Stereospecificity of monoacylglycerol acyltransferase activity from rat intestine and suckling rat liver

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Abstract The stereospecificity of monoacylglycerol acyltransferase from rat intestinal mucosa and suckling rat liver microsomes was examined using *sn*-1,2-diacylglycerol kinase from *Escherichia coli*. With 2-monooleoyl glycerol and palmitoyl-CoA, 88 and 87.9% of the diacylglycerol synthesized by the intestinal mucosa and suckling liver, respectively, was demonstrated to be the *sn*-1,2-isomer. Analysis of similar preparations of these diacylglycerol products by gas–liquid chromatography–mass spectrometry indicated that most of the remaining diacylglycerol was the 1,3-isomer that probably arose via acyl-migration. These results indicate that monoacylglycerol acyltransferase is stereospecific. Measurement of acyltransferase activities in microsomes using 1- and 2-monoacyl- and monoalkylglycerols as substrates indicated that the monoacylglycerol acyltransferases from suckling liver and intestinal mucosa have different substrate specificities. —**Coleman, R. A., J. P. Walsh, D. S. Millington, and D. A. Maltby.** Stereospecificity of monoacylglycerol acyltransferase activity from rat intestine and suckling rat liver. *J. Lipid Res.* 1986. **27:** 158–165.

Supplementary key words diacylglycerol • complex lipid synthesis • triacylglycerol • phospholipids • diacylglycerol kinase • gas–liquid chromatography–mass spectrometry

In rat intestinal mucosa and suckling rat liver, diacylglycerol can be synthesized by three microsomal routes, the glycerol phosphate, the dihydroxyacetone phosphate, and the monoacylglycerol pathways (1). The glycerol phosphate and dihydroxyacetone phosphate pathways synthesize *sn*-1,2-diacylglycerol, but the stereospecificity of the diacylglycerol product synthesized by monoacylglycerol acyltransferase activity (E.C. 2.3.1.22) has not been determined with certainty. In microsomes, monoacylglycerol acyltransferase from pig liver (2), rat liver (3), and hamster intestinal mucosa (4) was reported to synthesize *sn*-1,2-diacylglycerols. Later studies using intestinal mucosal cells and homogenates or everted intestinal sacs suggested that the intestinal activity lacks stereospecificity (5–8); between 29 and 40% of the diacylglycerols synthesized by these preparations were identified as *sn*-2,3-diacylglycerols. In order to determine the stereospecificity of the high hepatic monoacylglycerol acyltransferase activity of suckling rat liver (9) and to resolve the question of the stereospecificity of the monoacylglycerol acyltransferase activity from intestinal mucosa, we examined the products of the monoacylglycerol acyltransferases with an enzymatic assay that used the *sn*-1,2-diacylglycerol kinase from *Escherichia coli* (E.C.2.7.1.107).

EXPERIMENTAL PROCEDURES

Materials

Phosphatidylcholine (from egg lecithin), phosphatidylserine, *sn*-2-mono-C18:1 glycerol, *rac*-1-mono-C18:1 glycerol, *rac*-1,2-, *sn*-1,2-, and *sn*-1,3-diacylglycerols and *rac*-1 and 2-mono-C18:1 glycerol ethers were obtained from Serdary Research Laboratories, Inc., London, Ontario. *E. coli* cardiolipin was from Avanti Polar Lipids, Birmingham, AL. Bovine serum albumin (essentially fatty acid-free) was from Sigma Chemical Company, St. Louis, MO. ATP was from PL Biochemicals, Milwaukee, WI. [^{3}H]-Palmitic acid, Aquasol, and [γ^{32}P]ATP were obtained from New England Nuclear, Boston, MA. [^{3}H]Palmitoyl-CoA was synthesized enzymatically (10). Thin-layer chromatography plates were from Analtech, Inc., Newark, DE. Octyl-β-D-glucopyranoside was from Calbiochem Behring Corp., La Jolla, CA. *sn*-2,3-Diolein was prepared by exhaustive phosphorylation of *rac*-1,2-diolein by *E. coli* diacylglycerol kinase, followed by separation of *sn*-2,3-diolein from phosphatidic acid on preparative boric acid-impregnated silica gel plates (11, 12) or by high performance liquid chromatography (Walsh, J. P., and R. M. Bell, unpublished results).

Solvent systems for thin-layer chromatography

Solvent A: hepane–isopropyl ether–acetic acid 60:30:4 (v/v). Solvent B: $CHCl_3$–pyridine–88% formic acid 50:30:7 (v/v).

Abbreviations: HPLC, high performance liquid chromatography; BSTFA, bis(trimethylsilyl)-trifluoracetamide; GLC-MS, gas–liquid chromatography–mass spectrometry; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; TMS, trimethylsilyl.

