

Selective incorporation of various C-22 polyunsaturated fatty acids in Ehrlich ascites tumor cells

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Abstract Three ^{14}C -labeled 22-carbon polyunsaturated fatty acids, 7,10,13,16- ^{14}C docosatetraenoic acid (22:4(n-6)), 7,10,13,16,19- ^{14}C docosapentaenoic acid (22:5(n-3)), and 4,7,10,13,16,19- ^{14}C docosahexaenoic acid (22:6(n-3)), were compared with ^3H arachidonic acid (20:4(n-6)) and ^{14}C linoleic acid (18:2(n-6)) to characterize their incorporation into the lipids of Ehrlich ascites cells. The relatively rapid incorporation of the labeled 22-carbon acids into phosphatidic acid indicated that substantial amounts of these acids may be incorporated through the de novo pathway of phospholipid synthesis. In marked contrast to 20:4(n-6), the 22-carbon acids were incorporated much less into choline glycerophospholipids (CGP) and inositol glycerophospholipids (IGP). No selective preference was apparent for the (n-3) or (n-6) type of fatty acids. The amounts of the acids incorporated into diacylglycerophosphoethanolamine were in the order of: 22:6(n-3) > 20:4(n-6) >> 22:5(n-3) \geq 22:4(n-6) > 18:2(n-6), whereas for alkylacylglycerophosphoethanolamine they were in the order of: 22:4(n-6) > 22:6(n-3) > 22:5(n-3) >> 20:4(n-6) > 18:2(n-6). Of the mechanisms possibly responsible for the selective entry of 22-carbon acids into ethanolamine glycerophospholipids, the most reasonable explanation was that the cytidine-mediated ethanolamine phosphotransferase may have a unique double selectivity: 1) for hexaenoic species of diacylglycerol and 2) for 22-carbon polyunsaturated fatty acid-containing species of alkylacylglycerol. The relative distribution of fatty acids between newly incorporated and already maintained lipid classes suggested that IGP may function in Ehrlich cells as an intermediate pool for the retention of polyunsaturated fatty acids in glycerolipids.—Masuzawa, Y., S. Okano, K. Waku, H. Sprecher, and W. E. M. Lands. Selective incorporation of various C-22 polyunsaturated fatty acids in Ehrlich ascites tumor cells. *J. Lipid Res.* 1986. 27: 1145–1153.

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It has been recommended that human diets should contain polyunsaturated fatty acids of the linoleic (n-6) and linolenic (n-3) families, although there is little evidence that n-3 acids are essential for warm-blooded an-

imals (1–3). The essentiality of n-6 and n-3 fatty acids may be due to the functioning of arachidonic acid (20:4(n-6)) and eicosapentaenoic acid (20:5(n-3)) as precursors for the cyclooxygenase or lipoxygenase pathway (summarized in references 4 and 5). 20:5(n-3) may have a regulatory function in eicosanoid synthesis as a competitive inhibitor of the cyclooxygenase pathway (5–8). However, a part of the biological functions of the essential fatty acids may be due to 22-carbon (C-22) polyunsaturated fatty acids, since n-6 and n-3 C-22 polyunsaturated fatty acids are widely distributed in animal tissues and comparable to C-20 acids in the amounts found in nervous tissues (3, 9–11), testis (12), semen (13), ovary (12), and some malignant cells (14–16), even from animals fed normal diets. Actually, docosahexaenoic acid (22:6(n-3)), the most commonly encountered n-3 acid in terrestrial animals, is a stronger competitive inhibitor of prostaglandin synthesis than 20:5(n-3) (6, 17). Also 22:6(n-3) may be necessary for the development of nervous tissues (2, 18). These facts indicate that the requirement for n-3 acids may be due to such biological functions of 22:6(n-3). 22:6(n-3) (17, 19), docosapentaenoic acid (22:5(n-3)) (20), and docosatetraenoic acid (22:4(n-6)) (21) may also function as precursors of prostaglandins or hydroxy fatty acids. In addition, these C-22 polyunsaturated fatty acids esterified at the sn-2 position of phospholipids could influence the release and subsequent availability of 20:4(n-6) for eicosanoid synthesis (6). Therefore, it is important to clarify the mechanisms regulating the composition of

Abbreviations: CGP, choline glycerophospholipids; EGP, ethanolamine glycerophospholipids; IGP, inositol glycerophospholipids; SGP, serine glycerophospholipids; GPC, glycerol-3-phosphocholine; GPE, glycerol-3-phosphoethanolamine; GPI, glycerol-3-phosphoinositol; TLC, thin-layer chromatography; GLC, gas-liquid chromatography; HPLC, high performance liquid chromatography. Fatty acids are designated by number of carbon atoms:number of double bonds.

