The Effect of Phospholipid Fatty Acid Composition on Membranous Enzymes in Escherichia coli*

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RICHARD D. MAVIS‡ AND P. ROY VAGELOS

From the Department of Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri 63110

SUMMARY

The shape of an Arrhenius plot of glycerol 3-phosphate acyltransferase activity of an unsaturated fatty acid auxotroph of Escherichia coli is identical in membranes containing cis-vaccenic, oleic, linoleic, or linolenic acid as sole unsaturated fatty acid. The curve is linear at low temperatures with a continuous decrease in slope above 15°. In membranes containing trans-unsaturated fatty acids as the sole unsaturated fatty acid, the decrease in slope occurs at 20°. Membranes from cells grown in the presence of oleic acid and then grown for one generation in the absence of unsaturated fatty acids contained 79% saturated fatty acids and 21% oleic acid. Temperature dependence of the enzyme activity in these membranes was intermediate between that observed in membranes containing trans-unsaturated fatty acids and those containing normal amounts of cis-unsaturated fatty acids.

1-Acylglycerol 3-phosphate acyltransferase activity exhibited a linear Arrhenius plot identical in slope in membranes containing cis-unsaturated fatty acids of varying degrees of unsaturation. Membranes from cells deprived of unsaturated fatty acids for one generation or those containing trans-unsaturated fatty acids exhibited a steeper slope.

Membranous glycerol 3-phosphate dehydrogenase activity appeared to be independent of membrane fatty acid composition. Linear Arrhenius plots of identical slope were observed in all membrane preparations described above.

The difference in the dependence of the temperature characteristics of various membranous enzymes on membrane fatty acid composition suggests a heterogeneity in the relationship between membranous enzymes and membrane phospholipids.

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The critical function of the unsaturated fatty acids of phospholipids was illustrated by the isolation of auxotrophs. More important, the availability of these auxotrophs has made possible the manipulation of the membrane phospholipids since the phospholipid fatty acid composition reflected those fatty acids provided in the culture medium (2). Thus the effects of blocking phospholipid synthesis by deprivation of the required unsaturated fatty acid has been studied in order to determine whether simultaneous phospholipid synthesis is required during the synthesis of a functional β-galactoside transport system (3, 4). Another type of membrane study utilizing the unsaturated fatty acid auxotrophs took advantage of the fact that the unsaturated fatty acids of the phospholipids could be manipulated by varying the fatty acid supplement added to the growth medium. Investigations of the temperature characteristics of glycoside transport (5–8) as well as respiration and growth rate (7) indicated that Arrhenius plots for all these processes were biphasic, the slopes extrapolating to intersections at unique transition temperatures. The transition temperatures for all of these biological processes varied with the degree of unsaturation of the fatty acid supplement, being highest for trans-monoenoic, intermediate with cis-monoenoic, and lowest for cis-polyenoic acids. Similar transition temperatures were observed in studies of the isolated phospholipids in monolayers at an air-water interface (7), suggesting that the transition temperatures observed in the biological processes reflected transitions in the lipid portion of the membrane. A similar dependence of the temperature of phase transitions on fatty acid composition has been observed in lipid bilayers and biological membranes by calorimetric and x-ray diffraction techniques (9, 10). These studies together with studies using spectroscopic methods (11) suggest that a large portion of lipid in biological membranes is arranged in a bilayer structure, the phase properties of which are unperturbed by the protein present in the membrane. It thus becomes of interest to determine whether all membrane functions are dependent on the phase properties of membrane lipids.

One of the more important functions of the cell membrane is phospholipid biosynthesis. The initial step of phospholipid biosynthesis, acylation of glycerol-3-P to phosphatidic acid, has been studied previously in this laboratory (12, 13). Evidence has been obtained which suggests that a single membranous enzyme in E. coli catalyzes the acylation of glycerol-3-P with either palmityl- or oleyl-CoA to form monoeacglycerol-3-P. The positional specificity of this enzyme is consistent with the fatty

The involvement of lipids in membrane function has been investigated in recent years by the use of unsaturated fatty acid auxotrophs of Escherichia coli first isolated in this laboratory

(1).
acid distribution in naturally occurring phospholipids. A separate membranous enzyme appears responsible for the acylation of 1-acylglycerol-3-P to form phosphatidic acid, and this enzyme also displays specificity in regard to the thioester substrates. In the present study we selected these two membranous enzymes and the membranous glycerol-3-P dehydrogenase for investigation of temperature characteristics because sensitive optical assays are available. The effects of varying degrees of unsaturation of membrane fatty acids on the Arrhenius plots of these activities were determined in membranes of an E. coli unsaturated fatty acid auxotroph.