Subcellular fractionation

Livers from 8- to 13-day-old Charles River CD suckling rats were homogenized with six up-and-down strokes in a motor-driven Teflon–glass homogenizer at moderate speed in ice-cold Medium I (0.25 M sucrose, 1 mM EDTA, 10 mM Tris-Cl, pH 7.4). To obtain a microsomal preparation, the homogenate was centrifuged at 1,000 *g* for 10 min and the pellet was discarded. The supernatant was centrifuged at 25,000 *g* for 10 min. This pellet was discarded and the supernatant was centrifuged at 100,000 *g* for 1 hr. The microsomal pellet was resuspended in 0.5 M KCl, 0.25 M sucrose, and recentrifuged at 100,000 *g* for 1 hr. The KCl-washed microsomal pellet was resuspended in Medium I and stored at −80°C in small samples. Protein was determined by the method of Lowry et al. (13) using bovine serum albumin as the standard.

Intestinal mucosa was obtained by scraping the lumenal surface of the first 12 cm of the small intestines from 200-g female rats. Intestinal mucosa microsomes were prepared as described above for liver.

Assay of monoacylglycerol acyltransferase

Monoacylglycerol acyltransferase activity was determined at 23°C in a final volume of 200 μl as previously described (14), except that $MgCl_2$ was omitted and the pH was maintained at 7.0 to minimize acyl group migration of the mono-C18:1 glycerols (14–16). In both liver and intestine, monoacylglycerol acyltransferase activity at pH 7 is 90% of the activity observed at pH 8 (9). The reaction mixture contained 175 mM Tris-Cl, pH 7.0, 1 mg/ml of bovine serum albumin, 15 μg of 1:1 (w/w) mixture of phosphatidylcholine and phosphatidylserine sonicated in 10 mM Tris-Cl, pH 7.4, 1 mM dithiothreitol, 25 μM [^{3}H]-palmitoyl-CoA, and 50 μM mono-C18:1 glycerol dispersed in acetone. The final acetone concentration in the assay was 2.5%. The reaction was initiated by adding 0.05 to 4.0 μg of microsomal protein and was terminated after 5 min. The products were extracted into heptane and washed twice with alkaline ethanol (17). Aliquots of the final heptane phase were counted in 4 ml of Aquasol. The remaining heptane phase was concentrated under N_2 and chromatographed on 10-cm silica gel G plates in solvent A with carrier lipids. Lipids were visualized by exposure of the plates to I_2 vapor. The areas corresponding to diacylglycerol and triacylglycerol were scraped and counted. Monoacylglycerol acyltransferase specific activities were calculated by subtracting one-half of the counts that appeared as triacylglycerol. Diacylglycerol and triacylglycerol comprised more than 96% of the labeled product. For both intestinal mucosa and liver microsomes, this method used optimal amounts of the acyl-CoA and monoacylglycerol substrates. The amount of diacylglycerol synthesized was proportional to both the amount of microsomal protein and the time employed. In the absence of added monoacylglycerol, monoacylglycerol acyltransferase activities in liver and intestine were 0.7% and 3.0 to 6.0%, respectively, of those observed with 50 μM 2-mono-C18:1 glycerol. These low activities probably represent acylation of endogenous monoacylglycerol present in the microsomal preparations.

Gas–liquid chromatography–mass spectrometry

Gas–liquid chromatography was performed on a Varian 3700 gas chromatograph (Varian Associates, Palo Alto, CA) fitted with an on-column injector designed for use with fused-silica capillary columns (J & W Scientific, Rancho Cordova, CA). Separations were performed on a 15-m column (DB-1, J & W Scientific) with helium as carrier gas (1 ml/min). The oven temperature was 240°C for injection; it was then increased ballistically to 340°C and held for 10 min. The column was directly coupled to the ion source of a VG-7070-EHS mass spectrometer (VG Instruments, Inc., Stamford, CT) which was operated in the EI mode (electron energy 70 eV, trap current 100 μA, source temperature 250°C, resolution 1000, scan speed 1 s/decade from m/z 100 to m/z 750). Selected-ion monitoring was carried out at m/z 385, 397, and 411 to quantify the positional isomers of isomeric diacylglycerols relative to the internal standard, 1,2-diC18:1 glycerol.

Determination of diacylglycerol positional isomers

Commercially available 1- and 2-monoacylglycerols were derivatized with bis(trimethylsilyl) trifluoroacetamide (BSTFA) and analyzed by gas–liquid chromatography–mass spectrometry (GLC–MS) as described above. Storage at −20°C in acetone did not affect the relative isomeric content.

