Stereospecificity of Monoacylglycerol and Diacylglycerol Acyltransferases from Rat Intestine as Determined by Chiral Phase High-Performance Liquid Chromatography

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Using chiral phase high-performance liquid chromatography of diacylglycerols, we have redetermined the ratios of 1,2-/2,3-diacyl-*sn*-glycerols resulting from acylation of 2-monoacylglycerols by membrane bound and solubilized triacylglycerol synthetase of rat intestinal mucosa. With 2-oleoyl[-^{3}H]glycerol as the acyl acceptor and oleoyl-CoA as the acyl donor, 97–98% of the diacylglycerol product was 1,2(2,3)-dioleoyl-*sn*-glycerol, 90% of which was the *sn*-1,2- and 10% the *sn*-2,3-enantiomer. The remaining diacylglycerol (less than 3%) was the *sn*-1,3-isomer. The overall yield of acylation products was 70%, of which 60% were diacylglycerols and 40% triacylglycerols. With 2-oleylglycerol ether as the acyl acceptor and [1-^{14}C]oleoyl-CoA as the acyl donor, 90% of the diradylglycerol was 1-oleoyl-2-oleyl-*sn*-glycerol and 10% was the 2-oleyl-3-oleoyl-*sn*-glycerol. The diradylglycerols made up 96% and the triradylglycerols 4% of the radioactive product. With 1-palmitoyl-*sn*-glycerol as the acyl acceptor and [1-^{14}C]oleoyl-CoA as the acyl donor, the predominant reaction product was 1-palmitoyl-3-oleoyl-*sn*-glycerol. The 3-palmitoyl-*sn*-glycerol was not a suitable acyl acceptor. Both 1,2- and 2,3-diacyl-*sn*-glycerols were substrates for diacylglycerol acyltransferase as neither isomer was favored when 1,2-dioleoyl-*rac*-[2-^{3}H]glycerol was used as the acyl acceptor. There was a marked decrease in the acylation of the 1(3)-oleoyl-2-oleyl-*sn*-glycerol to the 1,3-dioleoyl-2-oleyl-*sn*-glycerol. It is concluded that neither monoacylglycerol nor diacylglycerol acyltransferase exhibit absolute stereospecificity for acylglycerols as fatty acid acceptors.
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During fat absorption, triacylglycerol synthesis in the intestinal mucosa proceeds predominantly *via* acylation of lumenal 2-acylglycerols, although X-1(3)-acylglycerols are also absorbed. The enzymes catalyzing the reesterification of 2-acylglycerols with fatty acids reside together in the endoplasmic reticulum of the enterocyte (1) and are believed to form a triacylglycerol synthetase complex (2) composed of acyl-CoA ligase, monoacylglycerol acyltransferase (MGAT) and diacylglycerol acyltransferase (DGAT). Because the resynthesis of the higher acylglycerols involves acylation of prochiral and chiral esters, the reaction could theoretically proceed either *via* enantiomeric or racemic intermediates. Previous work with whole cells (3), homogenates (3), isolated microsomal membranes (4,5) or purified enzyme preparations (6,7) has led to inconsistent and contradictory conclusions, possibly due to inadequacies in the analytical methodology used for determination of the enantiomeric ratios of synthesized diacylglycerols.

In the present study we have taken advantage of the discovery of a chiral phase high-performance liquid chromatography (HPLC) method for the separation of enantiomeric diacylglycerols (8) and of its adoption for natural diacylglycerol analysis (9). This approach avoids the uncertainties of the past and provides direct and unambiguous information with regards to separation, identification and quantitation of the acylglycerol acylation intermediates, as well as the initial substrates and final products.

