic acid. On the same chromatogram, more than 95% of the ^{32}P-labeled material in the final chloroform phase of DHAP acyltransferase assays migrated similar to acyl-DHAP. In a previous study of yeast membrane glycerol-P acyltransferase, Kuhn and Lynen (24) reported that the ratio of glycerol-P-dependent CoA release to [^{32}P]glycerol-P incorporation into lipid was 1.5, indicating a mixture of monoacyl and diacyl reaction products. However, their attempt to chromatograph the reaction products on Kieselgel G yielded only a single spot tentatively identified as lysophosphatidic acid.

Acyl-DHAP oxidoreductase activity was assessed by two different methods. In the first, the reduced nicotinamide adenine dinucleotide phosphate (NADPH) dependent conversion of [^{32}P]DHAP to [^{32}P]phosphatidic acid was followed (13, 25). Membranes were incubated in the standard DHAP acyl-

transferase assay mixtures, with and without 80 μM NADPH, for 10 min at 23°C. The radioactive lipid products, extracted as described previously (45, 46), were concentrated, applied to a silica gel HR thin-layer plate, and developed in the sodium metabisulfite system (26). Spots migrating similarly to acyl-DHAP, lysophosphatidic acid, and phosphatidic acid standards were localized by autoradiography, scraped into counting vials, and counted in 8 ml of Aquasol 2 (New England Nuclear Corp.). In the second method, the NADPH-dependent conversion of palmitoyl-[^{32}P]DHAP to [^{32}P]phosphatidic acid was monitored under conditions similar to those used by LaBelle and Hajra (25, 27). The assay mixture (final volume, 0.1 ml) contained 16 mM potassium phosphate buffer (pH 7.4), 10 mM NaF, 0.3 mM ethylenediaminetetraacetate, 2.5 μg of Triton X-100, 0.5 nmol of palmitoyl-[^{32}P]DHAP (5×10^4 cpm), 0 or 80 μM NADPH, and 0 or 50 μM oleoyl-CoA. After incubation at 23°C for 10 min, lipids were extracted and quantitated as for the first method.

When samples from glycerol-P acyltransferase, DHAP acyltransferase, and acyl-DHAP oxidoreductase (method 1) assays were chromatographed on Whatman no. 1 paper in the picric acid system (15), no conversion of [^{3}H]glycerol-P to [^{3}H]DHAP or of [^{32}P]DHAP to [^{32}P]glycerol-P could be detected under any of the reaction conditions employed. Any variations from the described reaction conditions for the various enzyme assays are noted in the appropriate figure and table legends.

Growth conditions of the organism and preparation of membrane fractions. *S. cerevisiae* S288c (the kind gift of M. Vickers Hershfield, Duke University Medical Center, Durham, N.C.) was grown, in 1-liter cultures at 26°C, anaerobically under a carbon dioxide atmosphere in GasPak 150 anaerobic culture systems. Methylene blue indicators were used to confirm complete anaerobiosis. The culture medium contained proteose peptone (3 g/liter), yeast extract (3 g/liter), and glucose (4 g/liter). Under these anaerobic, catabolite-repressing conditions, cells are devoid of mature mitochondria. They contain only dedifferentiated promitochondrial structures, which differ dramatically from normal mitochondria in several respects including morphology, enzyme content, protein and lipid contents, and potential for respiratory function (29, 30, 44). Thus, the growth conditions were chosen to facilitate investigations on the microsomal glycerol-P and DHAP acyltransferase activities without extensive subcellular fractionation.

Cells were harvested in the stationary phase, 36 to 40 h post-inoculation, after they had settled into a thin layer on the bottom of the culture flasks. Subsequent operations were carried out at 0 to 4°C. The culture medium was decanted, and cells were suspended in medium Ia (0.25 M sucrose; 10 mM Tris-hydrochloride, pH 7.4; 1 mM ethylenediaminetetraacetate; 1 mM dithiothreitol), isolated by centrifugation at 1,000 × *g* for 10 min, and resuspended in the same medium. The cells were then disrupted by three passages through a French pressure cell at 20,000 lb/in^2. After centrifugation at 1,000 × *g* for 15 min to remove unbroken cells and cell debris, membranes were isolated by centrifugation at 160,000 × *g* for 1 h. For some experiments, membranes were isolated in medium I (medium Ia with dithiothreitol omitted). Protein was determined by the method of Lowry et al. (31) with bovine serum albumin as the standard. Membrane preparations diluted to a protein concentration of 3 to 5 mg/ml in medium Ia were stored in 0.3-ml samples at −15°C. Once thawed, the unused portion of a given membrane sample was discarded. Under these conditions, the glycerol-P and DHAP acyltransferase activities were stable for at least 2 months.

