Specificity of acyl-CoA:phospholipid acyltransferases: solvent and temperature effects

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ABSTRACT Acyltransfer from CoA thiol esters to either the 1- or 2-position of monoacylglycerophosphoryl choline, which is catalyzed by a microsomal preparation from rat liver, had a temperature optimum of 30–35°C. No significant alteration was observed in the ability of the acyltransferases to distinguish among the various thiol esters tested in the range of 15–40°C.

Acyl-CoA:1-acylglycerophosphoryl choline acyltransferase activity is inhibited by urea, *N*-alkyl ureas, and short-chain alcohols. The effect is not equal for all acyl derivatives, and ethylene glycol has much less inhibitory effect on the transfer of acids with an $n - 6$ ($\omega 6$) double bond. On the other hand, this inhibition of acyltransfer was relatively insensitive to the configuration of the Δ^9-double bond of octadecadienoates.

The specificity of the enzyme-catalyzed transfer of different acids to the 2-position can be correlated in part with the dissociation constants for the urea clathrate complexes.

Added glycol does not appreciably alter the specificity of enzyme-catalyzed transfer to the 1-position, but it inhibits the transfer of all acids in a similar fashion.

KEY WORDS acyl-CoA:monoacylglycerophosphoryl choline acyltransferase · acyltransferase selectivity · 1- and 2-acylglycerophosphenyl cholines · solvent effects · temperature optimum · essential fatty acids · urea complexes

Liver acyl-CoA:acyl-GPC acyltransferases from a number of animals exhibit specificities which suggest that they could play a significant role in controlling the distribution of fatty acids in the lecithin fraction from this organ (2, 3). Acylation of the secondary hydroxyl at C-2 occurs mainly with unsaturated acyl-CoA derivatives, whereas acylation at the primary hydroxyl (C-1) is more rapid with saturated or *trans*-ethylenic fatty acyl-CoA's. The enzyme acting at the 2-position catalyzes esterification of Δ^9-*cis* and Δ^9-*trans* derivatives at similar rates, but both Δ^9-compounds react much more rapidly than the corresponding saturated derivative (3, 4). On this evidence the enzyme seemed able to detect the presence of the ethylenic bond in the fatty acid without being particularly influenced by its configuration. On the other hand, acylation of the 1-position was particularly slow with acids containing a 9-*cis*-ethylenic bond and rapid with the *trans* isomers. The purpose of the present study was to gain additional information about the properties of the acyltransferases that are related to such selectivities. We have altered several conditions in the reaction mixtures to determine what effect they have on the ability of the enzyme to respond to structural differences in the substrates.

EXPERIMENTAL METHODS

The isomeric 1- and 2-acylglycerophosphoryl choline (acyl-GPC) substrates were prepared by selective hydrolysis of diacyl-GPC (egg lecithin) and alkenyl acyl-GPC (choline plasmalogen) from bovine heart (4). Fatty acids obtained from The Hormel Institute with the aid of Dr. O. Privett were >99% pure. Acyl-CoA esters were synthesized as previously described (3). For acids with two or more double bonds, the CoA esters were

A preliminary report of these results was presented at the Seventh International Congress of Biochemistry, Tokyo (1).

Abbreviations: GPC, *sn*-glycero-3-phosphoryl choline; DTNB, 5,5′-dithiobis(2-nitrobenzoate). The isomeric 9,12-octadecadienoates are designated *cc* for *cis*-9,*cis*-12; *ct* for *cis*-9,*trans*-12; *tc* for *trans*-9,*cis*-12; and *tt* for *trans*-9,*trans*-12. Fatty acids are designated by chain length:number of double bonds; in some graphs, *c* and *t* refer to *cis* and *trans* double bonds.

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prepared and stored in the presence of the antioxidant 1,2-dihydro-6-ethoxy-2,2,4-trimethyl-quinoline (Santoquin). All solvents and chemicals used were of reagent grade and were obtained from commercial sources, except for the alkyl ureas, which had been recrystallized and were a gift of Dr. G. Zografi. Deionized water was used throughout.