MATERIALS AND METHODS

Chemicals. [1-^{14}C]Oleoyl-CoA (specific activity 52 mCi/mmol) and Aquasol were purchased from New England Nuclear/Dupont Canada Inc. (Lachine, Quebec, Canada). [2-^{3}H]Glycerol trioleate (specific activity 2 Ci/mmol) was obtained from ICN Biochemicals Canada Ltd. (Montreal, Quebec, Canada). Oleoyl-CoA, trioleoylglycerol, oleic anhydride and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma Chemical Co. (St. Louis, MO). Dithiothreitol (DTT) was from Bio-Rad Laboratories Canada Ltd. (Mississauga, Ontario, Canada). Silica gel H (Merck 60 H) and G (Merck 60 G) were obtained from Terochem Laboratories Ltd. (Mississauga, Ontario, Canada). Silica gel GF thin-layer chromatography (TLC) plates (20 × 20 cm, 0.25-mm thick) were purchased from Analtech Inc. (Newark, DE). 2-Oleylglycerol was from Serdary Research Laboratories (London, Ontario, Canada). The 1- and 3-palmitoyl-*sn*-glycerols were gifts from Dr. D. Buchnea (University of Toronto). All other chemicals and solvents were of reagent grade or better quality and were obtained from local suppliers.

Preparation of acyl acceptor substrates. 2-Oleoyl-[2-^{3}H]glycerol (specific activity 3.3 mCi/mmol) was prepared by digestion of [2-^{3}H]glycerol trioleate with porcine pancreatic lipase (10). The 1,2(2,3)-dioleoyl-*sn*-[2-^{3}H]-glycerol (specific activity 0.25 mCi/mmol) was prepared by Grignard degradation of [2-^{3}H]glycerol trioleate (10). 1,3-Dioleoyl-2-oleyl-*sn*-glycerol was prepared by chemical synthesis from 2-oleylglycerol and oleic anhydride as follows: four mg 4-dimethylaminopyridine and 90 μmol oleic anhydride were added to 90 μmol of 2-oleylglycerol in 150 μL dry toluene. After 5 min at room temperature the reaction mixture was dried under nitrogen, and the 1,3-dioleoyl-2-oleyl-*sn*-glycerol was recovered following

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Abbreviations: BCA, bicinchonic acid; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate; CI, chemical ionization; DG, diradylglycerol; DGAT, diacylglycerol acyltransferase; DNPU, dinitrophenylurethane; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetate (sodium salt); GLC, gas-liquid chromatography; HPLC, high-performance liquid chromatography; LC/MS, liquid chromatography/mass spectrometry; MG, monoradylglycerol; MGAT, monoacylglycerol acyltransferase; PMSF, phenylmethylsulfonyl fluoride; TG, triradylglycerol; TLC, thin-layer chromatography; Tris, tris[hydroxymethyl]aminomethane; UV, ultraviolet.

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TLC on silica gel H developed in heptane/diisopropyl ether/acetic acid (60:40:4, vol/vol/vol). X-1(3)-oleoyl-2-oleylglycerol was prepared by digestion of the triradylglycerol with pancreatic lipase (10). The purities and concentrations of the radiolabeled and unlabeled substrates were assessed by boric acid TLC and by gas-liquid chromatography (GLC) on a polar capillary column.

Animals. Male Wistar rats weighing 250–300 g were purchased from Charles River Canada Inc. (La Salle, Quebec, Canada) and were housed for 1–3 wk with free access to Purina Rodent Chow and water before being used in these studies.

Preparation of subcellular fractions. The animals were anesthetized with diethyl ether and exsanguinated *via* their abdominal aortae. The mucosal scrapings of the upper two-thirds of the small intestine were isolated as described by Hoffman and Kuksis (11). Supernatant devoid of brush border membranes, obtained after homogenization of the scrapings and low-speed centrifugation (12), was spun at 25,000 × *g* for 10 min, and the post-mitochondrial supernatant was further centrifuged at 106,000 × *g* for 60 min to pellet microsomal membranes. Microsomes were washed with 50 mM tris[hydroxymethyl]aminomethane (Tris)-HCl (pH 7.4), 0.5 M KCl, 1 mM ethylenediaminetetraacetate (EDTA), 2 mM DTT and sedimented by re-centrifugation at 106,000 × *g* for 60 min. All steps were carried out at 0–4°C. Washed microsomes were suspended in 50 mM K_2HPO_4 (pH 7.4), 1 mM EDTA, 1 mM DTT and 75 μg/mL PMSF to give a final concentration of protein between 7–8 mg/mL. Protein content was determined by bicinchonic acid (BCA) assay (Pierce Chemical Co., Rockford, IL.)