RESULTS

Preliminary experiments established that yeast membranes contained DHAP acyltransferase activity. The first systematic characterization of this activity, along with studies on the membranous glycerol-P acyltransferase activity to determine whether the glycerol-P and DHAP acyltransferase activities are associated with a single membrane-bound enzyme, is reported in this paper. DHAP appeared to be a competitive inhibitor of the glycerol-P acyltransferase (Fig. 1A). At 80 μM palmitoyl-CoA, an apparent K_m of 0.05 mM for glycerol-P, with a V_{max} of 3.41 nmol/min per mg of protein, was determined. A DHAP K_i of 0.91 mM was calculated. Likewise, glycerol-P was a competitive inhibitor of the DHAP acyltransferase activity (Fig. 1B). At 80 μM palmitoyl-CoA, an apparent K_m of 0.79 mM

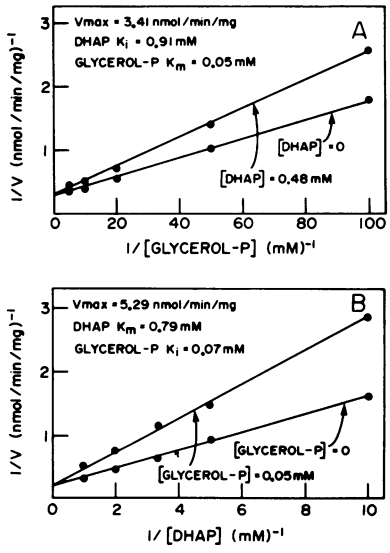

FIG. 1. *(A) Dependence of glycerol-P acyltransferase activity on the concentration of [^{3}H]glycerol-P in the absence and presence of DHAP. Assays, as described in the text, employed 22.4 μg of protein. (B) Dependence of DHAP acyltransferase activity on the concentration of [^{32}P]DHAP in the absence and presence of glycerol-P. Assays, as described in the text, employed 22.4 μg of protein. All data were plotted by the method of Lineweaver and Burk (28) and subjected to computer-assisted least-squares analysis.*

for DHAP, with a V_{max} of 5.29 nmol/min per mg of protein, was determined. A glycerol-P K_i of 0.07 mM was calculated. As expected, the apparent kinetic parameters determined for the two activities were strongly dependent on the level of acyl donor employed. However, at 50 μM palmitoyl-CoA, glycerol-P and DHAP still showed a pattern of reciprocal competitive inhibition (data not shown). At this level of palmitoyl-CoA, the glycerol-P acyltransferase activity had an apparent K_m of .03 mM for glycerol-P, with a V_{max} of 1.0 nmol/min per mg of protein and a DHAP K_i of 0.54 mM. Data on the DHAP acyltransferase activity at 50 μM palmitoyl-CoA showed an apparent K_m for DHAP of 0.53 mM, with a V_{max} of 2.0 nmol/min per mg of protein and a glycerol-P K_i of 0.03 mM. The changes in kinetic parameters for the glycerol-P and DHAP acyltransferase activities caused by varying the palmitoyl-CoA concentration were quite similar.

Acyl-CoA dependencies. Saturated acyl-CoA's containing four to eight carbon atoms were not substrates for either the yeast glycerol-P or DHAP acyltransferase activity. Figure 2A shows the relative glycerol-P and DHAP acyltransferase activities of various long-chain acyl-CoA's, which were employed at 80 μM. The two acyltransferase activities were quite similar in their dependence on acyl-CoA chain length. Data for the various acyl-CoA's at 50 μM (not shown) confirmed this similarity. The glycerol-P and DHAP acyltransferase activities were also quite similar in their dependence on the concentration of palmitoyl-CoA added to the assay (Fig. 2B). Both activities increased to a maximum at 80 μM and declined above this level.

pH dependence. The pH dependencies of the glycerol-P and DHAP acyltransferase activities were very similar over the pH range investigated (Fig. 3). Both activities were maximal about pH 7 and declined about 50% above pH 7.4. Control experiments demonstrated that the [^{32}P]DHAP substrate was stable throughout the pH range employed.