The enzyme preparations employed consisted of the 30,000–100,000 *g* pellet from liver homogenate prepared and centrifuged in 0.25 M sucrose. The rat liver enzyme preparation was treated with 10^{-3} M diisopropyl fluorophosphate at room temperature for 1 hr prior to use to inhibit the acyl-CoA hydrolase activity (2). The preparations obtained from pig liver had negligible hydrolase activity.

Acyl-CoA:acyl-GPC acyltransferase rates were determined by measurement of the sulfhydryl group in the liberated CoA with 5,5′-dithiobis(2-nitrobenzoate) (DTNB) (2). The reaction was measured in a Gilford model 2000 recording spectrophotometer so that all reported values for velocities result from a continual series of observations of the amount of product formed during the reactions. The assay mixture normally contained 1 mmole of DTNB, 100–175 mμmoles of acyl-GPC in 0.1 ml of distilled water, 4–25 μl of the enzyme preparation (0.18 mg of protein), 5–15 μl of acyl CoA (25 mμmoles), and 0.8 ml of 0.1 M Tris–chloride buffer, pH 7.4. When other additions to the system were made, the amount of buffer was diminished (but never to less than 0.1 ml) to maintain a constant volume. For experiments requiring specific temperatures, the temperature in the cuvette holder of the spectrophotometer was controlled by circulating water from an external constant-temperature bath through blocks around the sample changer. Under the usual assay conditions, when the temperature was not specifically controlled, the temperature in the cuvettes was 24–26°C.

RESULTS

Acyl-CoA : 1-acyl-GPC Acyltransferase

Temperature Effects. Acyltransferase rates for the *cis*-9,*cis*-12, the *trans*-9,*cis*-12, and the *trans*-9,*trans*-12 isomers of octadecadienoic acid and for stearic acid were measured with the rat liver enzyme at several temperatures from 13 to 43°C.

Rates with the different geometric isomers of 18:2 were similar at the lower temperatures, but became quite different as the temperature was increased; all reached a maximum value between 30 and 35°C (Fig. 1). Activity with the saturated thiol ester was always lower than that with any of the 18:2 isomers and all rates fell sharply at temperatures above 35°C. The increase in velocity with temperature was greatest with the *cc* isomer: about a 6-fold change from 13 to 33°C. The rate with *tc* was increased about 4-fold over the same range, while those with the *tt* and 18:0 thiol esters were only doubled at their maxima.

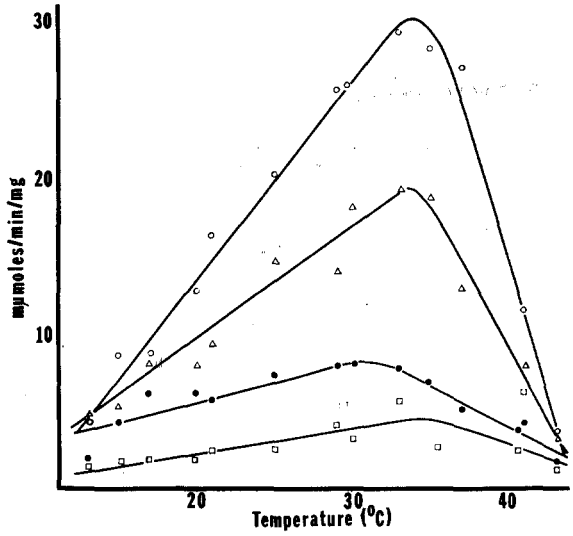

FIG. 1. Effect of temperature on acyl-CoA:1-acyl-GPC acyltransferase. ○, *cc*; △, *tc*; ●, *tt*; □, 18:0. Each value represents a separate assay in which the formation of product was continually recorded and is thus the result of a large number of observations. A preparation of rat liver microsomes was used for these measurements.

Effect of Altering Solvent Properties. The effects of altering the solvent of the reaction mixture were examined for several organic substances. The substance to be tested replaced a portion of the Tris–chloride buffer in the cuvette, so that the volume of the system was kept constant. A preliminary experiment showed that changes in the strength of the buffer would not significantly affect the transferase rates: alterations over the proposed experimental range (0.008–0.08 M) yielded variations of less than 5% in the reaction rate with the *cc* thiol ester when the pig liver enzyme was used.