Preparation of triacylglycerol synthetase. Microsomes were solubilized with 0.6% 3-[(3-cholamidopropyl)dimethylammonio]-propanesulfonate (CHAPS) for 30 min on ice, and the solubilized extract was loaded on a Cibacron Blue 3GA agarose column (1 × 5 cm) preequilibrated with 20 mM K_2HPO_4 (pH 7.4), 2 mM DTT and 0.5% CHAPS (Buffer A). The column was washed with Buffer A, followed by Buffer A containing 10 mM ATP, and the triacylglycerol synthetase activity was subsequently eluted with Buffer A containing 0.8 M NaCl (13).

Enzyme assays. The activities of MGAT and DGAT were determined using acetone dispersed acylglycerols (14). The enzyme fractions (20–25 μg protein) were incubated at 37°C for 10 min in 0.5 mL of 20 mM K_2HPO_4, pH 7.4, 1 mM DTT, 1 mM EDTA containing [1-^{14}C]oleoyl-CoA (30 μM), and various monoradylglycerol isomers (60 μM) or 1,2(2,3)-dioleoyl-*sn*-glycerol (250 μM) supplied in acetone (final conc. 2.5%). The reaction was stopped by addition of 4 mL of $CHCl_3/CH_3OH$ (2:1, vol/vol) and lipids were analyzed as described by O'Doherty *et al.* (15). The lipid extracts were separated by TLC on silica gel H employing heptane/diisopropyl ether/acetic acid (60:40:4, vol/vol/vol) as the mobile phase, visualized with iodine vapor, and bands corresponding to 1,2(2,3)-diacyl-*sn*-glycerol, X-1(3)-diacylglycerol and triacylglycerol were scraped off and counted for radioactivity.

Stereospecific analysis. Following enzymatic assay, the 1,2(2,3)-diradyl-*sn*-glycerols formed were separated on boric acid impregnated TLC (silica gel G) plates using chloroform/acetone (96:4, vol/vol) as the mobile phase. The recovered 1,2(2,3)-diradyl-*sn*-glycerols were converted to the corresponding dinitrophenylurethane (DNPU) derivatives according to the procedure of Takagi and Itabashi (8). The crude urethane derivatives were purified by TLC (silica gel GF) using hexane/ethylene dichloride/ethanol (40:10:3, vol/vol/vol). The DNPU derivatives of the enantiomeric diradylglycerols were resolved by HPLC as previously described (8) using *N*-(*R*)-1-(α-naphthyl)ethylaminocarbonyl-(*S*)-valine as the chiral phase and hexane/ethylene dichloride/ethanol (150:20:1, vol/vol/vol) as the mobile phase. For chiral phase liquid chromatography/mass spectrometry (LC/MS) (Hewlett-Packard 5985B quadrupole mass spectrometer combined with a Hewlett-Packard direct liquid inlet interface, Palo Alto, CA), the original mobile phase was replaced with isooctane/*t*-butyl methyl ether/isopropyl alcohol/acetonitrile (80:10:5:5, vol/vol/vol) containing 1% ethylene dichloride to enhance the negative ion response (16).

RESULTS

Overall utilization of substrate. Table 1 compares the overall synthesis of diradyl- (DG) and triradylglycerols (TG) from various monoradylglycerols (MG) and radiolabeled oleoyl-CoA by the microsomes of rat intestinal mucosa. Starting with 2-oleoylglycerol (60 μM) and 25 μg microsomal protein, 70% of the 2-acylglycerol was incorporated into higher acylglycerols within a 10-min incubation (specific activity 23 ± 4 nmol/mg protein/min). The major acylation products were 1,2(2,3)-dioleoyl-*sn*-glycerols (61.6%) and trioleoylglycerols (35.4%). About 3% of the total was recovered as X-1,3-dioleoylglycerols, which apparently arose from acyl migration during the experimental manipulations.

With 2-oleylglycerol ether (60 μM) as the acyl acceptor and 25 μg microsomal protein, the acylation proceeded at about 80% of the rate seen for 2-oleoylglycerol ester. The main product (96.9%) was 1(3)-oleoyl-2-oleyl-*sn*-glycerol, with only about 3% of the total acylation product being recovered as the 1,3-dioleoyl-2-oleyl-*sn*-glycerol.