C-22 polyunsaturated fatty acids in membrane phospholipids.

Special metabolic selectivities for the retention of C-22 acids, especially 22:6(n-3), have been indicated by the distributions of C-22 acids (3, 9, 10) and the results of feeding experiments (11, 22). This has been explained by the selective action of the enzymes for lipid synthesis. It was demonstrated, using Ehrlich ascites cell and liver microsomes, that, although acyl-CoA:lysophospholipid acyltransferase, an enzyme relatively selective for C-18 and C-20 polyunsaturated acyl-CoA (23, 24), does not prefer C-22 polyunsaturated acyl-CoA (25, 26), CDP-ethanolamine: diacylglycerol ethanolaminephosphotransferase shows a selectivity for the 22:6-containing molecular species of diacyl- and alkylacyl-glycerols (16, 27-30). CoA-independent transacylase between diacylglycerophospholipid and ether-linked lysophospholipids, which was considered to be a 20:4(n-6)-specific enzyme (31-33), can catalyze the transfer of 22:6(n-3) at a rate similar to those in the case of 20:4(n-6) in Ehrlich cells (25) and alveolar macrophages (34). However, it is still unclear whether the enzyme preference is for a 22-carbon chain length, the positions of double bonds (i.e., n-3 or n-6) or the total number of double bonds.

The selective synthesis of molecular species by the enzymes involved in lipid synthesis has been mainly investigated using rat liver (summarized in reference 35), which contains only negligible amounts of ether-linked lipids (36). However, most animal tissues contain appreciable amounts of ether-linked lipids (36). In this study, we compared the incorporation of three naturally occurring 22-carbon fatty acids (22:6(n-3), 22:5(n-3), and 22:6(n-6)) into diacyl and ether-linked lipids of Ehrlich ascites cells using 20:4(n-6) and 18:2(n-6) as controls, to examine the type of 22-carbon polyunsaturated fatty acid that is preferred by the enzymes for glycerolipid synthesis.

EXPERIMENTAL PROCEDURES

[¹⁴C]22:4(n-6) (21 Ci/mol), [¹⁴C]22:5(n-3) (47 Ci/mol), and [¹⁴C]22:6(n-3) (40 Ci/mol) were synthesized as previously described (19-21). [³H]20:4(n-6) (80 Ci/mmol) and [¹⁴C]18:2(n-6) (56 Ci/mol) were purchased from Amersham International, Amersham. Unlabeled 22:5(n-3) was obtained by means of total organic synthesis (21). The other unlabeled acids, 18:2, 20:4, and 22:6, were purchased from Nu-Chek-Prep, Elysian, MN. The labeled acids were purified when necessary by TLC with petroleum ether-diethyl ether-acetic acid 50:50:1 as the developing solvent system. Each of the labeled fatty acids, except for 22:4, was diluted with the corresponding unlabeled acid to 21 Ci/mol before use. The radiopurity of each fatty acid was over 98% when estimated by TLC,

and over 95% on HPLC of the methyl ester on Zorbax ODS reverse-phase column as described by Avelaño, VanRollins, and Horrocks (37).

Ehrlich ascites cells were maintained by weekly transplantation in male ddy-albino mice (Sankyo Laboratory, Shizuoka) fed a conventional laboratory chow. Peritoneal fluid was collected on the 11th day after inoculation and the ascites cells were washed with 20 mM HEPES/Ringer solution (pH 7.2). The washed cells were resuspended at a cell concentration of 10⁷ per ml in 20 mM HEPES/Eagle's minimum essential medium (pH 7.2) containing fatty acid-free bovine serum albumin (0.1%). One ml of the cell suspension was pipetted into each culture tube, followed by incubation with both [³H]20:4 (4.5 nmol/culture) and one of the ¹⁴C-labeled fatty acids (4.5 nmol/culture) for 2-30 min at 37°C. Each radioactive polyunsaturated fatty acid was combined with bovine serum albumin complexed with palmitic acid (25 nmol) and stearic acid (25 nmol), and then added to the cell culture.