MATERIALS AND METHODS

Materials

Unsaturated fatty acids and fatty acid methyl esters were purchased from Merck, Darmstadt, Germany. Sodium dodecyl sulfate, Triton detergents, sodium deoxycholate, Cutssem detergent, glycerol-3-P, 3-(4,5-dimethyl thiazolyl-2)-2,5-diphenyl tetrazolium bromide, phenazine methosulfate, phospholipase D, deoxyribonuclease, and 5,5'-dithiobis(2-nitrobenzoic acid) were from Sigma Chemical Company. 1-Acylglycerol-3-phosphoryl choline (lysolecithin prepared by phospholipase A) and 1-Acylglycerol-3-phosphoryl choline (lyssolecithin prepared by phospholipase D) were purchased from Emulsion Engineering, Inc., Elk Grove Village, Illinois. Sodium dodecyl sulfate, Triton detergents, sodium deoxycholate, Cutssem detergent, and 3-(4,5-dimethyl thiazolyl-2) 2,5-diphenyl tetrazolium bromide, phenazine methosulfate, phospholipase D, deoxyribonuclease, and 5,5'-dithiobis(2-nitrobenzoic acid) were from Sigma Chemical Company. 1-Acylglycerol-3-phosphoryl choline (lysolecithin prepared by phospholipase D) was purchased from Cyclo Chemical, Los Angeles, California.

Methods

Growth of Bacteria and Preparation of Membrane Fraction.—E. coli strain K-1060, generously provided by Peter Overath, has markers identical with strain K-1059 (7) and is unable to synthesize or degrade unsaturated fatty acids. K-1060 was grown in Medium E (15) supplemented with 0.5% glycerol, 0.3% casein hydrolysate (Difco, vitamin-free), 2% Brij 35, and 0.02% fatty acids. One-liter cultures of cells were grown to late exponential phase (200 Klett units, 54 filter) harvested, washed once with cold 0.02 M potassium phosphate buffer, pH 7.0, the membrane fraction was resuspended in the same buffer at a protein concentration of 10 mg per ml and stored frozen. The suspension was thawed and the cells were broken in a French pressure cell previously cooled in ice. The cell extract was made 2.5 mM in MgCl2 and a few crystals of deoxyribonuclease were added. The extract was incubated on ice for 30 min and then centrifuged 10 min at 6,000 × g to remove unbroken cells. The membrane fraction was then collected by centrifugation for 45 min at 50,000 × g. After washing twice by resuspension and homogenization in cold 0.02 M potassium phosphate buffer, pH 7.0, the membrane fraction was resuspended in the same buffer at a protein concentration of 10 mg per ml and stored frozen. Protein was determined by a microbiuret procedure (10).

Fatty Acid Analysis of Membrane Fraction.—Lipids were extracted from 0.4 ml of membrane fraction according to the method of Bligh and Dyer (17). The chloroform phase was evaporated to dryness under a stream of nitrogen, dissolved in 1 ml of 2% H2SO4 in methanol, and heated at 70° for 1 h. One milliliter of water was then added to the acidic methanol solution and the fatty acid methyl esters were extracted with diethyl ether. The ether extract was washed first with dilute sodium bicarbonate and then with water before analysis by gas liquid chromatography on a Varian model 2100 using a 4-foot glass column of 3% Silicone SE-30 on Varaport 30. Temperature was programmed from 140–180° at a rate of 4° per min with helium as carrier gas. Peak size was quantitated with an Infotronics model CIB-100 digital integrator. Fatty acids were identified by comparison of retention times with those of standard methyl esters. Identities were verified by chromatography on a column of 10% diethylene glycol adipate on Chromasorb W.