To quantitate the positional isomers synthesized from 1- and 2-mono-C18:1 glycerols, microsomes from liver or from intestinal mucosa were incubated under the assay conditions described above except that unlabeled palmitoyl-CoA was used. After various intervals the reaction was stopped with 1.5 ml of heptane–isopropanol–water 80:20:2 (v/v). Twenty μl of 0.025 mM *sn*-1,2-di-C18:1 glycerol in $CHCl_3$ was added, then 1 ml of heptane and 0.5 ml of H_2O were added. An aliquot of the heptane phase was evaporated under N_2 and 20 μl of BSTFA was added to derivatize the dry products. Diacylglycerol products were quantified by comparison of the response ratios of 1-C16:0, 2-C18:1 diacylglycerol, and 1-C18:1, 3-C16:0 diacylglycerol relative to 1,2-C18:1 diacylglycerol as determined by GLC–MS. Response ratios were calibrated using standard mixtures. The fragmentation and differentiation by mass spectrometry of 1,2- and 1,3-diacylglycerol-TMS ethers has been reported previously (18, 19). Products synthesized from the 1- and 2-C18:1 glycerol monoethers were also examined by GLC–MS.

Determination of stereospecificity

Microsomes from liver (1.5 to 30 μg of protein) or intestinal mucosa (3 to 15 μg of protein) were incubated for 5 min under the monoacylglycerol acyltransferase assay conditions as described above using either tritiated or unlabeled palmitoyl-CoA. The lipid-soluble products were extracted into heptane as described above, but the alkaline ethanol washes were omitted in order to avoid acyl-chain migration (14–16). An aliquot of the product was quantified by thin-layer chromatography on silica gel G in solvent A. Another aliquot of the heptane phase was evaporated in a Speed-Vac Concentrator (Savant Instruments, Inc., Hicksville, NY), taken up in $CHCl_3$, and passed over a 2.5-cm column of silicic acid in a Pasteur pipet to remove phospholipids and unreacted palmitoyl-CoA. The $CHCl_3$ was evaporated in a Speed-Vac Concentrator and the reagents for quantitative enzymatic conversion of *sn*-1,2 diacylglycerol to phosphatidic acid were added directly to the tube (Walsh, J. P., and R. M. Bell, unpublished results) (20). The mixture contained 1 mM cardiolipin, 1.5% octylglucoside, 1 mM EGTA, 2 mM dithiothreitol, 60 mM imidazole-HCl, pH 7.0, 60 mM NaCl, 12.5 mM $MgCl_2$, 1 μl of inner membranes from *E. coli* strain GK1/pJW1 (21) (13 μg of protein) and 5 mM of either [γ-^{32}P]ATP or unlabeled ATP. The *E. coli* membranes were prepared as described (21). After 10 min at 23°C, the reaction was terminated and the lipid products were extracted into $CHCl_3$ (22). An aliquot was dried and counted in Aquasol for ^{3}H and ^{32}P. The lipid products were identified by thin-layer chromatography on silica gel G in solvent A and on silica gel H in solvent B. Additional samples were applied to 2.5-cm silicic acid columns, and the neutral lipids were eluted with $CHCl_3$ and chromatographed on silica gel G in solvent A. Phospholipids were then eluted with $CHCl_3$–methanol 1:1 (v/v) and chromatographed on silica gel H in solvent B.

Under the conditions of this assay, *sn*-1,2-diacylglycerol is quantitatively converted to phosphatidic acid (Walsh, J. P., and R. M. Bell, unpublished results). Membranes from *E. coli* that greatly overproduce *sn*-1,2-diacylglycerol kinase also possess an *sn*-2,3-diacylglycerol kinase activity equal to 0.13% of that observed with the *sn*-1,2 isomer (data not shown). When 1 to 5 nmol of *sn*-2,3-diolein was treated with diacylglycerol kinase as described above, only 0.9 to 1.1% was converted to phosphatidic acid. No phosphorylation of *sn*-1,3-diolein could be detected. Thus, in the experiments reported in this study, virtually none of the phosphorylated product could have arisen from *sn*-2,3- or *sn*-1,3-diacylglycerol.

RESULTS

Products from 2-mono-C18:1 glycerol

The stereospecificity of monoacylglycerol acyltransferase from intestinal mucosa and suckling rat liver microsomes was investigated by subjecting the diacylglycerol products to phosphorylation by *E. coli* diacylglycerol kinase, which is specific for *sn*-1,2 diacylglycerols (23, 24). The percent of products measured varied minimally in four separate and independent determinations in which the time of the monoacylglycerol acyltransferase incubation was limited to 5 min but the amount of microsomal protein was varied from 1.5 to 30 μg. Product recovery was greater than 85% for each experiment. **Table 1** presents data from a repre-

TABLE 1. Phosphorylation of diacylglycerols synthesized by liver and intestinal microsomes

Organ	Monoacylglycerol	Conditions[a]	[^{3}H]DG	[^{3}H]TG nmol Product	[^{3}H]PA Synthesized	[^{3}H]Total[b]
Liver[c]	2-C18:1	MGAT	2.51	0.11		2.68
		DGK	0.29	0.13	2.10	2.62
Intestine[d]	2-C18:1	MGAT	0.97	0.56		1.70
		DGK	0.12	0.62	0.88	1.84
Liver[e]	1-C18:1	MGAT	0.87	0.13		1.27
		DGK	0.65	0.13	0.16	1.29
Intestine[f]	1-C18:1	MGAT	1.02	0.27		1.38
		DGK	0.90	0.29	0.10	1.40
Intestine[g]	none	MGAT	0.05	0.02		0.13
		DGK	0.02	0.03	0.02	0.19