The 1-palmitoyl-*sn*-glycerol was utilized even less effectively (34.6% of the acylation rate observed for 2-oleoylglycerol). The major product (81.8%) was 1-palmitoyl-3-oleoyl-*sn*-glycerol with a smaller proportion (18.2%) of 1-palmitoyl-2,3-dioleoyl-*sn*-glycerol. Since X-1,3-diacylglycerols are not acylated at the 2-position, the formation of the triacylglycerols (4.9% of the final product) must be attributed to partial isomerization of the 1-palmitoyl-*sn*-glycerol to 2-palmitoylglycerol, formation of 1,2(2,3)-

TABLE 1

Synthesis of Di- and Triradylglycerols from Monoradylglycerols and [1-^{14}C]Oleoyl-CoA by Microsomes from Rat Intestinal Mucosa[a]

Substrate	Percentage of total product synthesized			Relative activity (%)
	1,2(2,3) DG	1,3 DG	TG	
2-18:1-*sn*-glycerol	61.6	3.0	35.4	100.0
2-18:1-*sn*-glycerol ether	96.9	—[b]	3.1	80.4
1-16:0-*sn*-glycerol	13.3	81.8	4.9	34.6
3-16:0-*sn*-glycerol	58.1	17.4	24.4	0.4

[a]Values are averages of three independent experiments performed in duplicates.
[b]Not found.

palmitoyl-*sn*-glycerols and their further acylation to 1,3-dioleoyl-2-palmitoyl-*sn*-glycerols. Under the same conditions, 3-palmitoyl-*sn*-glycerol was not acylated to the *sn*-1,3-isomer.

Table 2 shows the results obtained with the triacylglycerol synthetase complex purified from rat intestinal microsomes (MGAT specific activity 165 ± 23 nmol/mg protein/min).

Enantiomeric specificity. For this analysis, which represents mass ratios of formed diacylglycerols, lipid extracts from five incubations were combined and applied to a TLC plate in the absence of lipid carriers. The 1,2(2,3)-diacyl-*sn*-glycerols were eluted from the gel and converted to the DNPU derivatives. The incubation blank contained 1,2(2,3)-diacyl-*sn*-glycerol DNPU derivatives from five incubations performed in the absence of exogenous monoradylglycerols and oleoyl-CoA, and represent endogenous levels of microsomal diacylglycerols. Figure 1 shows the ultraviolet (UV) absorption profile of the DNPU derivatives of standard diacylglycerols (A) and of the diacylglycerols (B) recovered from incubation of the microsomes with radiolabeled 2-oleoylglycerol and oleoyl-CoA. The incubation products are seen to consist largely of the 1,2-diacyl-*sn*-glycerols with smaller, but clearly recognizable amounts, of the *sn*-2,3-enantiomers. The incubation blank (C) contains UV absorbing peaks that coincide with the 1,2-diacyl-*sn*-glycerol standards of other common diacylglycerol species. In parallel experiments carried out to assess the radioactivity of the diacylglycerol enantiomers formed by microsomal MGAT, the peaks corresponding to the 1,2- and 2,3-diacyl-*sn*-glycerols were collected and rechromatographed under identical conditions (Fig. 2). It can be seen that the collected *sn*-1,2-enantiomer does not contain any *sn*-2,3-isomer (B), whereas the *sn*-2,3-isomer is devoid of the *sn*-1,2-enantiomer (C). Similar enantiomeric separations were obtained for the acylation products of the 2-oleylglycerol ether, which, however, eluted earlier than the corresponding diacylglycerols under the same chiral phase HPLC conditions (Fig. 3). The isolation of these acylalkylglycerol peaks required cochromatography with the corresponding non-radioactive 1-oleoyl-2-oleyl-*rac*-glycerols.

Table 3 shows that the 2-oleoylglycerol and its ether analog were preferentially esterified by the microsomal MGAT at the *sn*-1-position, giving an *sn*-1-/*sn*-3-acylation ratio of 9. Similar values were obtained for the acylation products of the solubilized triacylglycerol synthetase (Table 4). The radioactivity values are quite similar to those obtained by mass analysis (Figs. 1B and 2A).

