Englander, S. W., and Englander, J. J. (1965), *Proc. Nat. Acad. Sci. U. S. 53*, 370.
Englander, J. J., Kallenbach, N. R., and Englander, S. W. (1972), *J. Mol. Biol. 63*, 153.
Englander, J. J., and von Hippel, P. H. (1972), *J. Mol. Biol. 53*, 171.
Fiske, C. H., and Subbarow, Y. (1925), *J. Biol. Chem. 66*, 375.
Grunwald, E., and Price, E. J. (1964), *J. Amer. Chem. Soc. 86*, 2965.
Grunwald, E., and Puar, M. S. (1967), *J. Phys. Chem. 71*, 1842.
Hanson, C. V. (1971), *J. Mol. Biol. 58*, 847.
Hertz, H. G. (1960), *Z. Elektrochem. 64*, 53.
Hvidt, A., and Nielsen, S. O. (1966), *Advan. Protein Chem. 21*, 287.
Johnson, C. S., Jr. (1965), *Advan. Magn. Resonance 1*, 39.
Klotz, I. M., and Frank, B. H. (1965), *J. Amer. Chem. Soc. 87*, 2721.
Linderstrøm-Lang, K. U. (1955), *Chem. Soc. Sp. Publ. 2*, 1.
Marshall, T. H., and Grunwald, E. (1969), *J. Amer. Chem. Soc. 91*, 4541.
McConnell, B., Raszka, M., and Mandel, M. (1971), *Biophys. Soc. Meeting, 15th, Abstr.*, M-9.
McConnell, B., Raszka, M., and Mandel, M. (1972), *Biochem. Biophys. Res. Commun. 47*, 692.
McConnell, B., and von Hippel, P. H. (1970a), *J. Mol. Biol. 50*, 297.
McConnell, B., and von Hippel, P. H. (1970b), *J. Mol. Biol. 50*, 317.
McConnell, B., and von Hippel, P. H. (1971), *Procedures Nucl. Acid Res. 2*, 389.
Meiboom, S. (1960), *Z. Elektrochem. 64*, 50.
Meiboom, S. (1961), *J. Chem. Phys. 34*, 375.
Miles, H. T., Howard, F. B., and Frazier, J. (1963), *Science 14*, 1458.
Phillips, R., Eisenberg, S. J. P., George, P., and Rutman, R. J. (1965), *J. Biol. Chem. 240*, 4393.
Printz, M. (1970), *Biochemistry 9*, 3077.
Printz, M. P., and von Hippel, P. H. (1965), *Proc. Nat. Acad. Sci. U. S. 53*, 363.
Printz, M. P., and von Hippel, P. H. (1968), *Biochemistry 7*, 3194.
Robinson, D. R., and Grant, M. E. (1966), *J. Biol. Chem. 241*, 4030.
Ts'o, P. O. P., Schweizer, M. P., and Hollis, D. P. (1969), *Ann. N. Y. Acad. Sci. 158, Suppl. 1*, 256.
von Hippel, P. H., and Printz, M. P. (1965), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 23*, 1458.
Woodward, C. K., and Rosenberg, A. (1970), *Proc. Nat. Acad. Sci. U. S. 66*, 1067.
Woodward, C. K., and Rosenberg, A. (1971), *J. Biol. Chem. 246*, 4114.
Yamane, T. (1971), *Procedures Nucl. Acid Res. 2*, 262.

# Selective Transfers of *trans*-Ethylenic Acids by Acyl Coenzyme A. Phospholipid Acyltransferases†

Harumi Okuyama, William E. M. Lands,* Frank D. Gunstone, and J. A. Barve

ABSTRACT: *trans*-Octadecenoyl coenzyme esters are generally good substrates for acyl-CoA:phospholipid acyltransferase action to form phosphatidylethanolamine or choline derivatives. The CoA esters of the 9-, 10-, and 11-positional isomers, which are relatively common in processed dietary fats, have different activities in forming phospholipids. Comparison of the patterns of selectivity for the cis and trans series supports the concept that acyl transfer to the 1 position is quite sensitive to configurational differences in acids, whereas transfer to position 2 is not. The latter transfer, however, seems favorable for acids with $\pi$ bonds at positions 5, 9, and 12.

The unsaturated fatty acids of glycerides in nature are almost always of the cis configuration; some exceptions being vaccenic acid (*trans*-11-octadecenoic acid) in animal fats (Bertram, 1928) and *trans*-3-hexadecenoic acids at the 2 position of phosphatidylglycerol in plants (Haverkate and Van-Deenen, 1965). Although rumen bacteria can convert cis to trans configuration (Shorland *et al.*, 1955), the effect of the trans fatty acids from these origins on our diet is probably not very significant. However, the presence of a large amount of positional isomers of trans fatty acids in margarine and shortening prepared from hydrogenated fats (up to 40% of total fatty acids by Mabrouk and Brown, 1956) led many investigators to examine the nutritional and biochemical features of trans fatty acids. When ingested, trans fatty acids were absorbed, oxidized, and transported across the placental membrane at rates comparable to saturated acids or cis isomers (Ono and Fredrickson, 1964; Coots, 1964). Trans fatty acids were also incorporated into lipids of most tissues, although they were partly excluded from the lipids of the brain and testes (Sinclair, 1935). Esterified trans isomers were shown to occupy mostly the 1(3) position of triglycerides (Raulin *et al.*, 1965) and both the 1 and 2 positions of phospholipids, with significant enrichment of the 1 position (Raulin *et al.*, 1963; Selinger and Holman, 1965). While the physiological significance of the displacement of saturated and un-

† From the Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48104 (H. O. and W. E. M. L.), and from the Department of Chemistry, The University of St. Andrews, Scotland (F. D. G. and J. A. B.). *Received April 14, 1972.* This work was supported in part by a grant from the U. S. Public Health Service (NIH-AM05310).