[a] MGAT, Products of monoacylglycerol acyltransferase reaction; DGK, products following incubation of monoacylglycerol acyltransferase products with *E. coli* diacylglycerol kinase.
[b] Total tritiated nmol of fatty acid, acylglycerols, and phosphatidic acid.
[c] Three μg of microsomal protein.
[d] Three μg of microsomal protein.
[e] Thirty μg of microsomal protein.
[f] Three μg of microsomal protein.
[g] Three μM of microsomal protein. Results are the average of two independent experiments using two different microsomal preparations.

sentative experiment designed to maximize the amount of product formed.

With liver microsomes, 88.8 ± 1.5% (mean ± SD of four independent determinations) of the labeled lipid product from [^{3}H]palmitoyl-CoA and 2-mono-C18:1 glycerol was identified as [^{3}H]diacylglycerol. After phosphorylation by diacylglycerol kinase, 91.4 ± 2.5% of the diacylglycerol was recovered as [^{3}H]phosphatidic acid. This result agreed closely with quantitation of the ^{32}P-labeled product. Greater than 99.8% of the [^{32}P]phospholipid migrated with phosphatidic acid on thin-layer chromatography in Solvent B; the remainder migrated with lysophosphatidic acid.

Results were virtually identical whether [^{3}H]diacylglycerol was converted to [^{3}H]phosphatidic acid or [^{3}H/^{32}P]-phosphatidic acid or whether unlabeled diacylglycerol was converted to [^{32}P]phosphatidic acid. These results indicate that at least 90% of the diacylglycerol synthesized by liver microsomes from 2-mono-C18:1 glycerol is the *sn*-1,2-stereoisomer. The remaining 7.2 ± 3.0% of diacylglycerol that was not converted to phosphatidic acid could theoretically be either *sn*-2,3- or 1,3-diacylglycerol; GLC–MS analysis was therefore employed to determine the amount of the 1,3-isomer synthesized.

An example of the GLC–MS data from one of the samples is shown in **Fig. 1**. The integrated ion-current profile in Fig. 1a reveals all the mono- and diacylglycerol components after a 5-min incubation of the microsomal preparation. Residual 2-mono-C18:1 glycerol (peak 2) and the major product, 1-C16:0,2-C18:1 diacylglycerol (peak 4), are readily observed with their corresponding isomers (peaks 1 and 5). The ion-current traces from quantitative GLC–MS analysis of this particular sample, carried out by selected-ion monitoring after addition of the internal standard, are shown in Fig. 1b–d. The peak area ratios (response ratios) for each isomeric diacylglycerol relative to the internal standard were converted to concentration by reference to standard curves.

Fig. 2 summarizes the data obtained by GLC–MS quantitation of the diacylglycerol products synthesized by suckling rat liver monoacylglycerol acyltransferase activity. When the substrates were palmitoyl-CoA and 2-mono-C18:1 glycerol, 90% of the diacylglycerol product was 1(3)-C16:0, 2-C18:1 diacylglycerol and only 10% was 1(3)-C16:0, 3(1)-C18:1 diacylglycerol. These results suggest that the small amount of diacylglycerol that was not phosphorylated by *E. coli* diacylglycerol kinase was the 1,3-isomer that had arisen via acyl-migration.

The stereospecificity of monoacylglycerol acyltransferase from rat intestinal mucosa microsomes was examined similarly. Table 1 shows data from a representative experiment. Diacylglycerol and triacylglycerol comprised 46.6% and 53.4%, respectively, of the product synthesized from [^{3}H]palmitoyl-CoA and 2-mono-C18:1 glycerol. After phosphorylation by diacylglycerol kinase, 88% of the diacylglycerol was converted to phosphatidic acid, leaving about 12% unreacted diacylglycerol. This experiment was repeated seven times using varying amounts of microsomal protein from three different intestinal mucosa preparations. The range of diacylglycerol kinase phosphorylation was 81, 86 and 88% for the three microsomal preparations. Since GLC–MS analysis showed that with palmitoyl-CoA and 2-mono-C18:1 glycerol, more than 90% of the product was 1(3)-C16:0,2-C18:1 diacylglycerol and the remainder was the 1,3-isomer (Fig. 2B), virtually none of the product could be the *sn*-2,3-isomer.