The effects of several organic solvents on the transferase rate with *cc* were then determined (Table 1). The degree of inhibition increased with hydrocarbon chain-length for the series of *n*-alkanols tested at 5% concentration, *n*-butanol being completely inhibitory. The presence of hydroxyl groups on a molecule diminished or abolished this effect, whereas the presence of a chloro group apparently increased the inhibition. Conversion of the hydroxyl groups to methyl ethers also led to an increased inhibitory effect.

Inhibition of the transferase by 50% ethylene glycol could be completely or partially reversed by various degrees of dilution (Table 2). These reversal experiments were done over a short period of time (approximately 30 min) and the effects of prolonged treatment at room temperature were not investigated. However, when the

TABLE 1 EFFECT OF VARIOUS ORGANIC SOLVENTS ON LINOLEOYL-CoA:1-ACYL-GPC ACYLTRANSFERASE RATES

Addition	Rate*
	mμmoles/min/mg
None	17.3
5% Methanol	18.4
5% Ethanol	16.5
5% Propanol	1.4
5% *n*-Butanol	0
10% Ethylene glycol	17.1
10% Glycerol	17.6
10% Propane-1,2-diol	14.6
10% Isopropylidene glycerol	5.2
10% 3-Chloropropane-1,2-diol	1.3
10% Ethylene glycol monomethyl ether	0.5
10% Ethylene glycol dimethyl ether	0
10% Dioxane	8.1

* Average of two separate assays with pig liver microsomes.

TABLE 2 REVERSAL OF ETHYLENE GLYCOL INHIBITION OF LINOLEOYL-CoA:1-ACYL-GPC ACYLTRANSFERASE BY DILUTION

Dilution	Rate
	mμmoles/min/mg
Control (no glycol)	16.4
50% ethylene glycol	3.2
Diluted from 50% glycol to a final concn of 1%	16.9
Diluted from 50% glycol to a final concn of 20%	13.1
Diluted from 50% glycol to a final concn of 30%	9.7
Diluted from 50% glycol to a final concn of 40%	5.7

The enzyme was prepared from pig liver.

enzyme was stored at −10°C overnight in 50% ethylene glycol and subsequently diluted, no reversal of inhibition was obtained.

The effect of added ethylene glycol on the system was then tested, with the four geometric isomers of 9,12-octadecadienoyl-CoA as substrates. The pig liver enzyme, which was used throughout the remainder of the reported studies unless otherwise noted, does not discriminate as drastically between these acids under normal assay conditions as the rate liver enzyme (2, 3). However, the addition of ethylene glycol to the system altered the esterification rates with these isomers (Fig. 2). Rates with the *ct* and *tt* acids fell rapidly with increasing concentration of ethylene glycol, reaching about 20% of the control rates at 30% ethylene glycol. On the other hand, the rates with the *cc* and *tc* acids remained at about 80% of the control value up to 30% glycol. When the behavior of other unsaturated acids in the presence of ethylene glycol was examined, two types were distinguished (Fig. 3): those (20:4, 18:3) responding like the *cc* and *tc* acids, and those (*cis*-Δ⁹-16:1, *cis*-Δ⁹-18:1, and *trans*-Δ⁹-18:1) responding in the same manner as the *ct* and *tt* acids.