saturated fatty acids with trans fatty acids has not been wholly clarified, experiments *in vitro* have been carried out by using several trans isomers to see if geometrical isomers of fatty acids could be discriminated by acyl-CoA:phospholipid acyltransferases (Lands, 1965; Lands *et al.*, 1966; Jezyk and Lands, 1968). These enzymes are known to be highly selective in terms of chain length and degree of unsaturation (Lands and Hart, 1965). Earlier results (Lands, 1965; Jezyk and Lands, 1968) showed that cis-$\Delta^9$ and trans-$\Delta^9$ derivatives were esterified at similar rates by enzymes acting at the 2 position, whereas the trans-$\Delta^9$ isomer was esterified much faster than the cis-$\Delta^9$ isomer to the 1 position. This led to a hypothesis that $\pi$ bonds at certain positions were preferred by the acyltransferase(s) acting at the secondary hydroxyl. The hypothesis was supported earlier (Okuyama *et al.*, 1969) and is further supported by our present results.

In this paper, the whole series of *trans*-octadecenoyl-CoA isomers were examined as substrates for microsomal acyl-CoA:phospholipid acyltransferases, and the results were compared to those for the *cis*-octadecenoyl-CoA isomers and the methyleneoctadecanoyl-CoA (cyclopropane acyl-CoA) isomers reported previously (Reitz *et al.*, 1969; Okuyama *et al.*, 1969).

## Materials and Methods

*Preparation of trans-Octadecnoyl-CoA Isomers.* The complete series of positional isomers of *trans*-octadecenoic acid was synthesized by the catalytic conversion of cis isomers. The melting points and some chromatographic properties have been described (Gunstone and Ismail, 1967; Gunstone *et al.*, Barve *et al.*, 1972; Barve and Gunstone, 1971). The acyl-CoA esters were synthesized, with an average yield of 59%, from acyl chlorides and coenzyme A as described previously (Okuyama and Lands, 1969; Reitz *et al.*, 1969). The purity of the resulting acyl-CoAs was estimated by comparing the values obtained by the $A_{232}:A_{260}$ assay to the total amount of CoA released by the acyltransferase system (Lands and Hart, 1965) and to the total phosphorus content (Eibl and Lands, 1969). In all cases, the thiol esters showed no impurities beyond the accuracy limits of the methods used.

*Preparation of Isomeric Monoacyl-GPC and Monoacyl-GPE.*[1] 1-Acyl-GPC and 1-acyl-GPE were prepared by phospholipase $A_2$ (*Crotalus adamanteus* venom) hydrolysis of egg lecithin and egg phosphatidylethanolamine. They contained mostly saturated fatty acids; 16:0/18:0 ratios of 2 and 0.5, respectively.

Dioleoyl- and dilinoleoyl-GPC were prepared by acylation of GPC as described previously (Brandt and Lands, 1967). These were converted into dioleoyl- and dilinoleoyl-GPE by means of the transphosphatidylation reaction (Yang *et al.*, 1967). Using a partially purified phospholipase D from cabbage (acetone-precipitation step (see Davidson and Long, 1958) and an ethanolamine concentration of 3%, we obtained dioleoyl- and dilinoleoyl-GPE in a yield of about 45% after chromatography on DEAE-cellulose and silicic columns. To obtain 1-acyl-GPE, 50 $\mu$moles of dilinoleoyl-GPE was incubated with 30 mg of venom phospholipase A in the presence of 10 $\mu$moles of $CaCl_2$, 25 ml of 0.1 M borate (pH 6.5), and 25 ml of diethyl ether for 1 hr with constant shaking. After adding 25 ml of methanol, fatty acids were extracted from the reaction mixture four times each with 25 ml of petroleum ether (bp 30–60°)–ether (1:1) mixture. 1-Linoleoyl-GPE was then extracted from the water layer with 50 ml of chloroform–methanol (2:1) and was suspended in 0.1 M boric acid after evaporation of solvents.

2-Acyl-GPC and 2-acyl-GPE were prepared by iodine treatment of the corresponding choline and ethanolamine alkenyl derivatives from beef hearts. The method, which was a slight modification of that described previously (Lands, 1965; Eibl and Lands, 1970), was essentially the same as the one used in the preparation of 2-acylglycerophosphate (Okuyama *et al.*, 1971) except that a petroleum ether–diethyl ether mixture (1:1) was used instead of petroleum ether. Monacyl-GPC and monoacyl-GPE preparations showed single spots on silica gel G thin-layer chromatograms with $CHCl_3$–$CH_3OH$–$H_2O$ (65:25:4, v/v). The purity of the phospholipid acceptors was also examined by incubating them with radioactive acyl-CoA in the presence of liver microsomes, isolating the diacyl derivatives and then analyzing the radioactive fragments after phospholipase $A_2$ hydrolysis (Lands and Merkl, 1963). The fatty acids were principally 18:1 (44%) and 18:2 (32%).

Microsomes were prepared from rat liver by collecting the particulate fraction that sedimented between 20,000*g* (20 min) and 100,000*g* (90 min) from perfused organs. This fraction was then quickly frozen (with the aid of Dry Ice and acetone) and stored at −10°. In some experiments, the microsomes were prepared as described previously (Eibl *et al.*, 1969) and stored at −10°. A microsomal preparation with a higher specific activity of acyltransferase was obtained by deoxycholate treatment (Okuyama *et al.*, 1969; Reitz *et al.*, 1969).