Products from 1-mono-C18:1 glycerol

Similar experiments were performed using 1-mono-C18:1 glycerol. With the 1-isomer, monoacylglycerol acyltransferase activity from suckling rat liver was 10% of the activity observed with the 2-isomer (9, Table 1). Only 19.8% of this diacylglycerol product was converted to phosphatidic acid by *E. coli* diacylglycerol kinase, suggesting that 80% of the diacylglycerol formed was the *sn*-1,3- or 2,3-isomer. The GLC–MS data (Fig. 2A) showed that more than 60% of the product is the 1,3-isomer.

GLC–MS analysis showed that when the starting substrates were palmitoyl-CoA and *rac*-1-mono-C18:1 glycerol, the products were 1(3)-C16:0, 2-C18:1 diacylglycerol, and 1(3)-C18:1, 3(1)-C16:0 diacylglycerol. The total amount of diacylglycerol formed with the 1-isomer was only 13% of that formed with the 2-isomer when equal amounts of microsomal protein were used. These results suggest that acylation of the 1-monoacylglycerol proceeds readily only after the acyl group migrates to the 2-position. Lack of palmitate in the 2-position under any condition strongly suggests that hepatic monoacylglycerol acyltransferase does not acylate at the 2-position and that significant migration of 1,3-diacylglycerol to 1,2-diacylglycerol does not occur under these conditions. These results are consistent with results previously obtained using thin-layer chromatography of 1,2- and 1,3-diacylglycerols (9).

In order to confirm the position of acylation by the hepatic monoacylglycerol acyltransferase, the ether analogs of 1- and 2-mono-C18:1 glycerol were incubated with suckling rat liver microsomes under standard conditions using optimal amounts (50 μM) of monoalkylglycerol. These ether analogs have been shown to be good substrates for intestinal monoacylglycerol acyltransferase, whereas the *sn*-3-monoalkyl glycerol is not (25). The products were extracted into heptane but the alkaline ethanol wash was omitted. The product of palmitoyl-CoA and 1-mono-C18:1 glycerol ether revealed prominent ions corresponding to loss of 281 and 90 daltons from the molecular ion in the mass spectrum (**Fig. 3A**). This pattern is consistent with the identification of 1-C18:1