Thermolability of the glycerol-P and DHAP acyltransferase activities. Since the kinetic observations presented above supported the hypothesis that the yeast glycerol-P and DHAP acyltransferase activites are associated with a single enzyme, a number of possible inhibitory conditions were investigated to determine whether both activities were affected similarly. Membrane preparations were heated at 48°C, and samples were removed at various times for assay of the two acyltransferase activities at 23°C. The results (Fig. 4) demonstrate that both activities were inactivated with identical kinetics and declined to 30% of their initial values within 30 min. The two activities were also similar in their time courses of inactivation at 43°C (data not shown).

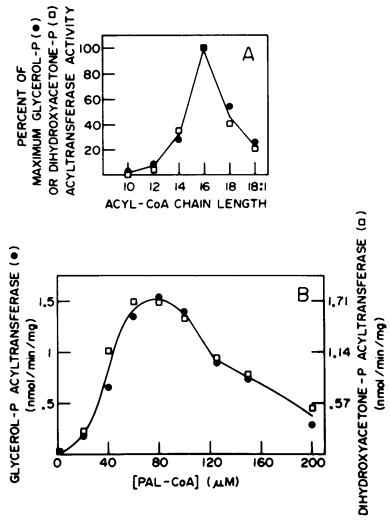

FIG. 2. *(A) Dependence of the glycerol-P and DHAP acyltransferase activities on acyl-CoA chain length. Assays containing 16.5 μg of protein were performed as described in the text, except that 80 μM acyl-CoA of the indicated chain length was substituted for C_{16}-CoA. (Because of the often confusing nomenclature identifying acyl-CoA's, we have elected to identify these chemicals by carbon atom number of the acyl chain and by the number of double bonds, where appropriate [45].) Maximum specific activities at C_{16}-CoA were 2.8 and 2.4 nmol/min per mg for the glycerol-P and DHAP acyltransferase activities, respectively. In this and all remaining figures, a filled square represents the superposition of the 100% control values for the two activities. (B) Dependence of glycerol-P and DHAP acyltransferase activities on the concentration of palmitoyl-CoA (PAL-CoA). Assays, as described in the text, contained 23.5 μg of protein and the indicated level of palmitoyl-CoA. The level of [^{3}H]glycerol-P in the glycerol-P acyltransferase assays was reduced to 0.1 mM.*

Inhibition by *N*-ethylmaleimide. When membrane preparations were incubated with 0.4 mM *N*-ethylmaleimide and samples were removed at various times for assay of the two acyltransferase activities, the results (Fig. 5A) confirmed Kuhn and Lynen's (24) report of the sensitivity of yeast membrane glycerol-P acyltransferase to inactivation by this reagent. This activity was inhibited 60% within 10 min. In addition, the time course for inactivation of the DHAP acyltransferase activity exactly paralleled that of the glycerol-P acyltransferase activity. When glycerol-P and DHAP acyltransferase activities were determined after 10 min of exposure to various concentrations of *N*-ethylmaleimide, both activities were again inactivated quite similarly (Fig. 5B).

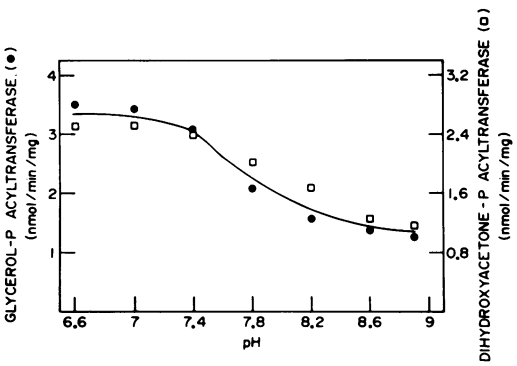

FIG. 3. *pH dependence of the glycerol-P and DHAP acyltransferase activities. Assays using 13.2 μg of protein were performed as described in the text, except that the pH of the Tris-hydrochloride buffer mixture was varied. The measured pH of reaction mixtures was as indicated.*

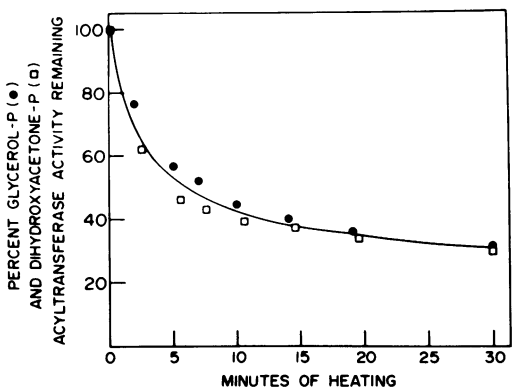

FIG. 4. *Thermolability of the glycerol-P and DHAP acyltransferase activities. Membranes (1 mg/ml) were incubated in medium Ia at 48°C for various times before determination of the activities. Assays, as described in the text, contained 17 μg of protein. Specific activities of unheated microsomes were 2.9 and 2.4 nmol/min per mg for the glycerol-P and DHAP acyltransferase activities, respectively.*