Incubation mixtures contained 80 $\mu$moles of Tris-Cl (pH 7.4), 0.1 ml of 0.01 M DTNB, 175 nmoles of 1-acyl-GPC (or 200 nmoles of 1-acyl-GPE or about 75 nmoles of either 2-acyl-GPC or 2-acyl-GPE) in 0.1 ml of 0.1 M boric acid, 20 nmoles of acyl-CoA, and 0.1–0.2 mg of microsomal protein in a final volume of 1 ml. The microsomes were preincubated for 1 min before starting the reaction by adding acyl-CoA. The acyl transfer rates were continuously recorded in a spectrophotometer with the use of DTNB to measure the released CoA (Lands and Hart, 1965; Ellman, 1959). The endogenous rate observed without added acceptor was subtracted to give a net acyl transfer rate. Since each assay involved a continual recording of the product, each velocity reported represents an average of a large number of recorded determinations.

## Results

2-Acyl-GPC and 2-acyl-GPE were prepared just before use and were stable at least for several hours during the assays. 1-Acyl-GPC is stable and can be kept in organic solvents for several months without significant isomerization (Eibl and Lands, 1970), whereas 1-acyl-GPE seems to be relatively labile under similar conditions. When 1-acyl-GPE (prepared from egg diacyl-GPE) was kept for several months in a $CHCl_3$–$CH_3OH$ (2:1) solution and was analyzed by using labeled acyl-CoA, a significant amount of radioactivity was found at the 1 position as well as at the 2 position of diacyl-GPE (Table I). Furthermore, the relatively lower solubility of 1-saturated acyl-GPE is apparent from the cloudy suspension in the incubation mixture. These difficulties could be diminished by using 1-linoleoyl-GPE prepared just before use (see Table I). When the effect of the concentration was examined with three preparations of 1-acyl-GPE (*e.g.*, 1-

[1] Abbreviations used are: GPC, *sn*-glyceryl-3-phosphorylcholine; GPE, *sn*-glyceryl-3-phosphorylethanolamine; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).

TABLE I: A Test for the Purity of Monoacyl-GPE.[a]

| Acyl-GPE | Acyl-CoA Used | % Radioactivity at the 2 Position |
|---|---|---|
| 1-(Saturated) acyl-GPE | 20:3 | 81 (7) |
| | 18:0 | 38 (7) |
| 1-Oleoyl-GPE | 20:3 | 95 (1) |
| | 18:0 | 68 (1) |
| 1-Linoleoyl-GPE | 20:3 | 98 (2) |
| | 18:0 | 84 (2) |
| 2-(Unsaturated) acyl-GPE | 20:3 | 18 (2) |
| | 18:0 | 1 (2) |

[a] The incubation mixture contained 75 nmoles of labeled stearoyl-CoA (18:0 CoA) or eicosatrienoyl-CoA (20:3 CoA), 200 nmoles of acyl-GPE, 0.4 mg of deoxycholate-treated microsomal protein (3), and 160 μmoles of Tris-Cl (pH 7.4) in a final volume of 2 ml. Incubations were carried out for 30 min at room temperature and the labeled diacyl-GPE was purified by preparative thin-layer chromatography. Radioactivity at both positions was then analyzed after phospholipase $A_2$ treatment. The number of samples analyzed is shown in parentheses.

saturated acyl-GPE, 1-oleoyl-GPE, and 1-linoleoyl-GPE), slight differences were observed in the apparent $K_m$ values whereas the maximum velocities were about the same (Figure 1). The crude microsomes showed relatively lower acyltransferase activities for 1-acyl-GPE and the specificity for acyl-CoAs with the 1-acyl-GPE was difficult to determine (Lands and Hart, 1965). Accordingly, deoxycholate-treated microsomes with a higher specific activity were used to examine the specificity of acyl-CoA:1-acyl-GPE acyl transfer reaction. As seen in Table II, unsaturated acyl-CoAs were the better substrates than the saturated acyl-CoAs examined. Higher activities for saturated acyl-CoAs observed with 1-saturated acyl-GPE might be explained by the presence of relatively larger amounts of 2-acyl-GPE contaminant in the preparation (see Table I). In all subsequent experiments, freshly prepared 1-linoleoyl-GPE was used.

The results in Table III show that the acyl-CoA concentration in routine assays (20 μM) was at a sufficiently high value

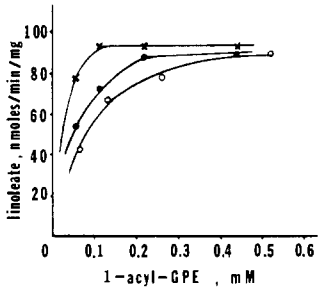


FIGURE 1: Effect of 1-acyl-GPE concentration on the initial velocities of the acylation of linoleoyl-CoA. The incubation system was the same as in Table I and experiments with different microsomal preparation showed similar features. Determinations of the velocities at very low 1-acyl-CPE concentrations were uncertain. (○) 1-Saturated acyl-GPE; (●) 1-oleoyl-GPE; (×) 1-linoleoyl-GPE.

TABLE II: Specificity of Acyl-CoA:1-Acyl-GPE Acyltransferase.[a]

| Acyl-CoA | Acyl Transfer Rate (nmoles/min per mg of Protein) | | |
|---|---|---|---|
| | 1-Saturated Acyl-GPE | 1-Oleoyl-GPE | 1-Linoleoyl-GPE |
| Palmitoyl | 20 | 8 | 8 |
| Stearoyl | 33 | 11 | 14 |
| Oleoyl | 33 | 24 | 24 |
| Linoleoyl | 90 | 88 | 92 |
| Arachidonyl | 77 | 89 | 103 |

[a] The incubator mixture contained 25 nmoles of acyl-CoA, 400 nmoles of 1-saturated acyl-GPE or 200 nmoles of 1-oleoyl-GPE or 200 nmoles of 1-linoleoyl-GPE, 0.1 ml of 0.01 M DTNB, and 0.1 M Tris-Cl (pH 7.4) in a final volume of 1 ml.