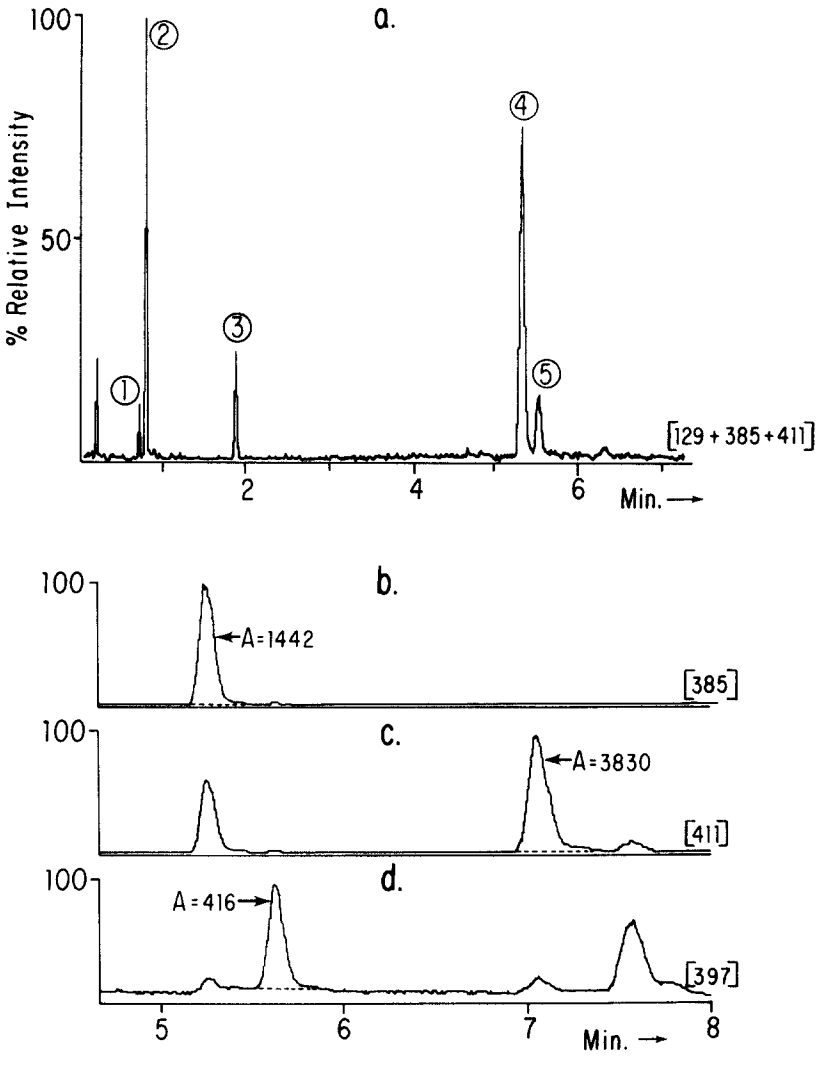

Fig. 1. a: GLC–MS integrated ion-current profile (m/z 129 + 385 + 411) of trimethylsilylated products from a 5-min incubation of liver microsomes from 13-day-old rats with 2-monoolein and palmitoyl-CoA. Peaks marked 1–5 correspond, respectively, to 1-mono-C18:1 glycerol, 2-mono-C18:1 glycerol, cholesterol, 1-C16:0,2-C18:1 glycerol, 1-C16:0,3-C18:1-glycerol. b–d: Selected ion-current profiles corresponding to the quantitative analysis of diacylglycerols in the same sample, using 1,2 di-C18:1 glycerol as the internal standard. The areas (arbitrary units) of the characteristic peaks for 1-C16:0, 2-C18:1 glycerol (m/z 385, panel b) and 1-C16:0,3-C18:1 glycerol (m/z 397, panel d) are compared with that of the internal standard (m/z 411, panel c).

alkyl, 3-C16:0 acyl glycerol. The product from the reaction of palmitoyl-CoA and 2-mono-C18:1 alkyl glycerol exhibited ions at m/z 239, 385 ($M-C_{18}H_{35}O$) and 549 ($M-CH_2TMS$) (Fig. 3B). This pattern is consistent with the structure of 1(3)-C16:0 acyl, 2-C18:1 alkyl glycerol. Since the ether linkage does not permit migration, these results support the lack of acylation at the *sn*-2 position by the liver monoacylglycerol acyltransferase.

In intestinal mucosa, only 10% of the diacylglycerol synthesized from 1-mono-C18:1 glycerol was phosphorylated by *E. coli* diacylglycerol kinase (Table 1). Thus, 90% was either the *sn*-2,3- or the 1,3-isomer. The GLC–MS data (Fig. 2B) indicate that the unphosphorylated diacylglycerol was the 1,3-isomer.

GLC–MS analysis indicated that when 1-mono-C18:1 glycerol was the substrate, 90% of the product was 1(3)-C18:1, 3(1)-C16:0 diacylglycerol. Thus, unlike the monoacylglycerol acyltransferase from liver, intestinal mucosa monoacylglycerol acyltransferase appears to acylate 1-monoacylglycerol readily, as has been previously reported (25). Furthermore, unlike the liver activity, intestinal monoacylglycerol acyltransferase specific activity was virtually identical with both the 1- and 2-monoacylglycerol substrates (compare Fig. 2A and B). These results suggest that either the monoacylglycerol acyltransferases of suckling rat liver and adult rat intestinal mucosa have different substrate specificities or that the intestine contains a second activity capable of acylating 1-monoacylglycerols.

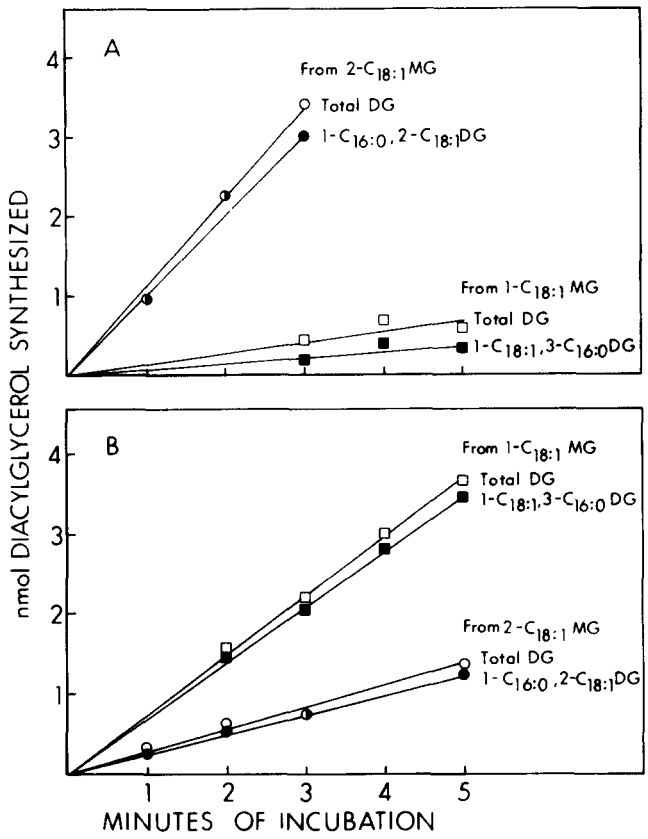

Fig. 2. GLC-MS analysis of diacylglycerol positional isomers synthesized by monoacylglycerol acyltransferase from (A) liver and (B) intestine. Microsomes were incubated with 1-C18:1 or 2-C18:1 monoacylglycerols for the times indicated. The 1-C16:0, 2-C18:1, and 1-C18:1, 3-C16:0 diacylglycerol products were identified by GLC-MS and quantified by comparison of response ratios to a known amount of added 1,2-C18:1 diacylglycerol as described in Experimental Procedures. In A, 2.0 and 6.0 μg (in two 3.0-μg aliquots) of microsomal protein were incubated with 2-C18:1 and 1-C18:1 monoacylglycerol, respectively. In B, 1.5 μg and 4.0 μg (in two 2.0-μg aliquots) of microsomal protein were incubated with 2-C18:1 and 1-C18:1 monoacylglycerol, respectively. DG, diacylglycerol; MG, monoacylglycerol.