Inhibition by trypsin and detergents. Incubation of membranes with 60 μg of trypsin per ml for various times before assay showed that both glycerol-P and DHAP acyltransferase activities were more than 80% inhibited within 10 min (Fig. 6). The kinetics of inactivation were similar for the two activities. When membranes were incubated with varying concentrations of trypsin for 5 min before assay, the glycerol-P and DHAP acyltransferase activities were again inhibited similarly (data not shown).

The addition of Triton X-100 to each of the acyltransferase assays showed that both glycerol-P and DHAP acyltransferase activities were mildly stimulated at a detergent level of 0.1 mg/ml. However, higher concentrations resulted in parallel and severe losses of both activities (Fig. 7A). When sodium deoxycholate was added to each acyltransferase assay, the mild stimulation of both activities persisted up to a detergent level of 0.3 mg/ml. Above this concentration,

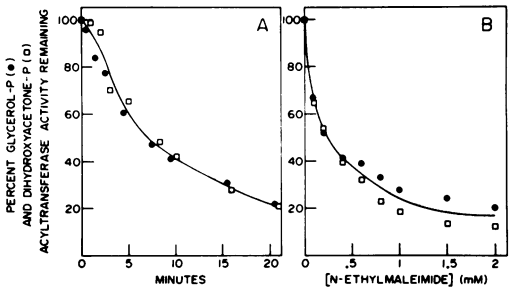

FIG. 5. *Inhibition of the glycerol-P and DHAP acyltransferase activities by N-ethylmaleimide. (A) Time dependence. Membranes (1 mg/ml) prepared in the absence of dithiothreitol were incubated with 0.4 mM N-ethylmaleimide in medium I at 23°C for various times before determination of the activities. (B) Dose dependence. Membranes (1 mg/ml) prepared in the absence of dithiothreitol were incubated with various levels of N-ethylmaleimide in medium I at 23°C for 10 (glycerol-P acyltransferase) or 10.5 (DHAP acyltransferase) min before determination of the activities. Assays containing 15 μg of protein were performed as described in the text. The 1 mM dithiothreitol contained in the assay mixture was sufficient to quench any unreacted N-ethylmaleimide. The specific activities of untreated microsomes were 2.0 and 1.5 nmol/min per mg for the glycerol-P and DHAP acyltransferase activities, respectively.*

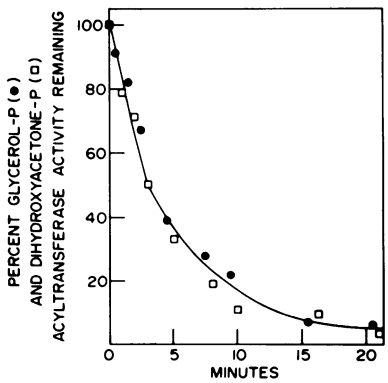

FIG. 6. *Inhibition of the glycerol-P and DHAP acyltransferase activities by trypsin. Membranes (1 mg/ml) were incubated with 60 μg of trypsin per ml in medium Ia at 23°C for various times before determination of the activities. Assays containing 15 μg of protein were carried out as described in the text. Specific activities of untreated microsomes were 2.2 and 1.8 nmol/min per mg for the glycerol-P and DHAP acyltransferase activities, respectively.*

the two acyltransferase activities declined markedly (Fig. 7B). (The critical micellar concentrations of Triton X-100 and sodium deoxycholate have been reported as 0.19 and 1.2 mg/ml, respectively [32].)

Yeast membranous acyl-DHAP oxidoreductase activity. Acyl-DHAP is an important intermediate in the biosynthesis of ether lipids (6, 25, 27, 38, 40, 49, 57). Although we observed a significant DHAP acyltransferase activity in membranes from *S. cerevisiae,* other workers have been unable to detect any trace of neutral or ionic alkoxylipids in this organism (34). Therefore, we investigated whether yeast membrane preparations contained detectable acyl-DHAP oxidoreductase, the enzyme activity required for further processing of acyl-DHAP into glycerol lipids (or of alkyl-DHAP into ether lipids).