to provide accurately reproducible initial velocities without an excessive interference by the detergent-like inhibitions (Lands and Hart, 1965) or by the binding effects of protein (Barden and Cleland, 1969; Okuyama and Lands, 1972) that can lower substrate availability at low concentrations. As with earlier studies (Reitz *et al.*, 1969; Okuyama *et al.*, 1969), the differences in velocity for 10, 20, and 40 μM thiol ester solutions were small compared to the differences in rates for the different isomers. Because of the consistency of this phe-

TABLE III: Acyl Transfer Rates with Varying Concentrations of Acyl-CoA.[a]

| Acyl-CoA (nmoles/ml) | | Acyl Transfer Rates (nmoles/min per mg) | |
|---|---|---|---|
| | | 1-Acyl-GPC | 2-Acyl-GPC |
| *trans*-10- | 5 | 5 | 7 |
| | 10 | 6 | 9 |
| | 20 | 5 | 11 |
| | 40 | 5 | 7 |
| *trans*-14- | 5 | 4 | 11 |
| | 10 | 5 | 13 |
| | 20 | 6 | 14 |
| | 40 | 6 | 18 |
| *cis*-9- | 5 | 8 | 10 |
| | 10 | 17 | 12 |
| | 20 | 15 | 12 |
| | 40 | 16 | 16 |
| *trans*-9- | 5 | 8 | 16 |
| | 10 | 10 | 22 |
| | 20 | 10 | 25 |
| | 40 | 13 | 28 |

[a] The reaction mixture contained 175 nmoles of 1-acyl-GPC or about 75 nmoles of 2-acyl-GPC, varying amounts of acyl-CoA and 0.2 mg of microsomal protein in a final volume of 1 ml (see Materials and Methods). All values are the average of two determinations and have been corrected for hydrolase activity.

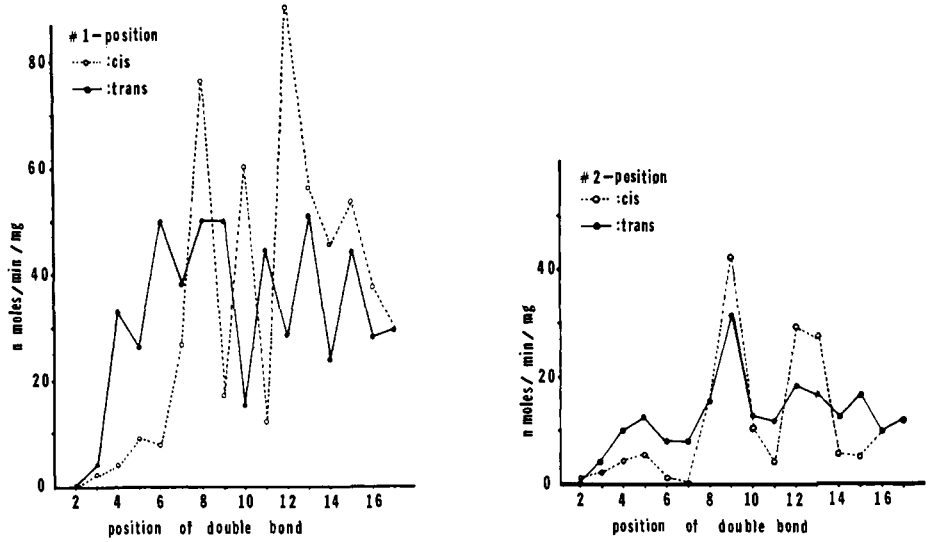


FIGURE 2: Acyltransferase specificities of rat liver microsomes with 2-acyl-GPC (A, left) or 1-acyl-GPC (B, right). The incubation system is the same as in Materials and Methods. Deoxycholate-treated microsomes corresponding to 0.1 mg of protein were used. All acyl transfer rates are corrected for hydrolase activity. Rates for 2-acyl-GPC (A) are the average of two determinations and those for 1-acyl-GPC (B) are the average of four determinations. Values (○) for *cis*-octadecenoate are provided for comparison to those (●) for *trans*-octadecenoate.

nomenon with the earlier studies, published *in extenso*, Table III contains results only for the more highly reactive and less reactive isomers (see Figures 2 and 3) to illustrate that those velocities were not an artifact of a particular acyl-CoA concentration. It should be noted that the very low activity observed for the trans $\Delta^{10}$ isomer with 2-acyl-GPC was not attributable to a higher $K_m$ value, because increasing the amount of acyl-CoA did not increase the velocity significantly.

TABLE IV: Acyl Transfer Rates of *trans*-Octadecenoyl-CoA Isomers with Crude Microsomes.[a]