Enzymic Assays.—The spectrophotometric assays for both the glycerol-3-P acyltransferase and the 1-acylglycerol-3-P acyltransferase have been described previously (13). Since these assays contain Tris buffer which is known to be affected by temperature, the pH of a typical reaction mixture was measured at 0° and 37°, the extreme limits of the temperatures used here. A change of 0.5 pH units, which would not significantly affect the activity of these enzymes, was observed in changing the temperature from 0° to 37°. Glycerol-3-P dehydrogenase was assayed according to Lin et al. (18) by measuring the rate of reduction of the tetrasodium dye, 3-(4,5-dimethyl thiazolyl-2) 2,5-diphenyl tetrazolium bromide, to its formazan, which absorbs at 550 nm. Temperature of the sample compartment of the spectrophotometer was controlled by a circulating constant temperature bath. Reaction temperatures were measured by insertion of a thermocouple directly into the cuvettes. The initial rate of reactions was linear for several minutes and proportional to the amount of membrane fraction used in all measurements reported.

RESULTS

Attempted Solubilization of Glycerol-3-P Aciyltransferase.—Treatment of the particulate fraction of E. coli with sodium dodecyl sulfate, Triton X-100, Triton X-165, sodium deoxycholate, Cutssem, 2-methyl-1-propanol, or 1-pentanol inactivated the glycerol 3-phosphate acyltransferase activity. Treatment with 1% Triton X-305 in the absence of buffer did not affect the acyltransferase activity, and differential centrifugation suggested the activity was associated with a light fraction of the Triton X-305-treated membrane. Partial resolution of the activity from a large portion of the membrane material was achieved by sucrose density centrifugation in the presence of 1% Triton X-305. One milliliter of membrane suspension in 1% Triton X-305 was layered on the top of a discontinuous gradient consisting of 1.5 ml of 70% sucrose, 1 ml of 40% sucrose, and 1 ml of 20% sucrose with 1% Triton X-305 throughout in a 4.5-ml centrifuge tube and centrifuged at 34,000 rpm for 2 hours in a Spinco SW 56 swinging bucket rotor. Two visible bands of turbidity resulted. The lighter band, which was located at the interface between 20 and 40% sucrose, contained approximately 65% of the recovered glycerol-3-P acyltransferase activity and roughly 15% of the recovered glycerol-3-P acyltransferase activity was located in this heavier band. The glycerol-3-P acyltransferase activity of the upper band could be pelletled by addition of 0.02 M potassium phosphate, pH 7.0, and centrifugation at 50,000 × g for 15 min. This procedure yielded a 10- to 15-fold purification of the glycerol-3-P acyltransferase. The activity was rapidly lost upon removal from the sucrose mixture, however, and attempts to further purify the enzyme failed. Due to the instability of this
partially purified acyltransferase activity, unresolved membrane fraction was used in the following experiments.

**Mutant Membrane Fatty Acid Composition**—Membrane fatty acid compositions resulting from supplementation of the double mutant, K-1060, with various fatty acids are shown in Table II together with fatty acid compositions of two wild type strains. In the supplemented mutant a single unsaturated fatty acid, that supplied in the growth media, was found in the membranes and these results are similar to those of Overath, Schuier, and Stoffel (7). The exception was supplementation with cis-vaccenic acid, which is synthesized in wild type *E. coli*; cis-vaccenic acid was partially converted to its cyclopropane derivative, cis-11,12-methylene octadecanoic acid, in the mutant. The effect of supplementation with various unsaturated fatty acids on chain length and percentage of saturated fatty acids in the membrane is generally consistent with the observations of this and other laboratories (2, 7, 19-21) and suggests that fatty acid composition of the membrane of *E. coli* is controlled with respect to the over-all physical properties of the hydrocarbon side chains. Linolenic acid (18:3) was incorporated into membrane to a lesser extent than other cis-unsaturated fatty acids, and its incorporation was not increased when the mutant was grown at a lower temperature. The amount of trans-unsaturated fatty acids incorporated was greater than the cis-analogues, and the largest amounts of shorter chain saturated fatty acids were found in membranes of the mutant grown in the presence of elaidic acid. Growth of the mutant for one generation in the absence of unsaturated fatty acids after prior growth in oleic acid-supplemented medium yielded membranes containing 79% saturated fatty acids consisting of equal amounts of myristic and palmitic