The identity of the HPLC peaks was confirmed by chiral phase LC/MS (Fig. 4). The total negative ion current profiles of the incubation product (A) and standard (B) are identical to those of the UV profiles. The bottom part of Figure 4 (C and D) shows the full mass spectra recorded for the DNPU derivatives of the 1,2- and 2,3-dioleoyl-*sn*-glycerols obtained from acylation of 2-oleoylglycerol with oleoyl-CoA in the presence of microsomal MGAT. The 1,2- and 2,3-dioleoyl-*sn*-glycerols gave the $[M]^-$ molecular

TABLE 2

Synthesis of Di- and Triradylglycerols from Monoradylglycerols and [1-^{14}C]Oleoyl-CoA by Solubilized Synthetase from Rat Intestinal Mucosa[a]

Substrate	Percentage of total product synthesized			Relative activity (%)
	1,2(2,3) DG	1,3 DG	TG	
2-18:1*sn*-glycerol	71.2	2.9	25.8	100.0
2-18:1-*sn*-glycerol ether	98.2	—[b]	1.8	77.4
1-16:0-*sn*-glycerol	8.6	87.2	4.3	33.6
3-16:0-*sn*-glycerol	46.3	21.6	32.1	0.6

[a]Values are averages of three independent experiments performed in duplicates.
[b]Not found.

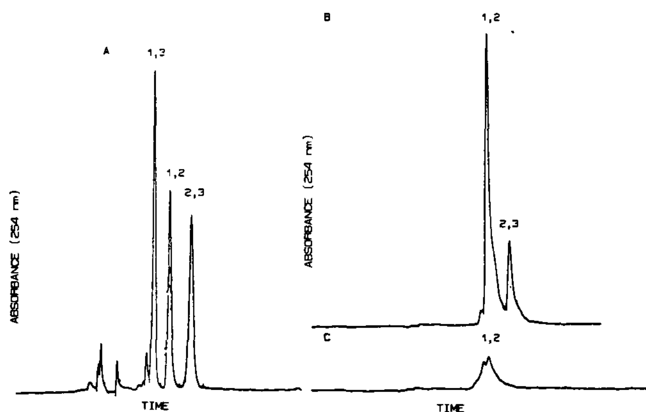

FIG. 1. Ultraviolet absorption profile of the 3,5-dinitrophenylurethane derivatives of standard diacylglycerols (A), of diacylglycerols recovered from incubation of the microsomes with radiolabeled 2-oleoylglycerol and oleoyl-CoA (B), and of endogenous diacylglycerols (C).

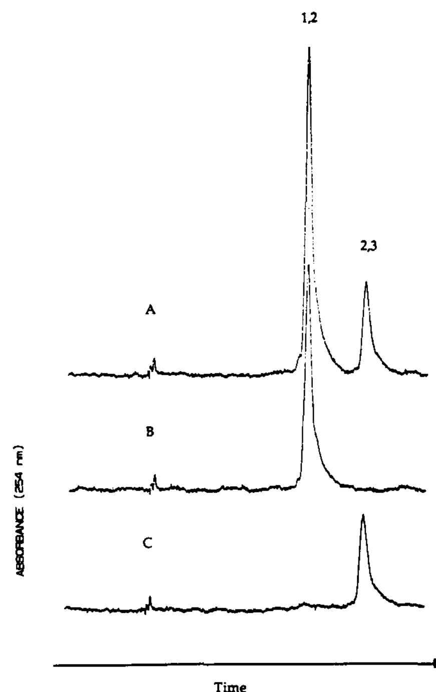

FIG. 2. Ultraviolet absorption profile of the 3,5-dinitrophenylurethane derivatives of diacylglycerols recovered from incubation of the microsomes with radiolabeled 2-oleoylglycerol and oleoyl-CoA (A), and of rechromatographed peaks corresponding to the *sn*-1,2-enantiomer (B) and *sn*-2,3-enantiomer (C).