| Acyl-CoA | Acyl Transfer Rate (nmoles/min per mg of Protein) 1-Acyl-GPC | 2-Acyl-GPC | Mp of Trans Acid[d] (°C) |
|---|---|---|---|
| $\Delta^2$ | 1.8 ± 0.9[b] | 0 ± 0.8[b] | 57.5–58.5 |
| $\Delta^3$ | 3.0 ± 0.6 | 1.8 ± 0.6 | 64.5–65.5 |
| $\Delta^4$ | 4.5 ± 0.3 | 16.4 ± 2.3 | 58.5–59.5 |
| $\Delta^5$ | 6.5 ± 1.8 | 14.1 ± 1.4 | 45.5–47.5 |
| $\Delta^6$ | 2.3 ± 1.3 | 21.2 ± 2.2 | 53.0–54.0 |
| $\Delta^7$ | 1.2 ± 0.4 | 14.2 ± 2.5 | 44.5–45.5 |
| $\Delta^8$ | 3.2 ± 1.1 | 21.8 ± 1.2 | 51.5–52.5 |
| $\Delta^9$ | 11.4 ± 1.1 | 23.4 ± 2.1 | 44.5–45.5 |
| $\Delta^{10}$ | 5.5 ± 0.8 | 4.4 ± 1.4 | 52.5–53.5 |
| $\Delta^{11}$ | 3.4 ± 0.4 | 25.4 ± 1.7 | 43.5–44.5 |
| $\Delta^{12}$ | 8.3 ± 0.6 | 13.3 ± 2.3 | 52.0–53.5 |
| $\Delta^{13}$ | 7.0 ± 0.9 | 22.1 ± 1.2 | 43.5–44.5 |
| $\Delta^{14}$ | 3.9 ± 1.0 | 11.7 ± 1.9 | 53.0–53.5 |
| $\Delta^{15}$ | 4.9 ± 1.2 | 15.9 ± 2.9 | 58.0–59.0 |
| $\Delta^{16}$ | 2.7 ± 0.7 | 10.5 ± 1.3 | 65.5–66.5 |
| $\Delta^{17}$[c] | 3.5 ± 0.8 | 13.1 ± 2.4 | 55.5–56.5 |

[a] The standard reaction mixture was used. The acyl transfer rates are the average of four to five experiments. [b] Standard error of the mean. [c] No configurational isomer for this acid. [d] Gunstone *et al.*, 1967.

In Table IV, the relative rates of the isomeric *trans*-octadecenoyl-CoA esters with 1-acyl-GPC and 2-acyl-GPC as acceptors in the presence of crude microsomes are shown together with the melting points of *trans*-octadecenoic acids (Gunstone *et al.*, 1967). The enzyme preparation showed different acyl transfer activities for the positional isomers. The enzyme(s) also discriminated among the positional isomers in a different manner when the 1 and 2 positions served as acceptors. Generally, trans isomers showed higher rates with 2-acyl-GPC than with 1-acyl isomers.

These features can be seen more clearly in Figures 2 and 3, in which acyl transfer rates observed (using the deoxycholate-treated microsomes with higher specific activity were shown) together with acyl transfer rates for isomeric *cis*-octadecenoyl-CoAs cited from the previous publications (Okuyama *et al.*, 1969; Reitz *et al.*, 1969). For the *trans*-octadecenoyl-CoA isomers, the patterns with 2-acyl-GPC and 2-acyl-GPE are almost completely identical. The pattern

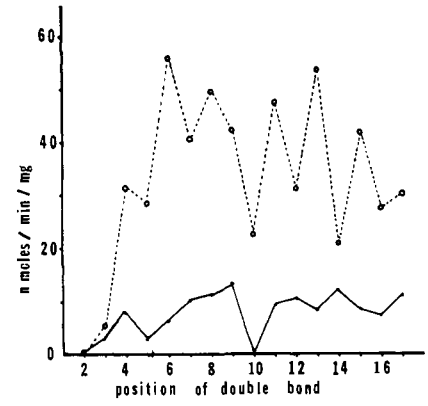


FIGURE 3: Acyltransferase specificities with 2-acyl-GPE or 1-acyl-GPE. The standard reaction system with 0.1 mg of deoxycholate-treated microscomes was used. The rates for 2-acyl-GPE are the average of two separate experiments and those for 1-acyl-GPE are the average of four determinations. (○) Acyl transfer rates at the 1 position; (●) acyl transfer rates at the 2 position.

TABLE V: Effect of *trans*-10- and *trans*-14-Octadecenoate on Acylation by Palmitate and Linoleate.[a]

| 1-Acyl-GPC | | | 2-Acyl-GPC | | |
|---|---|---|---|---|---|
| Mixed Acyl-CoA (nmoles/ml) | | Rates | Mixed Acyl-CoA (nmoles/ml) | | Rates |
| Linoleoyl- | Trans 10 | (nmoles/min per mg) | Palmitoyl | Trans 10 | (nmoles/min per mg) |
| 10 | 0 | 19 | 10 | 0 | 15 |
| 10 | 5 | | 10 | 5 | 17 |
| 10 | 10 | 16 | 10 | 10 | 20 |
| 10 | 15 | 14 | 10 | 15 | 18 |
| 15 | 5 | 14 | 15 | 5 | 17 |
| 0 | 10 | 6 | 0 | 10 | 9 |
| Linoleoyl- | Trans 14 | | Palmitoyl- | Trans 14 | |
| 0 | 10 | 5 | 0 | 10 | 13 |
| 10 | 0 | 19 | 10 | 0 | 15 |
| 10 | 5 | 12 | 10 | 5 | 15 |
| 10 | 10 | 17 | 10 | 10 | 14 |
| 10 | 15 | 17 | 10 | 15 | 12 |
| 15 | 5 | 18 | 15 | 5 | 17 |

[a] The reaction mixture contained 175 nmoles of 1-acyl-GPC or about 175 nmoles of 2-acyl-GPC, 10–15 nmoles of palmitoyl-CoA or 10–15 nmoles of linoleoyl-CoA with varying amounts of *trans*-10- or *trans*-14-octadecenoyl-CoA and 0.2 mg of microsomal protein in a final volume of 1 ml (see Materials and Methods). Values are the average of two separate determinations and have been corrected for hydrolase activity.

for 2-acyl acceptors is, however, completely different from the ones for 1-acyl acceptors. 1-Acyl-GPC and 1-acyl-GPE also showed fairly similar patterns (except $\Delta^5$, $\Delta^9$, and $\Delta^{10}$ isomers) although the relatively lower activity for 1-acyl-GPE makes a detailed comparison difficult.