### Table I

**Discontinuous sucrose density centrifugation of membrane fraction of *E. coli* in presence of Triton X-305**

The gradient is described under “Results.” Glycerol-3-P acyltransferase activity is presented in nanomoles per min.

| Fraction       | Volume | Total units | Δass | Δass X volume |
|----------------|--------|-------------|------|---------------|
| Water          | .000 1.1 0 0.04 0.044 |
| Water-20% sucrose interface | 0.25 | 0 | 0.04 | 0.010 |
| 20% Sucrose    | .000 0.45 2 0.05 0.023 |
| 20%:40% Interface | 0.45 | 11.0 | 0.135 | 0.061 |
| 40% Sucrose    | .000 0.2 | 1.171 | 0.034 |
| 40%:70% Interface | 0.65 | 1.5 | 0.492 | 0.320 |

### Table II

**Percentage of fatty acid compositions of membrane fraction from wild type *E. coli* and unsaturated fatty acid auxotroph supplemented with various fatty acids**

| Strain | Fatty Acid Growth | Total Saturated | cis | Linoleic | Total Unsaturated |
|--------|-------------------|-----------------|-----|---------|-----------------|
|        | Supplemented Temp. | 12:0 14:0 16:0 18:0 | 16:1 17:0<sup>a</sup> 18:1 18:2 18:3 19:0<sup>b</sup> |
| B      | None              | 37° 3 44 1 48 4 21 20 -- -- -- 7 52 |
| H139   | None              | 36° 3 32 1 36 29 5 30 -- -- -- -- 64 |
| K1060  | cis-vaccenic      | 37° 1 26 25 -- 53 -- -- -- 38 -- -- 9 47 |
|        | Oleic             | cis-<sup>Δ</sup><sub>κ</sub>18:1 | 37° 10 36 -- 46 -- -- 54 -- -- -- -- 54 |
|        | Linoleic          | cis-<sup>Δ</sup><sub>κ</sub>18:2 | 37° 10 25 -- 35 -- -- 65 -- -- -- -- 65 |
|        | Linolenic         | cis-<sup>Δ</sup><sub>κ</sub><sup>b</sup><sub>,11</sub>18:2 | 37° 3 44 -- 47 -- -- -- -- 53 -- -- 53 |
|        | Linolenic         | cis,cis-<sup>Δ</sup><sub>κ</sub><sup>b</sup><sub>,11</sub>18:2 | 37° 2 55 -- 57 -- -- -- -- 43 -- -- 43 |
|        | Elaidic           | cis-<sup>Δ</sup><sub>κ</sub>18:1 | 37° 3 21 11 -- 35 -- -- -- 65 -- -- -- 65 |
|        | trans-vaccenic    | trans-<sup>Δ</sup><sub>κ</sub>18:1 | 37° 8 7 -- 15 -- -- -- -- 84 -- -- -- 84 |
|        | cis-10-Methylene | cis-10-Methylene | hexadecanoic acid. |
|        | cis-11,12-Methylene | octadecanoic acid. |

<sup>a</sup> cis-9,10-Methylene hexadecanoic acid.

<sup>b</sup> cis-11,12-Methylene octadecanoic acid.
acids and a significant amount of lauric acid. These membranes are referred to as "starved" membranes.

Effect of Phospholipid Fatty Acid Composition on Glycerol-3-P Acyltransferase Activity—As shown in Table III, typical specific activities of glycerol 3 P acyltransferase at 25°C ranged from 1.0 to 2.2 nmol per min per mg of protein with palmityl-CoA as acyl donor in membranes prepared from cells supplemented with cis-unsaturated fatty acids. No significant deviation from normal specific activities was caused by supplementation with di- and transunsaturated fatty acids not normally found in E. coli membranes. On the other hand, membranes containing elaidic or trans-vaccenic acid had lower specific activities of 0.3 to 0.4 nmol per min per mg. Membranes prepared from cells starved for unsaturated fatty acids for one generation had a specific activity of 0.9 on the lower end of the normal range of specific activities.