Products formed in the absence of added monoacylglycerol

In order to determine the contribution of diacylglycerol formed via other microsomal pathways, microsomes were incubated under standard conditions (including 2.5% acetone) except that the monoacylglycerol substrate was omitted. The amounts of diacylglycerol formed by suckling rat liver under these conditions was 0.7% of that synthesized in the presence of 50 μM 2-mono-C18:1 glycerol; this amount of diacylglycerol did not contribute substantially to the total product and was not analyzed further.

Intestinal mucosa microsomes incubated without 2-monoacylglycerol synthesized 3 to 6% of the amount of diacylglycerol obtained when 50 μM 2-mono-C18:1 glycerol was present. These data suggest that endogenous monoacylglycerols present in the microsomes contributed minimally to the glycerolipid products formed. About 50% of the diacylglycerol formed in the absence of added monoacylglycerol was the 1,2-isomer, as indicated by its phosphorylation by *E. coli* diacylglycerol kinase (Table 1). Since it is uncertain how effectively the endogenous monoacylglycerol isomers would compete with added 2-mono-C18:1 glycerol, the results obtained in the presence of added substrate were not corrected for possible endogenously derived products. Thus, the amount of *sn*-1,2-diacylglycerol produced by intestinal monoacylglycerol acyltransferase may be underestimated.

GLC-MS identification of products formed in the presence of 50 μM monoacylglycerol also confirms the absence of diacylglycerol products other than those derived from 2-mono-C18:1-glycerol and palmitoyl-CoA (Fig. 1). In washed microsomes, the phosphatidic acid pathway would not contribute to diacylglycerol production unless glycerol 3-phosphate was added.

DISCUSSION

Our results were obtained under conditions in which acyl-migration of the mono- and diacylglycerols was minimized by maintaining a neutral pH during enzymatic syntheses and organic extractions and by avoiding non-neutral solvents that would tend to promote acyl-migration (15) during separation of 1,3- and 1,2-diacylglycerols by thin-layer chromatography. The short incubation time and non-coupled enzymatic reaction minimized chemical isomerization as well as possible enzymatic isomerization by, as yet undescribed, monoacylglycerol or diacylglycerol isomerases. With the 2-mono-C18:1 glycerol substrate, recovery of labeled fatty acid was less than 7% under any condition. Thus, quantitation of the di- and triacylglycerols synthesized could not have been impaired by product hydrolysis.[1]

These results are consistent with those reported for microsomes from pig and rat liver (2, 3) and hamster intestinal mucosa (4). In those studies stereospecificity was

[1]When the 1-mono-C18:1 glycerol substrate was incubated with hepatic microsomes under monoacylglycerol acyltransferase assay conditions, about 17% of the [^{3}H] product was recovered as fatty acid (Table 1 and unpublished data). If monoacylglycerol was omitted, little [^{3}H]fatty acid was found. These data suggest that hepatic microsomes contain a lipase that preferentially hydrolyzes 1,3- or *sn*-2,3-diacylglycerols.

[2]To confirm the reported ability of diacylglycerol acyltransferase to use *sn*-2,3-diacylglycerols, *sn*-2,3-diC18:1 glycerol was prepared by phosphorylating the 1,2-isomers of X-1,2-diC18:1 glycerol with *E. coli* diacylglycerol kinase and purifying the product by HPLC. Less than 2% of the purified product was the *sn*-1,2-isomer as measured by phosphorylation by *E. coli* diacylglycerol kinase. Dependencies showed that, at optimal diacylglycerol concentrations (150 to 200 μM), liver and intestinal mucosa microsomal diacylglycerol acyltransferase activity (28) with *sn*-1,2-di-C18:1-glycerol was 4 times higher than with *sn*-2,3-di-C18:1-glycerol.