The *trans*-10- and *trans*-14-octadecenoyl-CoAs showed very low activity compared to those with the double bond at the neighboring positions. A possibility that these preparations contained an inhibitor of acyltransferase reactions was then examined. As shown in Table V, trans 10 and trans 14 isomers only slightly inhibited the acylation of palmitate to 2-acyl-GPC and linoleate to 1-acyl-GPC. Thus, although the data do not exclude the possibility, it seems less probable that the observed low rates with *trans*-10- and *trans*-14-octadecenoate were caused by any inhibitor in the thiol ester preparation.

Both *cis*-9- and *trans*-9-octadecenoyl-CoAs showed higher activity than the other positional isomers in acylating the 2-hydroxyl of 1-acyl acceptors. A question arises as to whether or not the same enzyme catalyzed the transfer of both thiol esters. Four different microsomal preparations were examined to compare the relative acyl transfer rates for these two acyl-CoAs. As shown in Table VI, crude microsomes and three submicrosomal preparations obtained by deoxycholate treatment (Okuyama *et al.*, 1969; Reitz *et al.*, 1969) showed similar ratios of activity. Thus, it is possible that the transfer of cis $\Delta^9$ and trans $\Delta^9$ isomers are catalyzed by the same enzyme although further purification is needed to be certain.

## Discussion

A variety of earlier studies (Lauderbaugh, 1968) with 1-acyl-GPE proved to be vexatious by providing variable results with that particularly insoluble substrate. In the present work, the problem of turbid micellar dispersions due to the limited solubility was partially diminished by using the linoleoyl derivative. However, as work progressed, we found that the 1-acyl-GPE preparations must be prepared soon before use to avoid formation of the other positional isomer. In contrast of the high apparent stability of 1-acyl-GPC preparations, the 1-acyl-GPE underwent a relatively rapid isomerization that had been overlooked in previous work. The control information presented in Table I leads us to regard the current results on acyltransferase selectivity as more valid than any previously described. Further studies on the role of the primary amino group in catalyzing carboxy ester migration or altering the thermodynamic constants for the two types of carboxyl esters should prove useful in interpreting the unexpected instability of the ethanolamine derivative. The results are similar to those described for 1-acyl-GPE by Brandt and Lands (1967) (and also vandenBosch *et al.* (1967) and Barden and Cleland (1969)) in that differences in acyl chain of the acceptor have much less influence on reaction rates than differences in acyl chain of the thiol ester.

The microsomal acyltransferase(s) did not exclude the *trans*-acyl groups from esterification at the 2 position but did transfer most of them more rapidly to the 1 position. In general, the results agree with the experiments showing a greater accumulation *in vivo* of trans acids at the 1 than at the 2 position (Lands *et al.*, 1966; Raulin *et al.*, 1963). These selectivities probably provide the basis of Sinclair's (1935) observation that elaidate was incorporated at the expense of saturated rather than of oleic acid.

A variety of consequences may be considered as a result of trans acids replacing either the typically saturated acids at the 1 position or the typically unsaturated acids at the 2 position of phospholipids (*e.g.*, Chapman *et al.*, 1966). The quantities (Mabrouk and Brown, 1956) and specific positional isomers (Scholfield *et al.*, 1967) of *trans*-octadecenoates that may be expected in typical American diets have already been described. An undesirable metabolic and biochemical consequence could result either from certain trans acids inhibiting a needed biosynthetic process or by the various product lipids with trans configuration having inadequate functional abilities regardless of the prior biosynthetic selectivities. The present series of experiments was principally designed to examine whether or not the different types of acyltransferases which catalyze biosynthesis were capable of discriminating among configurational differences at every location along the acyl chain. We found that the synthetic system is certainly sensitive to the occurrence and location of trans ethylenic bonds. The observed low reactivity of the *trans*-10-octadecenoyl-CoA takes on greater significance when compared to the dominant abundance of that isomer in modified dietary oils (Scholfield *et al.*, 1967). If acyl transfer rates are useful indicators of physiological disposition, that isomer might be expected to be less effectively incorporated into membrane lipids than the less prevalent trans 9 isomer which, in turn, might be incorporated more efficiently than the trans 8 and 11 isomers.

Earlier studies had shown that the rates of acylation for *cis*-monoenoic acid at the 1 position may be partially correlated with alternating changes in the melting points for these acids (Reitz *et al.*, 1969). However, the pattern of acyl-

TABLE VI: Comparison of Acyl Transfer Rates of *cis*-9- and *trans*-9-Octadecenoyl-CoA and Linoleoyl-CoA with Four Microsomal Preparations from Rat Liver.[a]

| | Acyl Transfer Rate (nmoles/min per mg of Protein) | | | | | |
|---|---|---|---|---|---|---|
| | 1-Acyl-GPC | | | 2-Acyl-GPC | | |
| | Cis $\Delta^9$ | Trans $\Delta^9$ | Cis $\Delta^{9,12}$ | Cis $\Delta^9$ | Trans $\Delta^9$ | Cis $\Delta^{9,12}$ |
| Crude microsomes | 23.4 (1.0) | 19.7 (1.0) | 39.9 (1.0) | 15.4 (1.0) | 35.3 (1.0) | 6.8 (1.0) |
| EI | 24.1 (1.0) | 18.5 (0.9) | 41.3 (1.0) | 14.2 (0.9) | 26.2 (0.7) | 4.2 (0.6) |
| EII | 62.1 (2.7) | 99.8 (2.5) | 99.8 (2.5) | 20.0 (1.3) | 46.2 (1.3) | 11.0 (1.6) |
| EIII | 61.1 (2.6) | 42.9 (2.2) | 112.4 (2.8) | 32.1 (2.1) | 76.0 (2.2) | 11.2 (1.7) |

[a] The standard incubation mixture was used with 0.2 mg of crude microsomal protein, 0.2 mg of protein from pellet (EI), 0.2 mg of protein from 0.5 M sucrose fraction (EII) or 0.1 mg of protein from 0.25 M sucrose fraction (EIII) (see Okuyama *et al.*, 1969). The values are the average of two separate determinations and have been corrected for hydrolase activity. Relative specific activities are shown in parentheses.

transfer rates for *trans*-monoenoic acids at this position can not be correlated with melting points at all. Presumably the melting point, *per se*, is not the significant physical quality that regulates enzymic action since it is a property of the free acid in a bulk phase rather than of discrete solvated substrate chains attached to coenzyme A. Nevertheless, factors influencing the strength of acyl chain interactions, such as cis and trans configurations might be expected to have similar effects on interactions both in the bulk phase and between lipid substrate and enzymic protein.