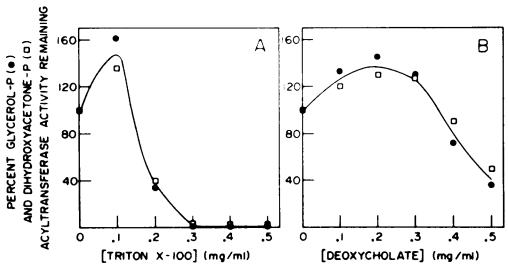

FIG. 7. *Inhibition of the glycerol-P and DHAP acyltransferase activities by detergents. (A) Triton X-100. Assays, as described in the text, contained 10 μg of protein and the indicated amount of Triton X-100. (B) Sodium deoxycholate. Assays, as described in the text, contained 10 μg of protein and the indicated amount of sodium deoxycholate. Specific activities of microsomes in the absence of detergent were 2.8 and 2.2 nmol/min per mg for the glycerol-P and DHAP acyltransferase activities, respectively.*

Acyl-DHAP constitutes the only radiolabeled product formed by the palmitoyl-CoA-dependent acylation of [^{32}P]DHAP by yeast membranes (see Materials and Methods) or liver microsomes (46) under the standard assay conditions. When 80 μM NADPH was added to the standard DHAP acyltransferase assays, more than 98% of the ^{32}P-labeled lipid product formed by yeast membranes still consisted of acyl-DHAP (Table 1, method 1). However, in the NADPH-supplemented assays, more than 70% of the product formed by liver microsomes consisted of phosphatidic acid, the remainder being acyl-DHAP. No lysophosphatidic acid was detected in either case. The total amount of lipid formed from [^{32}P]DHAP by either liver microsomes or yeast membranes was approximately the same in the presence and absence of NADPH.

Similarly, whereas liver microsomes could convert up to 15% of a sample of acyl-[^{32}P]-DHAP to [^{32}P]phosphatidic acid within 10 min (Table 1, method 2), dependent upon NADPH, yeast membranes were unable to perform this conversion. Again, no lysophosphatidic acid was detected in either case. That the conversion of acyl-DHAP to phosphatidic acid by liver microsomes did not depend on added acyl-CoA may be due to the ability of acyl-DHAP itself to serve as an acyl donor (14).

DISCUSSION

Estimates of the relative quantities of glycerolipid synthesized via the glycerol-P and DHAP pathways in various mammalian tissues remain

TABLE 1. *Formation of [^{32}P]phosphatidic acid from [^{32}P]DHAP or palmitoyl-[^{32}P]DHAP by yeast membranes and liver microsomes*[a]

Conditions	Yeast membranes			Liver microsomes		
	Acyl-DHAP (cpm)	LPA (cpm)	PA (cpm)	Acyl-DHAP (cpm)	LPA (cpm)	PA (cpm)
(1) Conversion of [^{32}P]DHAP to [^{32}P]phosphatidic acid						
Standard DHAP acyltransferase assay	9,541	0	103	6,593	0	91
Standard assay + 80 μM NADPH	8,145	0	137	2,661	0	6,501
(2) Conversion of palmitoyl-[^{32}P]DHAP to [^{32}P]phosphatidic acid						
Standard assay	8,733	0	114	7,712	0	133
Standard assay + 80 μM NADPH	6,741	0	185	6,228	0	1,098
Standard assay + 80 μM NADPH + 50 μM oleoyl-CoA	7,805	0	183	7,443	0	1,313

[a] Assays to measure acyl-DHAP oxidoreductase activity, performed as described in Materials and Methods, contained 23.5 μg of yeast membranes. Rat liver microsomes (40 μg), prepared as described previously (46), were also tested as a positive control. Results are expressed as counts per minute of radioactive lipid product migrating similar to acyl-DHAP, lysophosphatidic acid (LPA), and phosphatidic acid (PA).

controversial (9, 53, 55, 57) despite the numerous investigations addressing this question (1, 18, 35, 37, 38–40, 43). In previous studies of several tissues that prominently synthesize lipids (45, 46), we presented evidence strongly supporting the hypothesis that the microsomal glycerol-P and DHAP acyltransferase activities actually constitute dual catalytic functions of a single enzyme. This conclusion allowed a simplified view of metabolic regulation (45).