Lipids were extracted from the incubation mixtures by the method of Bligh and Dyer (38), and the chloroform layers were preserved by adding butylated hydroxytoluene. An aliquot of each chloroform layer was used for radioactivity estimation and the remainder was subjected to thin-layer chromatography for the separation of lipid classes on silica gel 60 TLC plates (Merck, Darmstadt). The alkyl diacylglycerol and triacylglycerol fractions were separated from fatty acids and phospholipids with petroleum ether-diethyl ether-acetic acid 80:20:1 as the developing solvent system (16). For detailed examination of the different phospholipid classes, a sample was separated by two-dimensional TLC with the solvent system described elsewhere (39). In the latter chromatography, an aliquot of phosphatidic acids obtained by cabbage phospholipase D (P-L Biochemicals, Milwaukee, WI) treatment of choline glycerophospholipids from Ehrlich ascites cells was added as a carrier to each sample. For further analysis of phospholipids in terms of the contents of the alkenylacyl, alkylacyl, and diacyl forms, 1-radyl-2-acyl-3-acetyl-glycerol derivatives were prepared from both the choline and ethanolamine phospholipid fractions as described by Waku et al. (40). Then the three types of diradylacetyl-glycerols were separated by TLC according to the method of Renkonen and Luukkonen (41).

The individual spots on the thin-layer plates were visualized with iodine vapor and then scraped into separate counting vials. The radioactivity was determined with a Packard 3320 liquid scintillation counter as previously described (16). The recovery of radioactivity from each thin-layer plate was over 90%.

The quantities of fatty acyl moieties of the separated lipid classes were estimated by GLC after methanolysis with sodium methoxide using methylheptadecanoate as

an internal standard. A 1.8 m column containing 5% diethylene glycolsuccinate on Chromosorb W (60–80 mesh) was used at 200°C with nitrogen as the carrier gas in a Shimadzu GC-mini gas–liquid chromatograph. The separation of fatty acid methyl esters by HPLC was performed as described above, according to the method of Aveldano et al. (37).

RESULTS AND DISCUSSION

Table 1 shows the mass distributions of the total fatty esters and polyunsaturated fatty acids among the glycerolipid classes in Ehrlich ascites cells. The cells harvested from mice fed the standard diet normally contained appreciable amounts of esterified 18:2(n–6) (30%), 20:4(n–6) (6.9%), and 22:6(n–3) (8.4%), whereas 22:4(n–6) and 22:5(n–3) were minor components. Each of the polyunsaturated fatty acids showed a distinctive distribution among the lipid classes. The preferential distribution of each acid may be determined by comparing the % distribution with the proportion of the total esters. The distribution of 18:2 among the neutral esters, diacyl-GPC, diacyl-GPE, and SGP was similar to the proportions of each of these glycerolipid classes, but it was relatively less in IGP and the ether-linked glycerolipids. On the other hand, the three 22-carbon polyunsaturated acids were located preferentially in ether-linked lipids as compared with the proportions of total esters. A higher percentage of 20:4 was found in ether-linked phospholipids and also in IGP, whereas 22:6 was relatively less in IGP.

The time-dependent incorporation of the five polyunsaturated fatty acids (18:2(n–6), 20:4(n–6), 22:4(n–3), 22:5(n–3), and 22:6(n–3)) into triacylglycerols and phos-

pholipids of Ehrlich ascites cells was determined. HPLC analysis of fatty acid methyl esters prepared from triacylglycerols and phospholipids (35) revealed that over 95% of each radioactive acid was not metabolized to other fatty acids. In all incubations, the incorporation rate of 20:4 was not affected by an equal amount of additional polyunsaturated fatty acid with which it was added. The overall amounts of the added fatty acids esterified after 30 min incubation ranged from 40% (in 18:2) to 75% (in 20:4), and most of the esterified fatty acids were in triacylglycerols and phospholipids (see Fig. 1). The incorporation of 18:2 and 22:6 into triacylglycerols was linear for 30 min, and that of 22:4 and 22:5 showed a slight limitation after 10 min incubation (Fig. 1a). On the other hand, the incorporation of 20:4 into triacylglycerols and that of all fatty acids into phospholipids were greatly limited after 10 min incubation with 4.5 nmol acid per 10⁷ cells (Fig. 1b). These limited incorporations were not observed in our previous study (16), in which a level of 1 nmol of acid per 10⁷ cells was used. This may be due to the consumption of endogenous acceptors or to other modifications of the incubation conditions. For example, in these experiments, glucose was added and stearic acid was used instead of oleic acid.