The very close agreement in patterns of reactivities for *cis*-alkenoic acids and the corresponding *cis*-cyclopropane acids (Okuyama *et al.*, 1969) suggested that the acyltransferase activity for the 1 position was in fact responding to the configuration of the acyl chain. The very pronounced lack of agreement between transfer rates for the cis and trans ethylene derivatives shown in Figure 2A also suggests that the synthetic activity that is possibly responsible for placing saturated acids at the 1 position, is very sensitive to configurational differences along the acyl chain. An unexpected feature of this selectivity is that the alternating pattern noted for cis derivatives seems shifted upward by one carbon atom for the trans. Thus, 8, 10, and 12 isomers are the favored cis ethylenic derivatives whereas the 8, 9, 11, and 13 trans isomers are rapidly esterified. The apparent frame shift at C-8 for effective enzymic interaction suggests that configurational features in addition to those influencing melting points must be perceived by the catalytic protein acting at the primary hydroxyl.

An additional concept to be tested by results with the trans series is that the acyltransferase activity for the 2-hydroxyl responds in a much different manner to the substrate acyl chain than the activity described above.

The catalyst(s) acting at the 2 position has been hypothesized to respond to the presence of $\pi$ bonds rather than to their configuration (Lands, 1965; Jezyk and Lands, 1968). This concept was supported by studies of cyclopropane derivatives (Okuyama *et al.*, 1969). The results in Figure 2B give further support to this concept in that the 5, 9, and 12-positional isomers, locations favored among the cis ethylene series, are also found optimal in the trans ethylene series of substrates. The similar results with positional isomers of both cis and trans acids are in marked contrast to those found for esterification at the primary hydroxyl. Experiments with cellular (Åkesson *et al.*, 1970; Kanoh, 1969) and subcellular systems (Waku and Lands, 1968; Hill and Lands, 1968) indicate that the acyl-CoA:phospholipid acyltransferases may play an important role in placing unsaturated acids at the 2 position of phospholipids. The present results suggest that for those enzymes, the $\pi$ bonds at the 9 position as well as those associated with essential fatty acids, that is the 12 (or $(n - 6)$) position and the 5 (which occurs in arachidonate) could play some role in the esterification of those favored substrates. No evidence is available at present as to what kind of structure in the enzyme could detect the $\pi$ bond of the acyl chain irrespective of its configuration.

Acknowledgments

The authors thank Mrs. Carol A. Pletcher for assistance in enzymatic assays.

References

Åkesson, B., Elovson, J., and Arvidson, G. A. E. (1970), *Biochim. Biophys. Acta 210*, 15.
Barden, R. E., and Cleland, W. W. (1969), *J. Biol. Chem. 244*, 3677.
Barve, J. A., and Gunstone, F. D. (1971), *Chem. Phys. Lipids 7*, 311.
Barve, J. A., Gunstone, F. D., Jacobsbey, F. R., and Winlow, P. (1972), *Chem. Phys. Lipids 8*, 117.
Bertram, S. H. (1928), *Biochem. Z. 197*, 433.
Brandt, A. E., and Lands, W. E. M. (1967), *Biochim. Biophys. Acta 144*, 605.
Chapman, D., Owens, N. F., and Walker, D. A. (1966), *Biochim. Biophys. Acta 120*, 148.
Coots, R. H. (1964), *J. Lipid Res. 5*, 468.
Davidson, F. M., and Long, C. (1958), *Biochem. J. 69*, 458.
Eibl, H., and Lands, W. E. M. (1969), *Anal. Biochem. 30*, 51.
Eibl, H., and Lands, W. E. M. (1970), *Biochemistry 9*, 423.
Ellman, G. L. (1959), *Arch. Biochem. Biophys. 82*, 70.
Gunstone, F. D., and Ismail, I. A. (1967), *Chem. Phys. Lipids 1*, 264.
Gunstone, F. D., Ismail, I. A., and Lie Ken Jie, M. (1967), *Chem. Phys. Lipids 1*, 376.
Haverkate, F., and Van Deenen, L. L. M. (1965), *Biochim. Biophys Acta 106*, 78.
Hill, E. E., and Lands, W. E. M. (1968), *Biochim. Biophys. Acta 152*, 645.