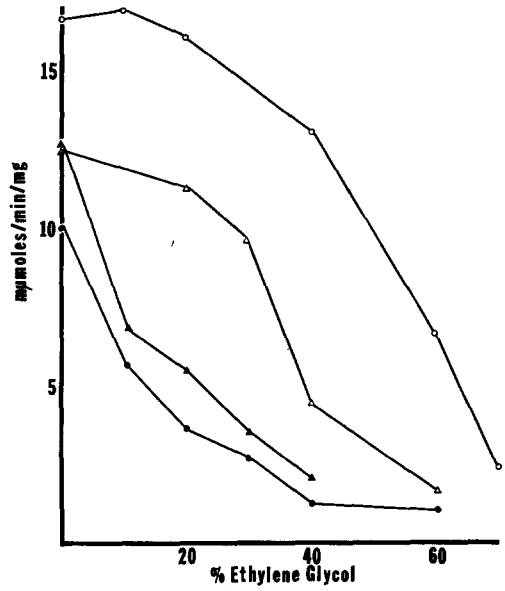

FIG. 2. Effect of ethylene glycol on the acyltransfer of geometric isomers of octadecadienoate by acyl-CoA:1-acyl-GPC acyltransferase from pig liver. ○, *cc*; △, *tc*; ▲, *ct*; ●, *tt*. Each value is the average of two to four separate assays.

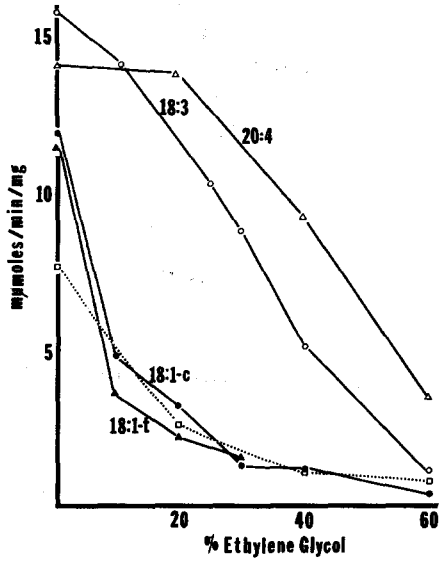

FIG. 3. Effect of ethylene glycol on the acyltransfer of several acids by acyl-CoA:1-acyl-GPC acyltransferase. 18:1-*c* and 18:1-*t* refer to *cis* and *trans*-Δ⁹-octadecenoate, respectively. The dotted line indicated values for palmitoleate, *cis*-Δ⁹-16:1. Each value is the result of two to three separate assays with pig liver microsomes.

In a similar experiment, increasing amounts of methanol rather than ethylene glycol were added (Fig. 4). The lowered transferase activities with the *ct* and *tt* acids were similar to those observed with ethylene glycol but occurred at somewhat lower concentration of added alcohol. On the other hand, with 10% methanol the reaction rate with the *cc* acid was increased to about

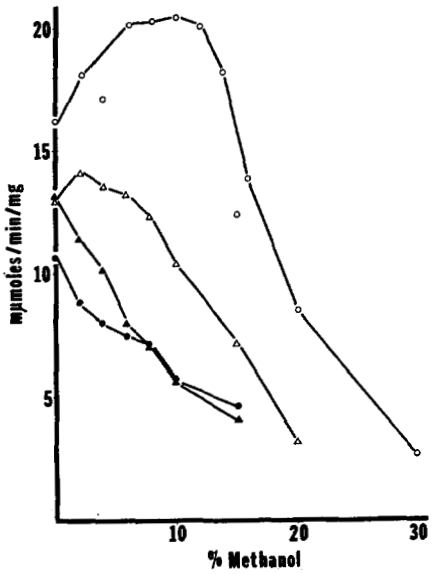

FIG. 4. Effect of methanol on the reaction of 18:2 isomers catalyzed by acyl-CoA:1-acyl-GPC acyltransferase from pig liver. ○, *cc*; △, *tc*; ▲, *ct*; ●, *tt*.

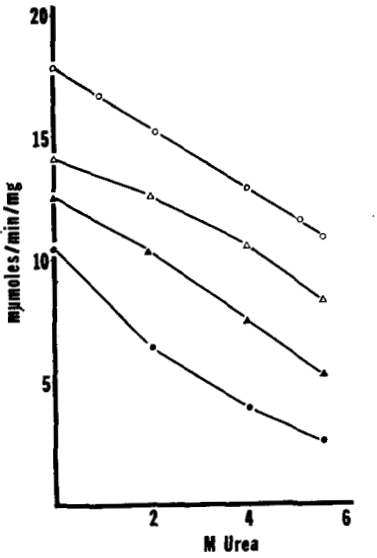

FIG. 5. Inhibition by added urea of the acyltransfer of geometric isomers of 18:2 by acyl-GPC acyltransferase from pig liver. ○, *cc*; △, *tc*; ▲, *ct*; ●, *tt*.