Arrhenius plots of glycerol-3-P acyltransferase activity in the various membrane preparations described above are shown in Figs. 1 and 2. The original data are presented in Figs. 1A and 2A; in Figs. 1B and 2B the resulting curves were normalized at 25°C in order to facilitate comparison of curve shapes. Each type of membrane was prepared at least twice. Curves obtained with all preparations of each type of membrane were indistinguishable. Using palmityl-CoA as the acyl donor (Fig. 1, A and B), the curves shown are identical in shape for membranes containing either cis-vaccenic acid or linolenic acid as the sole unsaturated fatty acid. Membranes prepared from wild type cells as well as from mutant cells supplemented with oleic or linoleic acid also yielded curves (not shown) identical with that shown for cis-vaccenic acid. The shape of these curves was independent of the cell growth temperatures ranging from 27-40°C. These "normal" curves are linear below 15°C, with a continuous decrease in slope as the temperature is raised above 15°C. Membranes containing trans-unsaturated fatty acids exhibit curves which are linear below 20°C, with a change in slope above 20°C. "Starved" membranes, containing only 21% unsaturated fatty acid, exhibited a shape intermediate between "normal" and trans-unsaturated fatty acid-containing membranes. A small decrease in slope occurred at 15°C, the rate of decrease being less than with "normal" membranes but more rapid than with the

| Membrane unsaturated fatty acid | Growth temperature | Specific activity with palmityl-CoA | Specific activity with oleyl-CoA |
|---------------------------------|--------------------|-----------------------------------|--------------------------------|
| cis-Vaccenic                    | 37°                | 2.21                              | 0.80                           |
| Oleic                           | 37°                | 2.00                              | 0.40                           |
| Oleic                           | 27°                | 1.00                              | 0.30                           |
| Linoleic                        | 40°                | 1.00                              | 0.80                           |
| Linolenic                       | 37°                | 1.40                              | 0.40                           |
| Linolenic                       | 31°                | 1.40                              | 0.50                           |
| Elaidic                         | 40°                | 0.30                              | 0.10                           |
| trans-Vaccenic                  | 40°                | 0.40                              | 0.30                           |
| Starved (21% oleic)             | 37°                | 0.90                              | 0.60                           |

a The mutant was grown for one generation in the absence of unsaturated fatty acid after prior growth in medium supplemented with oleic acid.

Fig. 1. Arrhenius plots of glycerol-3-P acyltransferase activity with palmityl-CoA as acyl donor in membrane fraction of E. coli mutant K-1060 supplemented with cis-vaccenic (O----O), linolenic (O--O), trans-vaccenic (Δ--Δ), or elaidic (Δ--Δ) acid, or starved for unsaturated fatty acids for one generation (□--□). A, plotted according to typical specific activities; B, curves from A normalized to 25°C to allow comparison of shapes. Curves virtually identical with "normal" curves shown for cis-vaccenic and linolenic acid supplemented membranes were obtained using membranes from the mutant supplemented with oleic or linoleic acids or from wild type cells.
FIG. 2. Arrhenius plots of glycerol-3-P acyltransferase activity with oleyl-CoA as acyl donor in membrane fractions described in Fig. 1. A, plotted according to typical specific activities; B, curves from A normalized to 25°C to allow comparison of shapes.

FIG. 3. Comparison of Arrhenius plots of glycerol-3-P acyltransferase activity in "normal" (-----) and trans-supplemented (-----) membranes using palmityl-CoA or oleyl-CoA as acyl donor. Curves from Figs. 1 and 2 are positioned arbitrarily on the vertical scale to allow comparison of slopes.

trans-fatty acid containing membranes. It should be noted that distinct transition points are not apparent in any of these curves.