Triacylglycerols incorporated relatively higher amounts of 22:4(n–6) and 22:5(n–3) in comparison with the other three acids, and the incorporation curves for these two 22-carbon fatty acids were almost identical to each other (Fig. 1a). A more desaturated 22-carbon acid, 22:6, was incorporated more extensively into triacylglycerols than 18:2 but less than the other 22-carbon fatty acids. The three 22-carbon fatty acids were incorporated into phospholipids in a manner similar to those in the case of 18:2, and the amounts were much lower than that of 20:4

TABLE 1. Distribution of polyunsaturated fatty acids among glycerolipids of Ehrlich ascites cells

Lipid Class	(Proportion)	18:2 (30%)	20:4 (6.9%)	22:4 (1.2%)	22:5 (1.3%)	22:6 (8.4%)
Neutral ester	(24%)	24	14	28	29	20
Diacyl-GPC	(42%)	47	25	20	20	16
Alkylacyl-GPC	(2.5%)	1.5	5.9	6.1	10	10
Alkenylacyl-GPC	(1.4%)	0.70	6.2	3.9	3.7	4.4
Diacyl-GPE	(18%)	18	19	8.8	7.3	12
Alkylacyl-GPE	(2.7%)	0.74	7.4	19	16	15
Alkenylacyl-GPE	(2.9%)	0.67	10	7.4	7.2	17
SGP	(4.9%)	5.2	1.4	3.2	3.6	4.0
IGP	(2.4%)	0.84	10	3.1	2.9	0.9
Phosphatidic acids	(0.29%)	0.22	0.14	0.21	0.47	0.17

Each lipid class was fractionated by two-dimensional TLC. Also, 1-*radyl*-2-acyl-3-acetylgllycerol derivatives of both EGP and CGP were prepared and separated as their alkenylacyl, alkylacyl, and diacyl acetates by TLC. After transmethylation with sodium methoxide, the amounts of the various fatty acids in each lipid class were estimated by GLC. The percentage distribution of each polyunsaturated fatty acid among the lipid classes was calculated from the average of two separate estimations, and the percentage of the acid among the total cellular esters is given in parentheses in the top row. Each lipid class also has its proportion of total ester given in parentheses adjacent to the left-hand column.

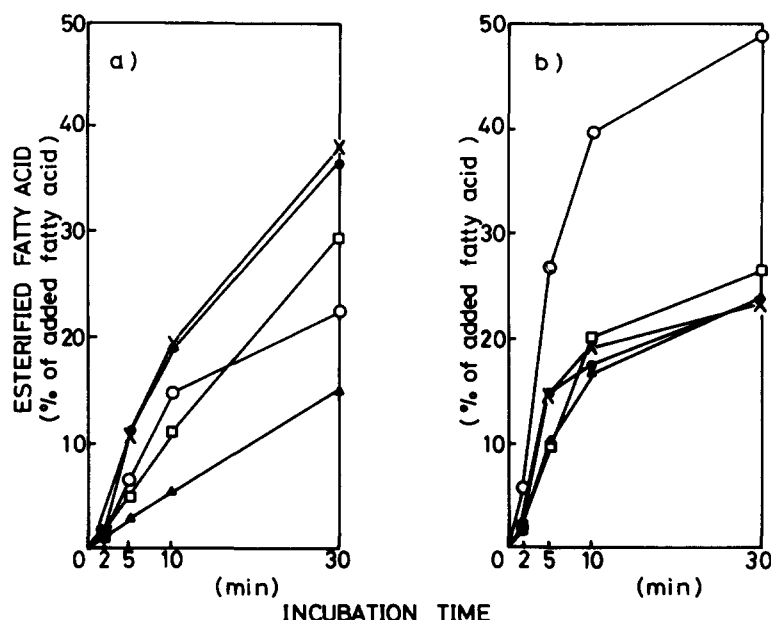


Fig. 1. Incorporation of various polyunsaturated fatty acids into triacylglycerols (a) and phospholipids (b). Cells were cultured for 2–30 min with [^3H]20:4 and one of the [^{14}C]-labeled polyunsaturated fatty acids (4.5 nmol/culture, respectively). The lipids were extracted, and triacylglycerols and phospholipids were separated by TLC as described in the text. The amounts incorporated into individual lipids were calculated from the specific radioactivities of the added fatty acids. Each value is the mean of four (18:2, 22:4 and 22:5, 22:6) or sixteen (20:4) cultures. The SEM (not shown) did not exceed 5% of the values. (\blacktriangle — \blacktriangle), 18:2; (\circ — \circ), 20:4; (\bullet — \bullet), 22:4; (\times — \times), 22:5; (\square — \square), 22:6.

for all the incubation times. This distribution was explained earlier in Ehrlich cells (16, 23, 25) and rat liver (24, 26), as being due to the high specificity for 20:4 in the deacylation–reacylation cycle of phospholipid biosynthesis. The limited incorporation of 20:4 into triacylglycerols after 10 min incubation may be due to consumption of the 20:4 available for de novo synthesis of glycerolipids.