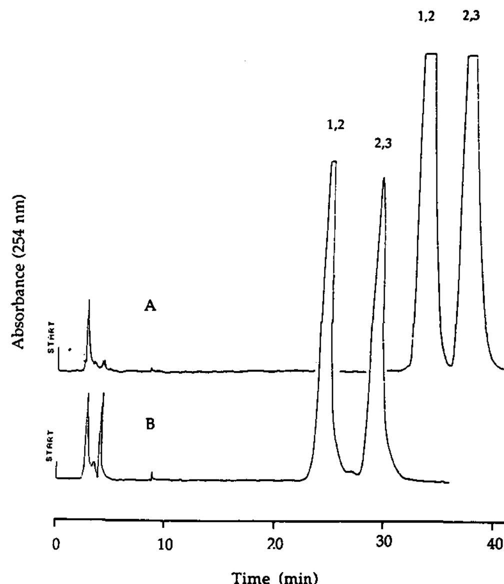

FIG. 3. Ultraviolet absorption profile of the 3,5-dinitrophenylurethane derivatives of diradylglycerols. (A), 1,2(2,3)-dioleoyl-*sn*-glycerol; (B), 1(3)-oleoyl-2-oleyl-*sn*-glycerol.

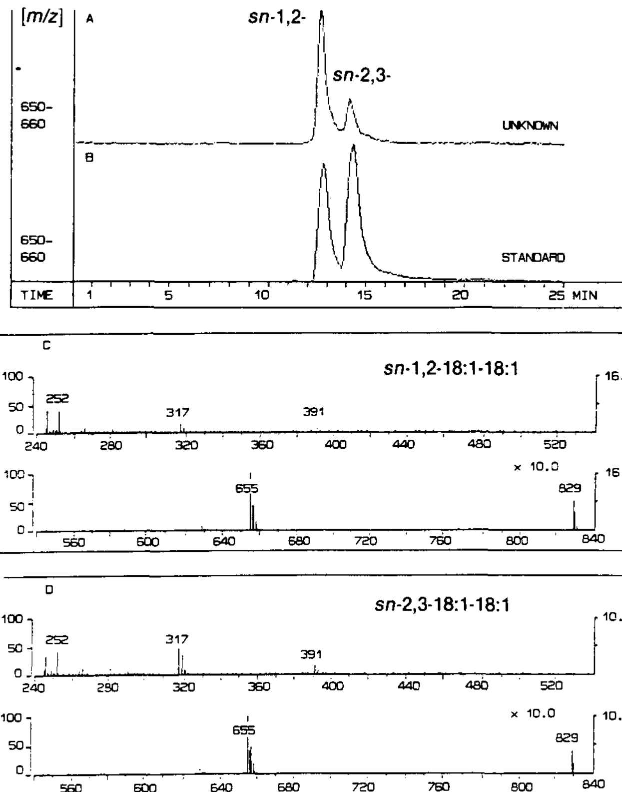

FIG. 4. Chiral phase liquid chromatography/mass spectrometry. The total negative ion current profiles of the incubation product (A) and standard (B). (C) and (D), full spectra recorded for the 1,2- and 2,3-dioleoyl-*sn*-glycerol dinitrophenylurethane derivatives obtained by acylation of 2-oleoylglycerol with oleoyl-CoA and microsomal monoacylglycerol acyltransferase.

TABLE 3

Enantiomeric Ratios of 1,2- and 2,3-Diradyl-*sn*-glycerol (DG) Products Synthesized by Microsomal Monoacylglycerol Acyltransferase from Rat Intestinal Mucosa

Substrate	1,2-DG (% total cpm)	2,3-DG (% total cpm)
2-18:1-*sn*-glycerol	90 ± 3 (6)[a]	10 ± 3 (6)
2-18:1-*sn*-glycerol ether	90 ± 1 (3)	10 ± 1 (3)

[a]Numbers in parentheses refer to number of independent determinations performed in duplicates.

TABLE 4

Enantiomeric Ratios of 1,2- and 2,3-Diradyl-*sn*-glycerol (DG) Products Synthesized by Solubilized Triacylglycerol Synthetase from Rat Intestinal Mucosa

Substrate	1,2-DG (% total cpm)	2,3-DG (% total cpm)
2-18:1-*sn*-glycerol	88 ± 2 (4)[a]	12 ± 2 (4)
2-18:1-*sn*-glycerol ether	91 ± 1 (2)	9 ± 1 (2)

[a]Numbers in parentheses refer to number of independent determinations performed in duplicates.

ion (*m/z* 829) and a characteristic $[M - DNPU + 35]^-$ fragment ion (*m/z* 655). This ion corresponds to the addition of a chlorine group to the dioleoylglycerol molecule. The negative chemical ionization (CI) was found to be about 100 times more sensitive than the positive CI current for these compounds under the chiral phase LC/MS conditions (16).