Jezyk, P., and Lands, W. E. M. (1968), *J. Lipids Res. 9*, 525.
Kanoh, H. (1969), *Biochim. Biophys. Acta 176*, 756.
Lands, W. E. M. (1965), *J. Amer. Oil Chem. Soc. 42*, 465.
Lands, W. E. M., Blank, M. L., Nutter, L. J., and Privett, O. (1966), *Lipids 1*, 224.
Lands, W. E. M., and Hart, P. (1965), *J. Biol. Chem. 240*, 1905.
Lands, W. E. M., and Merkl, I. (1963), *J. Biol. Chem. 238*, 898.
Lauderbaugh, T. (1968), Ph.D. Thesis, The University of Michigan.
Mabrouk, A. F., and Brown, J. B. (1956), *J. Amer. Oil Chem. Soc. 33*, 98.
Okuyama, H., Eibl, H., and Lands, W. E. M. (1971), *Biochim. Biophys. Acta 248*, 263.
Okuyama, H., and Lands, W. E. M. (1972), *J. Biol. Chem. 247*, 1414.
Okuyama, H., Lands, W. E. M., Christie, W. W., and Gunstone, F. D. (1969), *J. Biol. Chem. 244*, 6514.
Ono, K., and Fredrickson, D. S. (1964), *J. Biol. Chem. 239*, 2482.
Raulin, J., Lapous, D., Dauvillier, P., and Rérat, A. (1965), *C. R. Acad. Sci., Ser. A 260*, 344.
Raulin, J., Loriette, C. L., and Clément, G. (1963), *Biochim. Biophys. Acta 70*, 642.
Reitz, R. C., El-Sheikh, M., Lands, W. E. M., Ismail, I. A., and Gunstone, F. D. (1969), *Biochim. Biophys. Acta 176*, 480.
Scholfield, C. R., Davison, V. L., and Dutton, H. J. (1967), *J. Amer. Oil Chem. Soc. 44*, 648.
Selinger, Z., and Holman, R. T. (1965), *Biochim. Biophys. Acta 106*, 56.
Shorland, F. B., Weenink, R. O., and Johns, A. T. (1955), *Nature (London) 175*, 1129.
Sinclair, R. G. (1935), *J. Biol. Chem. 111*, 515.
vandenBosch, H., vanGolde, L. M. G., Eibl, H. and van Deenen, L. L. M. (1967), *Biochim. Biophys. Acta 144*, 613.
Waku, K., and Lands, W. E. M. (1968), *J. Lipid Res. 9*, 12.
Yang, S. F., Freer, S., and Benson, A. A. (1967), *J. Biol. Chem. 242*, 477.

# Structure and Biosynthesis of Diesters of Alkane-2,3-diols of the Uropygial Glands of Ring-Necked Pheasants†

Wajih N. Sawaya‡ and P. E. Kolattukudy*

ABSTRACT: The structure of the major components of the lipids excreted by the uropygial (preen) glands of chicken, quail, and ring-necked pheasants were determined by thin-layer chromatography and a combination of gas–liquid chromatography and mass spectrometry. Diacylalkane-2,3-diols constituted more than 90% of the lipids in all cases. In the pheasant $C_{18}$ diol was the major (85%) component whereas $C_{22}$, $C_{23}$, and $C_{24}$ diols predominated in chicken and quail. [1-$^{14}$C]Acetate injected under the gland was readily incorporated into preen oil in 24- to 72-hr experimental periods. The major part of the label was in the diol diesters and both acyl and diol portions were nearly equally labeled. The distribution of label among the $C_{10}$–$C_{20}$ acyl moieties and $C_{16}$–$C_{20}$ alkanediols, together with measurement of $^{14}$C located in C-3 position of the individual diols, indicated *de novo* synthesis of carbon chains from acetate. Injection of [1-$^{14}$C]-palmitic acid under the gland gave rise to labeled diol diesters in which the acyl moieties contained 1.5 times as much $^{14}$C as that in the diol portion. Distribution of the label among the diols and measurement of the $^{14}$C located at C-3 of individual diols indicated that palmitic acid was incorporated intact into $C_{18}$ diol and that the carboxyl carbon of the $C_{16}$ acid became C-3 of the $C_{18}$ diol. The data also indicated that exogenous $C_{16}$ acid was elongated to $C_{18}$ acid and subsequently incorporated into $C_{20}$ diol. The major labeled product in the acyl portion was $C_{18}$ acid, again suggesting elongation. Exogenous [1-$^{14}$C]stearic acid also labeled diol diesters but the acyl moiety was nearly four times as radioactive as the diol portion and incorporation into diol was much less than that observed with [1-$^{14}$C]palmitic acid. However, the major part of the label derived from the labeled $C_{18}$ acid was found in $C_{20}$ diol as expected from a condensation of the $C_{18}$ with a $C_2$ unit. These results are consistent with the hypothesis that alkane-2,3-diols are synthesized by reduction of the acyloin formed by the condensation of a fatty aldehyde with active acetaldehyde. However, attempts to specifically label C-1 and C-2 of the diol with exogenous [2-$^{14}$C]pyruvate, [U-$^{14}$C]alanine, and [2-$^{14}$C]lactate failed probably because these substrates were readily incorporated into alkane chain of the diols *via* acetate.

A wide variety of alkane diols occur in microorganisms, animals and plants. Three major types are alkane-$\alpha,\omega$-diols (Murray and Schoenfield, 1955; Mazliak, 1962), alkane-1,2-diols (Horn and Hougen, 1953; Fieser *et al.*, 1957; Downing *et al.*, 1961; Karkkainen *et al.*, 1965; Nicolaides, 1965) and alkane-2,3-diols (Haahti and Fales, 1967; Hansen *et al.*, 1969; Saito and Gamo, 1970). No biosynthetic studies on these diols have been reported. However, the first two types are structurally closely related to $\omega$-hydroxy acids and $\alpha$-hydroxy acids, respectively, and were therefore suggested as arising by reduction of the corresponding acids (Kolattukudy, 1970a). On the other hand the mechanism of synthesis of the 2,3-diols is more intriguing because unlike the other diols no

† From the Department of Agricultural Chemistry, Washington State University, Pullman, Washington. *Received June 8, 1972.* This is scientific paper no. 3884, Project 4001, College of Agriculture Research Center. This work was supported in part by Grant GM-18278 from U. S. Public Health Service.

‡ Recipient of a scholarship from Lebanese National Council for Scientific Research.