125% that of the control. Above this concentration of alcohol, the degree of inhibition increased more rapidly with increasing concentration of alcohol than with the glycol. For the *tc* acid, the rate also increased slightly and then fell rapidly with increasing levels of alcohol.

Additional studies showed that inhibition at a given concentration was also greater with ethanol than with ethylene glycol, for both the *cc* and *tt* acids. With the *tt* acid the rate of the inhibition was greatest in the range of of 0 to 5% ethanol, whereas the reaction with the *cc* isomer was more rapidly inhibited as the concentration of ethanol increased from 7 to 15%.

The acyltransferase activity was also inhibited by the addition of urea (Fig. 5). In this case, the inhibition was similar for all isomers tested. For the *cc* acid, alkylated derivatives of urea yielded results analogous to those found with the *n*-alkanols (Table 3). With 1 M solutions, both urea and methylurea gave less than 10% inhibition whereas ethylurea produced about 50% inhibition and *n*-butylurea, complete inhibition. For both the *cc* and *tt* acids, ethylurea caused 100% inhibition at a concentration of 3 M.

TABLE 3 INHIBITION OF LINOLEOYL-COA:1-ACYL-GPC ACYLTRANSFERASE BY ADDED UREA AND ALKYLATED UREAS

Addition	Rate
	mμmoles/min/mg
None	17.7
1 M Urea	17.3
1 M Methylurea	16.8
1 M Ethylurea	9.7
1 M Butylurea	0

The enzyme was prepared from pig liver.

Influence of Position and Configuration of the Ethylenic Bond. Acyltransferase rates were determined for a series of acids differing in the position and configuration of a single ethylenic bond (Table 4). The naturally-occurring *cis*-Δ^9-18:1 acid was the most active substrate of those tested, but the rate was only slightly slower with the *trans* isomer of this acid. With *cis*-Δ^{11}-18:1 acid, the rate was about one-half that of the Δ^9-acid; the *trans* isomer was 15% slower than the *cis* in this case. A *cis* double bond at the Δ^6-position increased the rate over that of the saturated C_{18} acid very little compared to the higher rates seen for other 18:1 acids. Palmitoleic acid (*cis*-Δ^9-16:1) was a better substrate than the unnatural 18:1 isomers tested, but not as good as the Δ^9-18:1 acids.

Acyl-CoA:2-acyl-GPC Acyltransferase

Temperature Effects. Acyltransferase rates with the four 18:2 isomers were determined at temperatures from 15 to 39°C (Fig. 6). The rate with the *tt* acid was the most rapid at all temperatures, reaching a maximum near 30°C. The *tc* acid showed a similar response, but the maximum rate with this isomer was only two-thirds that with the *tt* acid. Rates with the *cc* and *ct* acids were similar to each other over the entire temperature range and showed maxima that were only about one-fifth of that with *tt*.

Effect of Ethylene Glycol. Enzymatic activity with several acids was inhibited by addition of ethylene glycol (Fig. 7). However, there were no marked differences in the rates of inhibition similar to those found with the

TABLE 4 EFFECT OF POSITION AND CONFIGURATION OF DOUBLE BOND ON ESTERIFICATION RATE

Fatty Acid	Rate
	mμmoles/min/mg
18:0	2.5
cis-Δ^6-18:1	3.3
cis-Δ^9-18:1	12.0
trans-Δ^9-18:1	11.8
cis-Δ^{11}-18:1	6.6
trans-Δ^{11}-18:1	5.5
cis-Δ^9-16:1	8.0

The values are averages of two to six separate assays with pig liver microsomes.

enzyme acylating the 2-position. One phenomenon was similar, in that inhibition from 40 to 60% ethylene glycol was greater than that from 0 to 40% with those acids having the more rapid rates in the absence of glycol.