Utilization of the diacylglycerol intermediates for triacylglycerol synthesis was also examined by incubating microsomes with racemic 1,2(2,3)-dioleoyl-*sn*-[2-^{3}H]-glycerol and oleoyl-CoA. Some 25–30% of the radiolabeled substrate was converted to triacylglycerols (30–40 nmoles). The substrate remaining after incubation was subjected again to chiral HPLC, and the residual *sn*-1,2- and *sn*-2,3-enantiomers resolved and the peaks collected for assay of radioactivity. There was no change in the relative proportions of the mass (Fig. 5) or in the radioactivity in the two enantiomers (Table 5).

DISCUSSION

The present study clearly demonstrated that membrane-bound, as well as solubilized, triacylglycerol synthetase of rat intestinal mucosa is capable of acylating both *sn*-1- and *sn*-3-positions of the 2-acylglycerols, although the *sn*-1-position is preferred. The *sn*-2-position of the 1- or 3-acyl-*sn*-glycerols or X-1,3-diacylglycerols is not acylated. The 1-acyl-*sn*-glycerol yielded exclusively 1,3-diacyl-*sn*-glycerol, and 3-acyl-*sn*-glycerol was not acylated. The above results, which were obtained with substrates and products of known enantiomeric composition and purity, allow critical comparisons with previous results. Thus, the studies confirmed the positional requirements established previously for rat intestine (17), which converts 2-acylglycerols to X-1,2-diacylglycerols and triacylglycerols, and 1-acyl-*sn*-glycerols to 1,3-diacyl-*sn*-glycerols, whereas 3-acyl-*sn*-glycerols are not acylated. Furthermore, the

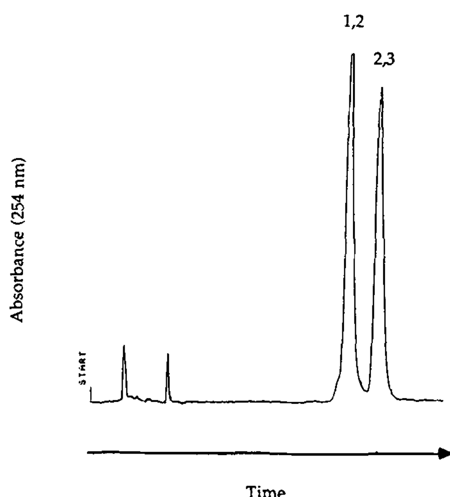

FIG. 5. Ultraviolet absorption profile of the 3,5-dinitrophenylurethane derivatives of the residual 1,2(2,3)-dioleoyl-*sn*-[2-^{3}H]glycerol after incubation of microsomal diacylglycerol acyltransferase with the radiolabeled diacylglycerol and oleoyl-CoA.

TABLE 5

Utilization of 1,2(2,3)-Dioleoyl-*sn*-glycerol (DG) by Microsomal Diacylglycerol Acyltransferase from Rat Intestinal Mucosa

Substrate	1,2-DG (% total cpm)	2,3-DG (% total cpm)
1,2(2,3)-18:1-*sn*-glycerol	53 ± 2 (2)[a]	47 ± 2 (2)

[a]Numbers in parentheses refer to number of independent determinations performed in duplicates.

present study showed that X-1,3-diacylglycerols and X-1-acyl-2-alkylglycerols are not suitable substrates for DGAT. Previous work (17) had shown that DGAT of hamster microsomes utilizes X-1,3-diacylglycerols as well as acylalkylglycerols for triacylglycerol biosynthesis. However, more recent work has shown that the hamster also utilizes the 2-acylglycerols at similar rates as compared to the rat (18). Our microsomal preparations yielded closely similar MGAT specific activities (23 ± 4 nmol/mg protein/min) to those reported by Coleman and Haynes (14) in the absence of activating phospholipids and albumin (28–45 nmol/mg protein/min). Acylation of X-1-acylglycerols yields X-1,3-diacylglycerols which are not esterified further. The earlier studies (17,19) apparently did not eliminate all the uncertainties about the identity and composition of either substrates or products. The present results also differ from the findings in chicken embryos, where 1-oleoylglycerol and 2- and X-1-oleylglycerols and their amide analogs were much less efficiently utilized when compared to 2-oleoylglycerol (19). However, in all instances, a lower activity with 2-alkyl than found with 2-acylglycerol was observed in the present study, as reported previously (17,19).