Yeast membranes possess an easily measurable DHAP acyltransferase activity. Several lines of evidence strongly support the hypothesis that yeast membrane glycerol-P and DHAP acyltransferase activities are catalyzed by a single enzyme. Glycerol-P was a competitive inhibitor of the DHAP acyltransferase activity, and its K_i was similar to its K_m as a substrate for acylation. Likewise, DHAP was a competitive inhibitor of the glycerol-P acyltransferase activity, and its K_i was similar to its K_m. This pattern of reciprocal competitive inhibition observed with glycerol-P and DHAP is consistent with acylation occurring at a single active site. In addition, shifts in kinetic parameters caused by varying the acyl-CoA concentration in the assay were similar for the two activities. Finally, the glycerol-P and DHAP acyltransferase activities showed great similarities in their pH dependence, fatty acyl-CoA chain length and concentration dependencies, thermolability, and patterns of inactivation by *N*-ethylmaleimide, trypsin, and detergents. The present data are similar to those previously reported for the microsomal glycerol-P and DHAP acyltransferase activities present in isolated fat cells (45), liver (46), and several other tissues (46). However, in a recent report, Rock et al. observed that microsomes from rabbit harderian gland contained latent DHAP acyltransferase activity distinct from glycerol-P acyltransferase activity by a number of criteria (42). The growth conditions employed were presumed essentially to inhibit the biogenesis of mitochondria in yeast. The quantitative similarity of the glycerol-P and DHAP acyltransferase activities in the membrane preparation also suggests that under these conditions microsomal enzyme activities are quantitatively predominant or that any mitochondrial glycerol-P or DHAP acyltransferase activity present behaves similar to the corresponding microsomal activity.

Although our assay procedures employed 20- to 50-fold less protein and a lower temperature (23 instead of 30°C) than previous investigations (8, 21, 24, 51, 56), the observed glycerol-P acyltransferase reaction velocities are in good agreement with those of previous reports in which the incorporation of radioactive glycerol-P into lipids was measured (8, 51, 56). Other investigators, who assessed glycerol-P acyltransferase activity by spectrophotometrically monitoring CoA released from palmitoyl-CoA during the reaction (50), found values approximately fivefold higher than we observed (21, 24). The single previous report of DHAP acyltransferase activity in *S. carlsbergensis* agrees reasonably well with our reaction velocities (21), even though an assay that measured CoA release was employed. The pH and acyl-CoA chain length dependencies observed for the glycerol-P acyltransferase are in substantial agreement with previous data (24, 56). However, the optimal concentrations of glycerol-P and palmitoyl-CoA required were considerably lower than the values reported previously (24, 56).

The cellular contents of yeast metabolites are dependent on the culture medium, state of oxygenation, and other environmental factors (17). Glycerol-P and DHAP in *S. cerevisiae* growing in the presence of glucose have been estimated to be in the ranges 1.2 to 2.0 and 0.8 to 1.0 μmol/g of fresh anaerobically grown yeast, respectively (19). Assuming that 71% of a yeast is aqueous (19) and that there is no compartmentalization of substrates, intracellular glycerol-P and DHAP concentration ranges of 1.7 to 2.8 and 1.1 to 1.4 mM, respectively, can be calculated. Using these estimated cellular glycerol-P and DHAP concentrations and the kinetic parameters (K_m, V_{max}, K_i) determined (Fig. 1) for the yeast membranous glycerol-P and DHAP acyltransferase activities, the ratio of glycerol-P to DHAP acylation would be greater than 12:1. Thus, the glycerol-P pathway for phospholipid synthesis would be expected to be predominant in yeast cells in vivo in comparison to the DHAP pathway.

The failure to detect acyl-DHAP oxidoreductase activity in our membrane preparations might reflect repression of this activity under the growth conditions employed. However, the lack of this enzyme activity coupled with the previously reported absence of alkyl-linked lipids in yeast (34) suggests that the DHAP pathway may not operate in *S. cerevisiae*. If the DHAP acyltransferase activity detected in vitro functions in vivo, the acyl-DHAP formed might serve as an acyl donor (14) in biosynthetic reactions for the formation of complex lipids in the cell.

The data suggest that *S. cerevisiae* can provide a system for genetic analysis of the eucaryotic glycerol-P and DHAP acyltransferase activities. In addition, experiments employing hybrid *E. coli* plasmids bearing yeast DNA to correct the *E. coli plsB* glycerol-P acyltransferase lesion (3, 4) might provide a system with

which to investigate the expression of a eucaryotic membrane enzyme in *E. coli*. Since this organism does not contain significant DHAP acyltransferase activity (54), expression of the yeast enzyme should be relatively easy to demonstrate.

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