Influence of Chain Length and Configuration of the Acyl Group. The enzyme exhibited a marked specificity for chain length and configuration (Fig. 8). With saturated C_{12}–C_{20} acids, the maximum rate occurred with palmitic acid. Addition of *trans* double bonds to the C_{18} acyl group also caused an increase in the rate of reaction, *tt* reacting even more rapidly than 16:0. Addition of *cis* double bonds to the C_{18} chain however, caused a drastic decrease in the reaction rate.

Comparison of Transferases from Different Animals

The behavior of the acyl-CoA:1-acyl-GPC acyltransferases from different animals were compared to determine whether the results with the pig liver enzyme could be applied to the liver enzyme from other animals. The rates with several acids for the pig liver enzyme and for the enzyme from the liver of a young caiman (presumed

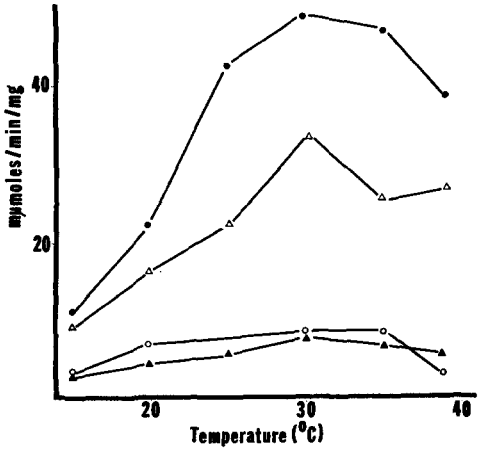

FIG. 6. Effect of temperature on activity of acyl-CoA:2-acyl-GPC acyltransferase from pig liver. ○, *cc;* △, *tc;* ▲, *ct;* ●, *tt.*

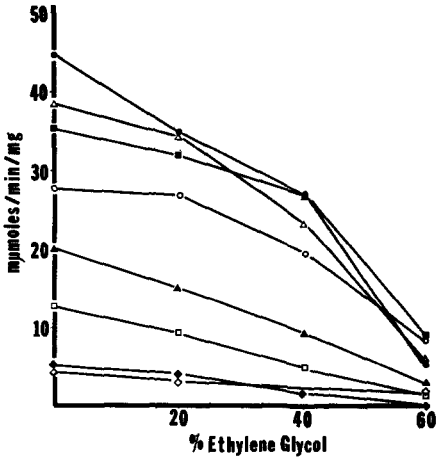

FIG. 7. Effect of ethylene glycol on activity of acyl-CoA:2-acyl-GPC acyltransferase from pig liver. ●, *tt;* △, 16:0; ■, *trans*-Δ^9-18:1; ○, 18:0; ▲, *tc;* □, 12:0; ◆, *ct;* ◇, *cis*-Δ^9-18:1.

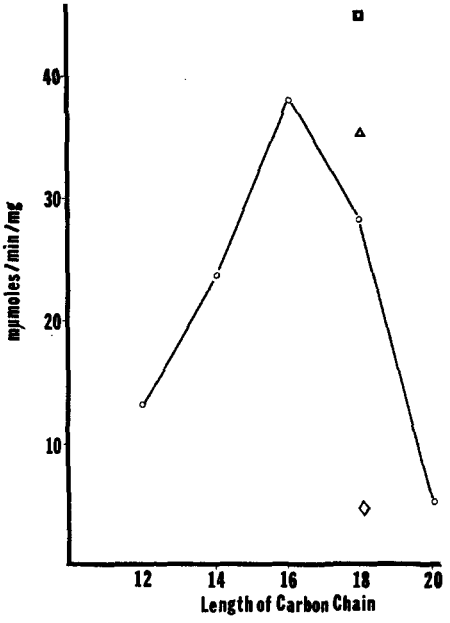

FIG. 8. Effect of acyl chain length and configuration on activity of acyl-CoA:2-acyl-GPC acyltransferase from pig liver. ○, saturated acids; △, *trans*-Δ^9-18:1; □, *tt;* ◇, *cc.*

to be a *Caiman sclerops*),[1] as well as published values for the rat liver enzyme, are shown in Table 5. Velocities with the caiman liver enzyme were generally slightly higher than those with the pig enzyme, but the relationships among the rates were similar for the two enzymes. Interestingly, 20:3 (homo-γ-linolenic acid), normally a minor constituent of the liver phospholipid acids, gave the highest rate with both enzymes. The *cc* and 18:3 yielded the next highest rates, nearly identical in each case, but only slightly higher than with 20:4. On the

[1] The caiman was generously provided by Mr. A. Brandt.