Using oleyl-CoA as acyl donor (Fig. 2, A and B), membranes containing cis-vaccenic, linolenic, oleic, or linoleic acid, as well as membranes containing trans-vaccenic and linolenic acid-supplemented membranes were obtained using membranes from the mutant supplemented with oleic or linoleic acids or from wild type cells.

FIG. 4. Arrhenius plots of 1-acylglycerol-3-P acyltransferase activity in membrane fraction of E. coli mutant K-1060 supplemented with oleic (O), linolenic (O), trans-vaccenic (Δ), or elaidic (A) acid, or starved for unsaturated fatty acids for one generation (□). Each plot is located arbitrarily in a vertical direction to allow comparison of slopes. Specific activities ranged from 1.0 to 4.0 nmoles per min per mg of protein at 25°C. Identical plots were obtained using either oleyl-CoA or palmityl-CoA as acyl donor.
as membranes of wild type cells (data for the latter three not shown), gave identical Arrhenius plots of glycerol-3-P acyltransferase activity. "Starved" membranes exhibited a curve nearly identical with "normal" curves, in contrast to the observations with palmitoyl-CoA as acyl donor. The shape of "normal" curves with oleyl-CoA as acyl donor is unlike "normal" curves with palmitoyl-CoA as acyl donor. Comparison in Fig. 3 shows that glycerol-3-P acyltransferase activity decreased less upon lowering temperature with oleyl-CoA than with palmitoyl-CoA as acyl donor. The ratio of palmitoyl-CoA activity to oleyl-CoA activity at 25°C is 40% higher than the ratio at 10°C. On the other hand, Arrhenius plots of glycerol-3-P acyltransferase activity in trans-fatty acid-containing membranes are very similar in shape with palmitoyl-CoA and oleyl-CoA as acyl donor at temperatures over 12°C. The low specific activities of the membranes containing trans-fatty acids made measurements at lower temperatures difficult.

**Effect of Phospholipid Fatty Acid Composition on 1-Acylglycerol-3-P Acyltransferase Activity**—The effect of temperature on 1-acylglycerol-3-P acyltransferase activity was also investigated in those membrane preparations of varying fatty acid composition. As shown in Fig. 4, Arrhenius plots of this activity are linear with all membrane preparations studied over the temperature range 0-37°C. The curves with membranes containing oleic or linolenic as well as linoleic (not shown) acids are virtually identical in shape. Membrane preparations from trans-fatty acid-supplemented cells, however, exhibit a higher slope which is very similar to that observed with "starved" membranes. In contrast to the data obtained with glycerol-3-P acyltransferase, the effect of temperature on 1-acylglycerol-3-P acyltransferase was identical when palmitoyl-CoA or oleyl-CoA was used as acyl donor.

**Effect of Phospholipid Fatty Acid Composition on Glycerol-3-P Dehydrogenase Activity**—A third membranous enzyme, glycerol 3-phosphate dehydrogenase, was also studied in these membrane preparations. As shown in Fig. 5, linear Arrhenius plots of virtually identical slope were obtained with membrane preparations containing oleic, linolenic, trans-vaccenic, or elaidic acid as well as "starved" membrane preparations.

**DISCUSSION**

The procedure utilized in this work in an attempt to solubilize the *E. coli* glycerol-3-P acyltransferase is similar to a procedure recently reported by Schnaitman (22) in which he separated two discrete fractions of the cell envelope by isopycnic centrifugation. The more dense fraction was shown by him to be enriched in components of the cell wall; the lighter fraction contained the components of the cytoplasmic membrane. Enrichment of the glycerol-3-P acyltransferase in the lighter fraction obtained here suggests that this enzyme is localized in the inner, cytoplasmic membrane of the *E. coli* envelope. Our failure to solubilize this enzyme by using many of the procedures that have succeeded in solubilizing other membranous enzymes emphasizes the fact that new methods are required for studies of membranous enzymes. The finding that glycerol-3-P acyltransferase was inactivated by several delipidating or membrane solubilizing agents suggests that this enzyme has a requirement for some component of intact membrane structure. Work now in progress indicates that the enzyme is inactivated by pure phospholipase C, suggesting a phospholipid requirement. However, attempts to reactivate a delipidated, inactive preparation by the addition of phospholipids have not succeeded thus far.