Fig. 2 shows the time-dependent changes in the amounts of radioactive polyunsaturated fatty acids incorporated into each phospholipid class. The incorporation curves for phosphatidic acids showed peaks at 5 min (Fig. 2a), reflecting the role of phosphatidic acid as a common precursor for glycerolipid synthesis, and major differences were not observed among the incorporations of the five fatty acids at 2 min incubation. Each of the 22-carbon fatty acids or 18:2 was incorporated in greater amounts into phosphatidic acids than that into other glycerolipids at 2 min incubation (compare Fig. 2a with Fig. 2b,c). That 20:4 among the five acids showed the lowest accumulation in phosphatidic acids after 10 min incubation may be due to the rapid consumption of 20:4, which reduced the amount available for de novo synthesis of glycerolipids, as suggested above for the incorporation into triacylglycerols. On the other hand, the three 22-carbon fatty acids were present more than 20:4 and 18:2 in the phosphatidic acids after a 10-min incubation, and presumably went on to form triacylglycerols as shown in Fig. 1a.

The amounts of all the polyunsaturated fatty acids incorporated into other glycerophospholipids increased during the experimental period, although the mode of the time-dependent increase differed. The CGP fraction incorporated considerable amounts of 20:4 even at 2 min incubation, and it retained this polyunsaturated fatty acid in the greatest amount among the five added acids during the experimental period (Fig. 2b). The selectivity for incorporating 20:4 seemed to be stronger with IGP than that observed in the case of CGP (compare Fig. 2b with Fig. 2d). These two glycerophospholipids acquired relatively lower amounts of the 22-carbon polyunsaturated fatty acids, and CGP acquired less of the 22-carbon fatty acids than 18:2. On the other hand, IGP incorporated more 22:4 and 22:5 than 18:2 throughout the experimental period, whereas 22:6 seemed not to be utilized for IGP synthesis, especially within 5 min of incubation. While the amount of each fatty acid incorporated into EGP (Fig. 2c) was lower than that into CGP (Fig. 2b), a unique selectivity was observed. The EGP fraction acquired not only 20:4, but also 22:6, on 5-min incubations (Fig. 2c). After a 10-min incubation, 22:6 became the polyunsaturated fatty acid most selectively incorporated into EGP among those added. Throughout the experimental period, the amounts of 22:4 and 22:5 incorporated into EGP were less than that of 20:4, but they were more than that of 18:2.