TABLE 5 COMPARISON OF LIVER ACYL-CoA:1-ACYL-GPC ACYLTRANSFERASE ACTIVITIES FROM DIFFERENT ANIMALS

Fatty Acid	Caiman	Pig	Rat*
		mμmoles/min/mg	
12:0	6.7	9.2	6.5
14:0	6.3	5.5	4.3
16:0	6.2	5.1	3.5
cis-16:1	10.8	8.0	6.0
18:0	4.2	2.5	1.7
cis-Δ^6-18:1	4.3	3.3	—
cis-Δ^9-18:1	13.0	12.0	12.4
trans-Δ^9-18:1	—	11.8	10.3
trans-Δ^{11}-18:1	10.8	6.6	—
18:2*cc*	20.5	16.6	23.1
18:2*ct*	17.6	12.6	9.1
18:2*tc*	16.2	12.4	13.7
18:2*tt*	13.5	10.2	6.1
18:3	20.6	15.3	14.6
20:3	33.7	28.0	—
20:4	17.7	14.0	19.6

* Values from reference 3.

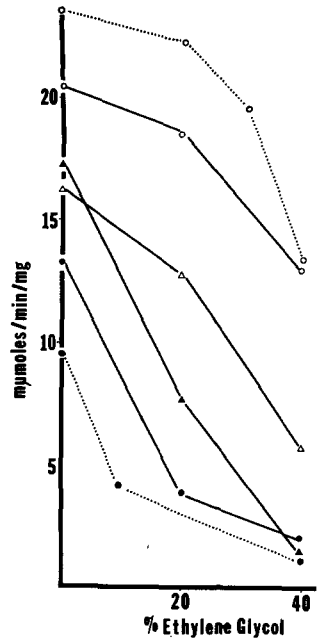

FIG. 9. Effect of ethylene glycol on acylation of the isomers of octadecadienoate by acyl-CoA:1-acyl-GPC acyltransferases. Solid lines indicate results with caiman liver enzyme and dotted lines those with rat liver enzymes. ○, *cc*; △, *tc*; ▲, *ct*; ●, *tt*.

other hand, the rat liver enzyme transferred 20:4 more rapidly than 18:3 and both rates were lower than with *cc*. Both the pig and caiman enzymes esterified the four 18:2 isomers at different rates, but the poorest substrate of the four, the *tt* acid, went 60–70% as fast as the best substrate, the *cc* acid. The rat liver enzyme has been found to be more selective, *tt* reacting only 25% as rapidly as *cc*. With all of the enzyme preparations, the rate of acylation of 1-acyl-GPC by saturated acids increased with decreasing chain length.

When the rates of reaction for the 18:2 isomers in the presence of ethylene glycol were determined with the caiman and rat liver enzymes, the behavior was found to be similar to that with the pig liver enzyme (Fig. 9). That is, acylation with the *cc* and *tc* acids was inhibited to a lesser degree at a given concentration than with the *ct* and *tt* acids.

DISCUSSION

The long-chain saturated acids and the higher-melting *trans* unsaturated derivatives are rapidly esterified by microsomal acyltransferase(s) to the primary position of glycerolipids (3, 4). This observation suggested that these high-melting derivatives might be "recognized" by the enzyme through some physical property related to the melting point or the dispersion forces between hydrocarbon chains. The physical properties studied in the bulk phase have shown differences in molecular interactions for derivatives of the different acids (5, 6) that might be related to the physiological properties of such derivatives (7, 8). The possibility existed that the enzymatic discrimination between substrates containing high- and low-melting acids would be minimized at high temperatures and enhanced at lower temperatures. In our experiments, however, change of temperature over the range of 15–40°C caused no significant alteration in the ability of the acyltransferases to distinguish between the various thiol esters. Thus, at all temperatures tested, the *trans,trans*-dienoate was rapidly transferred to the 1-position and slowly transferred to the 2-position. An important consideration in this study is that the acyltransferase reaction may occur with highly dispersed or solvated substrates rather than micellar or "bulk phase" materials. In such a case, the interactions of two hydrocarbon chains may not be as significant as the interactions of a chain with its surrounding layer of solvent. Some of the differences observed in the rates of esterification could be due to different stabilities of the solvent layer that must be removed to permit reaction.