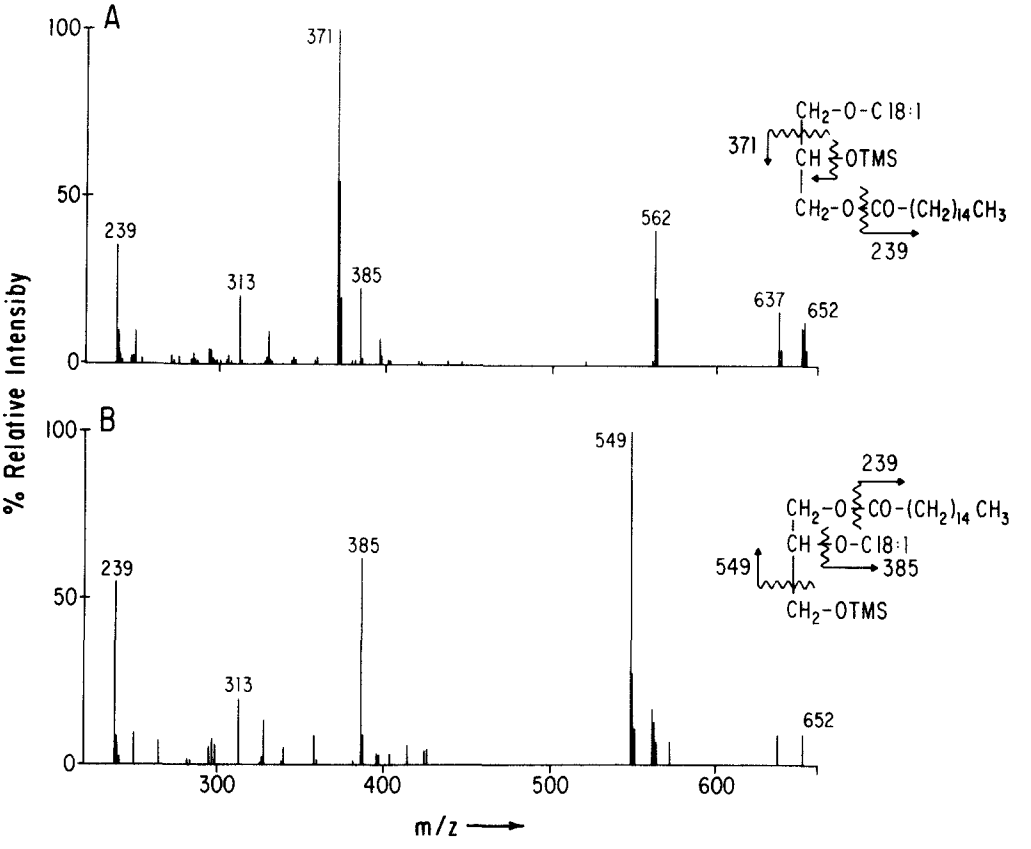

Fig. 3. Mass spectra of A, 1-C18:1 alkyl, 3-C16:0 acyl glycerol and B, 1(3)-C16:0 acyl, 2-C18:1 alkyl glycerol. When the TMS group is at the 2-position, the M-90 is prominent; when the TMS group is at the 3-position, M-103 is prominent.

determined after chemical conversion of diacylglycerol to phosphatidylcholine and release of labeled fatty acid by phospholipase A_2. More recent studies using intestinal mucosa homogenates, isolated mucosal cells, or everted intestinal sacs have reported that as much as 40% of the monoacylglycerol product is the *sn*-2,3-isomer (5–8). These latter studies used long incubation times and a coupled assay that required synthesis of the acyl-CoA substrate. It is likely that acyl migration of both the mono- and diacylglycerols occurred during the incubations. Furthermore, the amount of triacylglycerol synthesized was not reported. Intestinal diacylglycerol acyltransferase activity is high and, even during the brief incubation times employed here, 50% of the labeled product from 2-monoacylglycerol was triacylglycerol (Table 1). Since diacylglycerol acyltransferase activity is minimal with 1,3-diacylglycerol (17, 26, 27), and is several[2] times higher (17, 27) with *sn*-1,2-diacylglycerols than with *sn*-2,3-diacylglycerols, analysis of only the unreacted diacylglycerols favors measurement of 1,3- and 2,3-diacylglycerols that are poor substrates for the diacylglycerol acyltransferase.

Without taking into account the 1,2-diacylglycerols that were converted to triacylglycerol during the incubations, our results indicate that 88% of the diacylglycerol synthesized from 2-monoacylglycerol by intestinal and liver monoacylglycerol acyltransferases can be converted to phosphatidic acid by *E. coli* *sn*-1,2-diacylglycerol kinase and that the unphosphorylated diacylglycerol is predominantly the 1,3-isomer that probably arose via acyl-migration. These results strongly indicate that both intestinal and liver monoacylglycerol acyltransferases synthesize only *sn*-1,2-diacylglycerols.

Supported by grants HD19068 (RAC) and GM20015 (JPW) from the National Institutes of Health and grant I-850 (RAC) from the March of Dimes Birth Defects Foundation. We thank Drs. R. M. Bell and C. M. Mansbach for helpful discussions, Dr. B. R. Ganong for the HPLC purification of 2,3-diCl8:1 glycerol, and Ms. Tessie Stinson for secretarial assistance.

Manuscript received 29 May 1985.

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