Earlier studies of the temperature characteristics of glycoside transport with a similar unsaturated fatty acid auxotroph of *E. coli* have indicated that transport, as well as respiration and growth, reflected the degree of unsaturation of the fatty acid supplied in the growth medium; these biological processes were all characterized by distinct transition temperatures that were determined by the temperatures of the phase transitions of the bulk membrane phospholipids (4-8). Neither dependence of temperature characteristics on the degree of unsaturation of the phospholipid fatty acids nor distinct transition temperatures were observed in studies of the three membrane enzymes investigated in this study. Thus membrane enzymes may or may not reflect the physical properties of the membrane phospholipids, and the differences disclosed by studies of temperature characteristics suggest a heterogeneity among membrane enzymes with regard to dependence upon the membrane phospholipids.

The similarity of slope of the linear portions of all Arrhenius plots shown for glycerol-3-P acyltransferase suggests that at lower temperatures the enzyme exists in the same form independent of the membrane fatty acid composition. The temperature of departure from linearity was increased, however, by the presence of trans-unsaturated fatty acids in the membrane, implicating lipid-protein interactions in the action of this enzyme, and suggesting an increase in the phase transition temperature of the lipid affecting this enzyme. Trans-Unsaturated fatty acids have been shown to raise the transition temperatures of phospholipid monolayers relative to cis-unsaturated fatty acids (7, 23), as well as the transport transition temperatures of biological membranes (4-8). The identical shape of the curves reported here for membranes containing cis-mono-, cis-di-, or cis-triun-}

\footnote{R. Mavis, R. Bell and P. R. Vagelos, unpublished data.}
saturated fatty acids, however, indicates that the glycerol-3-P acyltransferase activity is not dependent on the over-all phase properties of membrane lipids, in contrast to the dependencies reported for various transport systems (4–8). A possible explanation for this could be selective association of this enzyme with lipids of appropriate fatty acid composition to maintain its normal temperature profile. Alternatively, the protein could interact with lipids around it in a way which affects their phase properties.

A role for glycerol-3-P acyltransferase in the temperature regulation of the membrane fatty acid composition has been suggested by the work of Sinensky (24). A relative increase in glycerol-3-P acyltransferase activity with unsaturated as opposed to saturated fatty acid thiosterols at lower temperatures was shown to correlate with an increased proportion of unsaturated fatty acids found in phospholipids of E. coli grown at lower temperatures. The decrease in the ratio of enzyme activity with palmitoyl-CoA to that with oleyl-CoA at lower temperatures as reported here is also consistent with such a role. It would thus be of importance to maintain the temperature profile of this enzyme. Maintenance of a normal Arrhenius plot (Fig. 2B) of glycerol-3-P acyltransferase activity with oleyl-CoA as acyl donor in membranes of cells starved for unsaturated fatty acid, and therefore containing only 21% cis-unsaturated fatty acid, supports the hypothesis of selective association of this enzyme with lipids whose physical properties permit a normal temperature profile independent of the over-all phase properties of the membrane. Enzyme activity with palmitoyl-CoA as acyl donor (Fig. 1B), in contrast, exhibits an Arrhenius plot in membranes from the cells starved for unsaturated fatty acid which is similar to that observed with membranes containing trans-unsaturated fatty acids, consistent with expected similarities in the physical properties of the two membrane preparations. This contrasting behavior between activities with oleyl-CoA and palmitoyl-CoA suggests that the physical properties of the lipids associated with this enzyme in “starved” membranes are intermediate between those of the lipids normally associated with the enzyme and those containing only trans-unsaturated fatty acids. The abnormal Arrhenius plots obtained with membranes containing trans-unsaturated fatty acids suggests that maintenance of the normal temperature-dependent substrate specificities depends on the presence of cis-unsaturated fatty acids in the lipids associated with this enzyme. The similarity of these plots (Fig. 3) when either palmitoyl or oleyl-CoA was used as acyl donor in trans-unsaturated fatty acid-containing membranes suggests that the ability to distinguish between saturated and unsaturated acyl donors is lost in the presence of only trans-unsaturated fatty acids. An involvement of the unsaturated fatty acid hydrocarbon side chains in the recognition of substrate by the active site of this enzyme is thus suggested.