We considered the possibility that the enzyme acting at the 2-position could distinguish the existence of a 9-ethylenic bond by interacting with the π-bonds irrespective of geometric configuration. The nature of the solvent could then considerably influence this interaction in a manner similar to that described for charge-transfer complexes (9). This effect would then be more closely related to the solvent–solute interaction parameter, Z, than the more commonly used property of the bulk solvent, the dielectric constant. As the Z value of the solvent decreased, there might be an increase in relative reactivity of those acids containing π-bonds at position 9. The studies with added methanol and ethylene glycol,

however, showed decreased activities that correlated not with the configuration of the 9-ethylenic bond, but rather with that of the 12-ethylenic bond. Comparison of the curves for the *ct* and *tc* isomers (Figs. 2 and 3) shows that the acids containing a *cis*-12 ethylenic bond and arachidonate (all $n - 6$ acids) were still transferred rapidly to the 2-position of 1-acyl-GPC in the presence of 20% ethylene glycol. The lack of an inhibitory effect of solvent on acids containing the ethylenic bond 6 carbon atoms from the methyl end is interesting in view of the fact that this bond is considered to confer "essentiality" upon a fatty acid whereas the 9-ethylenic bond is "nonessential" (10, 11). The effect of added urea is also interesting in this regard since it appeared to diminish the transfer of all four $\Delta^{9,12}$-isomers in an unselective manner. This may be related to an increased solubility of hydrocarbon in aqueous urea solutions reported by Wetlaufer, Malik, Stoller, and Coffin (12); the observation may be interpreted in terms of a structured solvent (13). Of course the solvent may also directly affect the protein catalyst in some fashion.

One physical property that in part resembles the selectivity of the enzyme-catalyzed transfer to the 2-position is the dissociation of urea complexes of the free acids (14). Although the values have no direct relationship, the similarities are apparent in Table 6. The stability of the hydrocarbon structure within the organized urea complex was surprisingly independent of the configuration of the 9-ethylenic bond. Thus the clathrate complex acts in the manner expected of a π-bond "detector" that is not sensitive to configuration. Nevertheless, even though the dissociations for the two monoeneoates differ by an order of magnitude from that for either the 18:0 or 18:2 acid, the urea complexes are unlikely to involve a positionally specific π-bond interaction. A somewhat similar relationship between clathrate stability and rate of transfer to the 2-position can be seen for the acids of medium chain length, but these similarities should not be overemphasized. At the present time, they serve best to indicate the possible role that solvent structure might play in influencing the transfer of acyl groups from their solvated thiol esters to the 2-position of 1-acyl-GPC. In addition, the specificity of the transfer to the 1-position differs in being highly dependent upon the configuration of the 9-ethylenic bond, and does not seem to be appreciably regulated by the solvent changes studied. Different forces are undoubtedly involved in the "recognition" processes of the two enzymic activities.

TABLE 6 COMPARISON OF DISSOCIATION CONSTANTS OF UREA COMPLEXES WITH ENZYME-CATALYZED ESTERIFICATION RATES FOR DIFFERENT FATTY ACIDS

Acid	K^* × 10^4	Rate†
		mμmoles/min/mg
18:0	2	1.7
trans-Δ^9-18:1	15	10.3
cis-Δ^9-18:1	21	12.4
18:2*cc*	190	23.1
16:0	17	3.5
14:0	29	4.3
12:0	63	6.5

* Values from reference 14.
† The enzyme was prepared from rat liver.

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