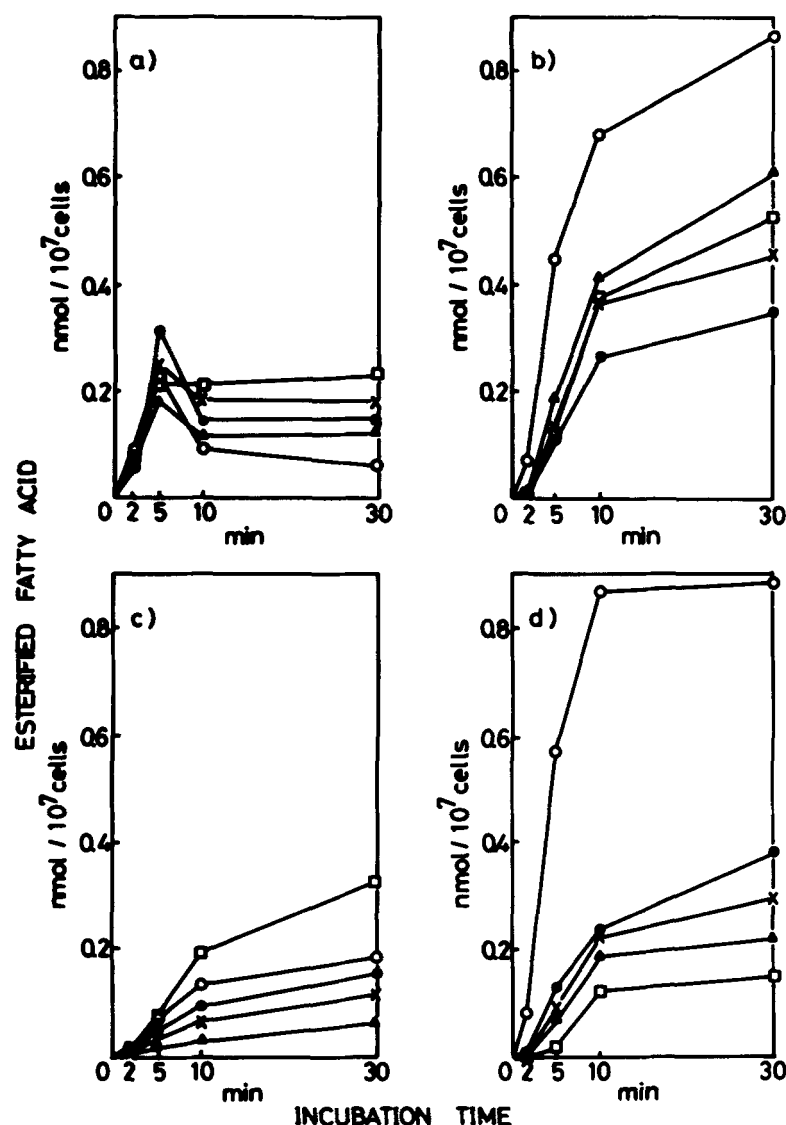


Fig. 2. Incorporation of polyunsaturated fatty acids into various phospholipid classes. a) Phosphatidic acids; b) choline glycerophospholipids; c) ethanolamine glycerophospholipids; and d) inositol glycerophospholipids. The lipid extract from each cell culture was separated into phospholipid classes by two-dimensional TLC. In this separation, unlabeled phosphatidic acids were added as a carrier. Each value is the mean of four (18:2, 22:4, 22:5 and 22:6) or sixteen (20:4) cultures. The SEM (not shown) did not exceed 5% (CGP and IGP) or 10% (phosphatidic acids and EGP) of the values. (\blacktriangle — \blacktriangle), 18:2; (\circ — \circ), 20:4; (\bullet — \bullet), 22:4; (\times — \times), 22:5; (\square — \square), 22:6.

Since Ehrlich ascites cells contain large amounts of ether-linked lipids (Table 1), we separated the alkylacyl, alkenylacyl, and diacyl types of lipids and determined the distributions of radioactive polyunsaturated fatty acids among each glycerophospholipid class at 10 and 30 min incubation. As previously reported for 20:4 and 22:4 (16), over 95% of the radioactive fatty acids were distributed in the diacyl forms in the triacyl-glycerol and CGP fractions (data not shown). On the other hand, each of the fatty acids studied showed a distinctive distribution among the three EGP subfractions, which incorporated relatively

greater amounts of the 22-carbon fatty acids. We compared the amounts and the % distributions of radioactive fatty acids among the various types of EGP (Table 2). The amounts of fatty acids incorporated into the diacyl-GPE and alkenylacyl-GPE were in the order of: 22:6 > 20:4 >> 22:5 \geq 22:4 > 18:2, while those into alkylacyl-GPE were in the order of: 22:4 > 22:6 > 22:5 >> 20:4 > 18:2. These data indicate that, among the three 22-carbon acids, 22:6(n-3) was the one most selectively incorporated into diacyl-GPE whereas 22:4(n-6) was most selectively incorporated into alkylacyl-GPE. In addition, all three 22-

TABLE 2. Incorporation of polyunsaturated fatty acids into three types of ethanolamine glycerophospholipids

Incubation		18:2	20:4	22:4	22:5	22:6
		<i>pmol / 10⁷ cells</i>				
Alkenylacyl	10 min	1.5 (4.2)	4.5 (3.7)	3.2 (3.4)	2.7 (3.6)	4.9 (3.6)
	30 min	1.2 (1.6)	9.1 (4.2)	4.6 (3.0)	4.3 (3.8)	9.4 (3.6)
Alkylacyl	10 min	2.2 (6.1)	8.1 (6.6)	39 (42)	22 (29)	25 (15)
	30 min	3.4 (4.4)	9.3 (4.3)	60 (39)	32 (28)	39 (15)
Diacyl	10 min	32 (90)	110 (90)	52 (55)	52 (68)	132 (82)
	30 min	72 (94)	200 (92)	88 (58)	78 (68)	213 (82)

1-*Radyl*-2-acyl-3-acetyl-glycerol derivatives were prepared from the EGP fraction and separated as their alkenylacyl, alkylacyl, and diacyl acetates by TLC. The amounts of incorporated acids were calculated from the specific radioactivities of the added fatty acids. Each value is the mean of three separate cultures incubated for 10 or 30 min. Values in parentheses are % distributions of each radioactive fatty acid among the three types of EGP.

carbon polyunsaturated fatty acids were incorporated into alkylacyl-GPE ten times more than 18:2 and three times more than 20:4. In contrast, 22:4 and 22:5 were incorporated into diacyl-GPE only a little more than 18:2 (1.1–1.6 times 18:2).