The effect of temperature on 1-acylglycerol 3-phosphate acyltransferase activity as a function of membrane fatty acid composition is different from that observed with glycerol-3-P acyltransferase. A linear Arrhenius plot was obtained which is identical to that for membranes containing cis-mono-, cis-di-, or cis-trunsaturated fatty acids but has a steeper slope in membranes from cells starved for unsaturated fatty acids or containing trans-unsaturated fatty acids (Fig. 4). This suggests that this enzyme exists in a different form in the presence of trans-unsaturated or a higher proportion of saturated fatty acids, in contrast to the glycerol-3-P acyltransferase, which exhibits a similar slope in all membrane preparations at lower temperatures. No evidence of a change in slope or phase transition is observed with the I-acylglycerol 3-P acyltransferase, yet interaction of lipid and protein is suggested by the steeper slope of the Arrhenius plot in membranes containing trans-unsaturated or membranes from cells starved for unsaturated fatty acids. Thus a highly specific association of lipids with this enzyme may be postulated in which the phase transitional properties of the lipids are not expressed. Only the over-all temperature characteristic of the lipoprotein complex is affected by the physical properties of the lipid hydrocarbon side chains. The higher slope of the plots with membranes containing trans-unsaturated or predominantly saturated fatty acids suggests that the enzyme is a less effective catalyst when cis-unsaturated fatty acids are not available.

The possibility that the temperature characteristics observed in studies of the two acyltransferases were influenced by temperature effects on the substrates acyl-CoA or 1-acylglycerol-3-P cannot be entirely discounted. However, substrate saturation curves of acyl-CoA were similar at high and low temperatures; thus no obvious differences were detected at this level. Moreover, since major differences in temperature characteristics were noted between enzyme preparation made from membranes containing different fatty acids, although the assays were carried out under identical conditions, it is likely that the temperature profiles largely reflected the properties of the membranous enzymes.

Glycerol-3-P dehydrogenase activity, in contrast to the acyltransferase activities, appears to be independent of membrane fatty acid composition. If lipid-protein interaction is essential to the action of this enzyme, the requirement is either nonspecific with respect to the over-all physical properties of hydrocarbon side chains or exclusive for saturated fatty acids. A third, more obvious possibility is a total lack of lipid-protein interaction in the action of this membranous enzyme.

The unique response of each of three membranous enzyme activities to variations in membrane fatty acid composition indicates a heterogeneity of membrane proteins with respect to lipid dependence. In addition to transport systems, which exhibit changes in temperature characteristics and distinct breaks in Arrhenius plots at temperatures corresponding to temperatures of over-all phase transition of the bulk of membrane lipid (4–6), at least three additional classes of membrane proteins appear to exist. Lack of response of these enzymes to changes in over-all phase properties of the membrane affected by the physical properties of the lipid hydrocarbon side chains in the immediate environment by the protein itself. In either case, a microheterogeneity of membrane lipids with respect to phase properties is indicated.

Temperature studies of other membranous enzymes have been recently reported. Raison, Lyons, and Thomson (25) found that disruption of mitochondria from rat liver and sweet potato by sonication, hypotonic swelling, and freezing and thawing did not alter the discontinuity in the Arrhenius plot exhibited by mitochondrial respiratory enzymes, including the succinate oxidase system, succinate dehydrogenase, and cytochrome c oxidase. Thus intact membrane structures are not required for the demonstration of transition temperatures. Disruption of the mito-
chondrial membranes with detergent brought about a change in the temperature characteristics of all three enzyme systems with a loss of the discontinuity in the Arrhenius plot. The authors interpreted these results as suggesting that the temperature-induced change in activation energy of these membranous enzymes was associated with a phase change in the lipid component of membranes. On the other hand Zeylemaker et al. (26) noted a breakpoint in the Arrhenius plot with both particulate and soluble succinate dehydrogenase of heart muscle which they interpreted as indicating temperature-dependent conformational changes in the enzyme.

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