The acylation of 2-acylglycerol yielded 90% *sn*-1,2- and 10% *sn*-2,3-enantiomers. The results obtained with solubilized MGAT indicate that the 2,3-diacyl-*sn*-glycerol formation cannot be attributed to the slower reaction rate of the microsomal preparations. A similar enantiomer proportion was observed for the acylation of 2-alkylglycerol, although the overall yield of the diacylglycerols was only about 80% of that obtained for the 2-acylglycerol. Furthermore, the 1-acyl-2-alkyl-*sn*-glycerol was only partly converted to the 1,3-diacyl-2-alkyl-*sn*-glycerol, which indicated that the DGAT discriminated more against the 2-alkylglycerol derivatives than did MGAT. The 90% yield of 1,2-diacyl-*sn*-glycerols is similar to the 85–90% originally reported by Johnston *et al.* (20), O'Doherty and Kuksis (4) and, more recently, by Coleman *et al.* (5), but the conclusions differed. Whereas Johnston *et al.* (20) and Coleman *et al.* (5) felt that the enzyme was completely stereospecific, O'Doherty and Kuksis (4) allowed for the possibility that the *sn*-2,3-enantiomers recovered were also products of direct acylation of 2-monoacylglycerols. All previous methods used for enantiomeric determinations employed indirect procedures that required 100% completion of enzymatic conversions of 1,2-diacyl-*sn*-glycerols to phosphatidic acid by diacylglycerol kinase (5) or hydrolysis of 1,2-diacyl-*sn*-glycerophosphophenols by phospholipase A_2 (4). The present study established by direct and unambiguous analysis that about 10% 2,3-diacyl-*sn*-glycerols occur as intermediates during triacylglycerol synthesis from 2-acylglycerols and 2-alkylglycerols. This is also the first instance where both products of enzymatic reaction have been directly measured, rather than estimated by difference from incomplete or partial reactions. The present finding is similar to the 15–19% 2,3-diacyl-*sn*-glycerol yield reported (4) for rat intestinal microsomes. The somewhat higher proportions of 2,3-diacyl-*sn*-glycerols recovered during biosynthesis in isolated cells and mucosal homogenates (3) also may have reflected partial lipolysis of the radioactive triacylglycerols by the endogenous lipases which participate in the lipolysis/reacylation cycle during lipoprotein secretion (21–23). The much higher yield (40%) of 2,3-diacyl-*sn*-glycerols obtained with the 37 kDa polypeptide of MGAT (7) was not confirmed in the present study. The acylation of 2-acylglycerols to 2,3-diacyl-*sn*-glycerols is supported by the isolation of 2-alkyl-3-acyl-*sn*-glycerols during the acylation of 2-alkylglycerols. Because the 2-alkylglycerol is not subject to acyl migration, the recovery of 2-alkyl-3-acyl-*sn*-glycerols cannot be attributed to X-1,3-alkylglycerols.

Finally, this study demonstrated that both 1,2- and 2,3-diacyl-*sn*-glycerols are acylated to triacylglycerols at comparable rates, which would explain why 2,3-diacyl-*sn*-glycerols did not accumulate during the acylation of 2-acylglycerols to triacylglycerols. Direct acylation of enantiomeric diacylglycerols was recognized in the early work of Weiss *et al.* (24), who noted greater than 50% utilization of 1,2-diacyl-*rac*-glycerols by chicken liver particles. As the acylation of 1,2(2,3)-alkylacyl-*sn*-glycerols to alkyldiacylglycerols is very slow, it was not possible to use this enantiomer mixture to confirm the utilization of the racemic diradylglycerols by DGAT. The present findings and the proposed interpretation of the data reconcile essentially all previous experimental findings and corrects earlier conclusions about the stereochemical course of acylation of 2-acylglycerols to triacylglycerols by rat intestinal mucosa.

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