Although it is difficult to elucidate the biochemical mechanisms responsible for the preferential incorporation of these fatty acids only from the results of this experiment, the above data and the results of a series of previous experiments (14–16, 23, 25, 27, 42, 43) allow the assumption of the existence of enzymes that play a role in the selective retention of these polyunsaturated acids in intact cells. That the greatest amount of 22-carbon fatty acids was incorporated into phosphatidic acids on 2-min incubations (Fig. 2) suggests that the 22-carbon polyunsaturated fatty acids were more rapidly esterified during *de novo* phosphatidic acid synthesis compared to the retailoring process of phospholipid synthesis. The rapid incorporation of 20:4(n-6) into IGP and CGP (shown in Fig. 2b and 2d) seems to be due to acyl-CoA:1-acyl-GPI/GPC acyltransferase(s), as demonstrated in Ehrlich cells (23, 25), rat liver (24, 26), and brain (44). On the other hand, such a retailoring process may not function so effectively for the selective retention of 22-carbon polyunsaturated fatty acids as for 20:4. In Ehrlich cell microsomes, 22:6-CoA (25) and 22:4-CoA were not preferentially utilized by acyl-CoA:1-acyl-GPC acyltransferase (Masuzawa, Y., S. Okano, and K. Waku, unpublished results).

Although the three enzymes involved in EGP biosynthesis, i.e., 1-*radyl*-glycerophosphate acyltransferase, ethanolamine phosphotransferase and 1-*radyl*-GPE acyltransferase, are possibly responsible for the selective synthesis of unsaturated molecular species of EGP, the most reasonable explanation for the selective entry of 22:6 into diacyl-GPE (Fig. 2c and Table 2) is that ethanolamine

phosphotransferase preferentially utilizes 22:6-containing diacylglycerols as previously demonstrated in Ehrlich cells (27) and liver (29, 30) microsomes. Since 22:4 and 22:5 were not so strongly selected as 22:6 for the synthesis of diacyl-GPE, the ethanolamine phosphotransferase appeared to show greater preference for hexaenoic diacylglycerols with little selectivity for the 22-carbon chain length per se. Nakagawa and Waku (27) demonstrated that the ethanolamine phosphotransferase also prefers 22:6-containing alkylacylglycerols in Ehrlich ascites cells. Our present results (Table 2) further indicate that, when the substrate contains a 1-O-alkyl bond, the selectivity of the ethanolamine phosphotransferase may favor the 22-carbon chain length at position two, and that this enzyme may prefer 22:4-containing alkylacylglycerols to the species containing more unsaturated 22-carbon acids.

In addition to ethanolamine phosphotransferase, two other enzymes may also contribute to the different patterns of incorporation of 22-carbon polyunsaturated fatty acids between diacyl-GPE and alkylacyl-GPE. 1) 1-Alkylglycerophosphate acyltransferase might have a selectivity that is dependent on the substrate chain length, and 2) 1-alkyl-GPE acyltransferase may differ in the substrate selectivity for C-22 polyunsaturated fatty acids from 1-acyl-GPC acyltransferase (25). However, as previously noted (16), such a selectivity of 1-alkylglycerophosphate acyltransferase could not explain why the preferential incorporation of C-22 fatty acids was not observed into alkylacylglycerols and alkylacyl-GPC. Although a 22:6-CoA:1-alkyl-GPE acyltransferase activity was not detected in Ehrlich ascites cell microsomes (25), we detected the direct transacylation of 1-alkyl-GPE by 22:6 and 20:4 in the microsomes (25). This acyltransferase may participate in the synthesis of 22-carbon acid-containing species of alkylacyl-GPE. However, the much higher distributions of exogenously added labeled C-22 acids than 20:4 in

TABLE 3. Percent distribution of radioactive polyunsaturated fatty acids among glycerolipids on 10 min incubation

	18:2	20:4	22:4	22:5	22:6
Neutral ester	32 ± 1 (1.3)	33 ± 2 (2.4)	56 ± 3 (2.0)	53 ± 1 (1.8)	40 ± 1 (1.9)
Diacyl-GPC	37 ± 1 (0.77)	25 ± 2 (0.98)	15 ± 1 (0.74)	20 ± 1 (0.98)	25 ± 1 (1.5)
Alkylacyl-GPC	0.14 ± 0.09 (0.17)	0.41 ± 0.19 (0.07)	0.25 ± 0.17 (0.04)	0.21 ± 0.06 (0.02)	0.37 ± 0.05 (0.04)
Alkenylacyl-GPC	0.05 ± 0.02 (0.07)	0.37 ± 0.11 (0.06)	0.05 ± 0.04 (0.01)	0.04 ± 0.03 (0.01)	0.26 ± 0.09 (0.06)
Diacyl-GPE	2.8 ± 0.2 (0.15)	4.0 ± 0.5 (0.21)	2.8 ± 0.2 (0.31)	2.6 ± 0.1 (0.35)	10 ± 1 (0.87)
Alkylacyl-GPE	0.18 ± 0.05 (0.24)	0.25 ± 0.11 (0.03)	2.1 ± 0.2 (0.11)	1.1 ± 0 (0.07)	1.9 ± 0.2 (0.13)
Alkenylacyl-GPE	0.12 ± 0.02 (0.18)	0.24 ± 0.16 (0.02)	0.17 ± 0.03 (0.02)	0.14 ± 0.02 (0.02)	0.37 ± 0.09 (0.02)
SGP	0.34 ± 0.01 (0.06)	0.66 ± 0.08 (0.46)	0.51 ± 0.12 (0.16)	0.44 ± 0.11 (0.12)	0.38 ± 0.03 (0.09)
IGP	18 ± 1 (21)	33 ± 1 (3.2)	14 ± 1 (4.4)	13 ± 0 (4.6)	8.2 ± 0.3 (9.5)
Phosphatidic acid	11 ± 1 (48)	3.5 ± 1.2 (25)	8.0 ± 1.6 (38)	9.8 ± 0.3 (21)	14 ± 0 (81)
Esterification of added acids ^a (%)	25 ± 1	59 ± 4	39 ± 2	42 ± 3	34 ± 1

Isotope distributions were determined among the spots separated by two-dimensional TLC and among the subtypes of CGP or EGP, respectively, and the distribution among all classes was recalculated. Each value is the mean ± SEM of four (18:2, 22:4, 22:5 and 22:6) or sixteen (20:4) cultures. Figures in parentheses are relative specific activities (% distribution of isotope shown in this table/% distribution of the mass shown in Table 1).

^a Values were calculated as (total radioactivity found in glycerolipids/added radioactivity) × 100.

alkylacyl-GPE (Table 2) cannot necessarily be explained by this enzyme, since the activity of Ehrlich cell microsomes seems to have similar selectivity for 20:4 and 22:6 (25), and labeled 20:4 was found in much higher amounts than C-22 fatty acids in diacyl-GPC (Fig. 2b), a probable acyl donor for the transacylase reaction (25).

Table 3 shows the isotope distributions among the lipid classes and the relative specific activities (% distribution of isotope/% distribution of the mass) calculated from the above data at 10 min incubation (Tables 1 and 2, and Figs. 1 and 2). The results permit comparison of the overall distributions of fatty acids between those newly incorporated and those already maintained, and evaluation of the role of the selective retention of fatty acids by the enzymes for glycerolipid synthesis in the regulation of the fatty acid composition of membrane phospholipids. All of the acids were incorporated at higher relative percentages into phosphatidic acids and IGP compared with their mass distributions shown in Table 1, resulting in high relative specific radioactivities of all acids in these lipids. The high relative specific activities of phosphatidic acids reflect that this lipid class turns over rapidly as a common metabolic intermediate in glycerolipid synthesis. Although the physiological function of IGP in Ehrlich ascites cells is still unclear, the high relative specific activities indicate that this glycerolipid might function as an intermediate pool for the retention of polyunsaturated fatty acids in glycerophospholipids. IGP rapidly turns over in Ehrlich ascites cells (42). Perhaps the diacylglycerol derived from IGP through the action of a phospholipase C can be utilized for the synthesis of triacylglycerols, CGP and EGP, permitting fatty acid translocation without the need for an acyltransferase activity.

Our previous study demonstrated that the specific radioactivities of [³H]glycerol incorporated into Ehrlich ascites cells were in the order of, alkylacyl-GPE ≥ diacyl-GPC > diacyl-GPE ≥ alkylacyl-GPC > alkenylacyl-GPE, with the glycerol backbone of alkylacyl-GPE turning over most rapidly among the subtypes of CGP and EGP (15, 43). This pattern occurs in the fatty acid specific activities, with the marked exception of alkylacyl-GPE. Therefore, the very low relative specific activities of fatty acids in the alkylacyl-GPE may reflect the nonutilization of the exogenous acids under these conditions and not a slow turnover of ether-linked phospholipids. The retailoring process may differ between the diacyl and ether-linked types of phospholipids. The microsomal activity of acyl-CoA:1-alkyl-GPC acyltransferase is much lower than that of acyl-CoA:1-acyl-GPC acyltransferase in Ehrlich ascites cells (23, 25). Furthermore, in the retailoring process, the ether-linked phospholipids may utilize an acyl donor pool other than that derived from exogenously added fatty acids. Recently, we found a transacylase activity that may participate in the synthesis of polyunsaturated acid-containing species of ether-linked phospholipids in Ehrlich cells (25). Such a transfer would permit the retailoring of glycerol-labeled molecules with endogenous fatty acids retained at the second position of diacylphospholipids, and it may account for the relatively low specific activities observed for exogenous polyunsaturated acids in this study. ■

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