[Chem. Pharm. Bull. 36(6)2109—2117(1988)]

Kinetic Parameters of Lysophospholipid Acyltransferase Systems in Diet-Induced Modifications of Platelet Phospholipid Acyl Chains

SHIGEO MURASE,* KAZUYO YAMADA, and HARUMI OKUYAMA

Faculty of Pharmaceutical Sciences, Nagoya City University, 3–1 Tanabedori, Mizuhoku, Nagoya 467, Japan

(Received December 8, 1987)

Competitive effectiveness and relative K_m values of polyunsaturated fatty acids were determined in the acyl-CoA: lysophospholipid acyltransferase systems in platelet membranes. Some CoA esters of fatty acids such as 18:2n-6 (linoleic), 18:3n-3 (α-linolenic), 18:3n-6, 20:3n-6 (dihomo-γ-linolenic) and 20:5n-3 (eicosapentaenoic) were found *in vitro* to be relatively good competitive inhibitors of arachidonate (20:4n-6) incorporation into phosphatidylcholine, while in the acylation of lysophosphatidylinositol, 18:2n-6, 20:2n-6, 20:3n-6, 20:5n-3 and 22:4n-6 were relatively good inhibitors. When vegetable oil-supplemented diets rich in 18:2n-6 or 18:3n-3 were fed to rats, these fatty acids were converted to highly unsaturated fatty acids such as 20:4n-6 and 20:5n-3, and were esterified to phospholipids in platelets. The kinetic parameters determined *in vitro* were useful in predicting grossly the relative abundance of eicosanoid precursors (20:4n-6 and 20:5n-3), but not that of 18-carbon polyunsaturated fatty acids. It was found that arachidonate was conserved but n-3 fatty acids were excluded relatively more strictly in phosphatidylinositol than in phosphatidylcholine in platelets of rats on diets containing different proportions of 18:2n-6 and 18:3n-3.

Keywords——competitive inhibitor; arachidonate; acyltransferase; polyunsaturated fatty acid; phospholipid; platelet

Fatty acid patterns of phospholipids are known to be quite different among different tissues or organs (high organ specificity) but very similar in the same organs of different species (low species specificity). Polyunsaturated fatty acids such as arachidonate (20:4n-6) and eicosapentaenoate (20:5n-3) are esterified mainly at the 2-position of phospholipids in platelets as well as in liver[1-3] and this selective esterification has been shown in liver to be catalyzed mainly by acyl-CoA: lysophospholipid acyltransferase systems.[4,5] We and others have determined the specificities for acyl-CoAs of these acyltransferase systems in platelets,[6-8] and these enzyme systems appeared to assimilate polyunsaturated fatty acids into phospholipids in platelets as well.

When platelets are stimulated, these polyunsaturated fatty acids are liberated from phospholipids, which then serve as substrates and inhibitors of eicosanoid synthesis.[9-13] Three pathways have been postulated for the liberation of polyunsaturated fatty acids from phospholipids: a) by direct action of phospholipase A_2,[14,15] b) by the action of phospholipase C followed by diacylglycerol lipase and monoacylglycerol lipase,[16-18] or c) by the action of phospholipase C followed by diacylglycerol kinase, phosphatidate-specific phospholipase A_1 and lysophospholipase or phosphatidate-specific phospholipase A_2.[19-21] So far, the relative importance of these pathways in the liberation of polyunsaturated fatty acids in stimulated platelets has not been defined.[18,22-28] Among the liberated fatty acids, arachidonate is converted to thromboxane (TX)A_2 with potent aggregatory activity, while eicosapentaenoate (20:5n-3) may be converted to TXA_3 with little aggregatory activity.[29,30] Moreover, 20:5n-3 competitively inhibits the conversion of arachidonate to TXA_2 at the step of

NII-Electronic Library Service

cyclooxygenase.[10-13] The competition between arachidonate and other polyunsaturated fatty acids is assumed to occur also at the step of esterification into phospholipids.[31] Therefore, the relative abundance of these fatty acids can influence the aggregability of platelets. Since higher animals depend entirely on the diet for the supply of n-3 and n-6 series polyunsaturated fatty acids, dietary fatty acids can influence platelet aggregability. Recent findings on the preventive effects of fish oil rich in eicosapentaenoic acid against thrombosis clearly proved the presence of such competitive mechanisms.[9,13,29]

The purposes of the present experiments were: (i) to define how various polyunsaturated fatty acids are esterified selectively through 1-acyl-glycerophospholipid acyltransferase systems, (ii) to find out from a practical point of view which fatty acids are good competitive inhibitors of arachidonate incorporation through the acyltransferase systems *in vitro*, and (iii) to examine if the kinetic parameters determined *in vitro* have any significance in predicting the diet-induced changes in platelet phospholipid acyl chains.

Materials and Methods

Fatty acids used were products of Serdary Research Laboratories. [1-^{14}C]Arachidonic acid was obtained from Amersham International and [2-^{3}H]glycerol was from New England Nuclear. Coenzyme A was from Kyowa Hakko Co., Ltd. (Tokyo). Coenzyme A thiol esters of unsaturated acids and [^{14}C]arachidonic acid were synthesized by a modification[32] of Seubert's procedure.[33] 1-Acylglycerol-3-phosphocholine (1-acyl-GPC) and 1-acylglycerol-3-phosphoinositol (1-acyl-GPI) labeled with tritiated glycerol were prepared biosynthetically using *Saccharomyces cerevisiae* culture as described previously.[34] Phosphorus was determined as described by Eibl and Lands.[35] Specific radioactivities were 3500 cpm/nmol for [^{3}H]1-acyl-GPC, 4000 cpm/nmol for [^{3}H]1-acyl-GPI and 2000 cpm/nmol for [^{14}C]arachidonoyl-CoA as determined by using a liquid scintillation spectrometer (Searle Mark II) with a toluene/Triton X-100/water scintillant.[36]

Platelet membranes were prepared as described previously.[6] Protein was determined by the method of Lowry *et al.*[37] Maximum velocities for various acyl-CoAs were determined in the presence of a single acyl-CoA and a single acceptor under the optimized conditions. Competitive effectiveness of acyl-CoA was determined in the presence of ^{3}H-labeled acceptor, ^{14}C-labeled arachidonoyl-CoA, various amounts of non-labeled other acyl-CoA and enzyme protein. The assay mixture for the acyl-CoA : 1-acyl-GPC acyltransferase system consisted of 150 μM [^{3}H]1-acyl-GPC, 20 μM [^{14}C]arachidonoyl-CoA, 20, 40 or 80 μM other acyl-CoA and 0.1 mg of platelet membrane protein in 0.5 ml of 0.1 M Tris–HCl (pH 7.5). Under the optimized conditions, reactions were linear for at least 5 min. After incubation at 25 °C for 3 min, reactions were terminated by adding chloroform–methanol (1 : 2). Products extracted by the Bligh and Dyer method[38] were separated by thin layer chromatography (TLC) on Silica gel G with chloroform–methanol–water (65 : 25 : 4) as a solvent. The assay mixture for the acyl-CoA : 1-acyl-GPI acyltransferase system consisted of 100 μM [^{3}H]1-acyl-GPI, 15 μM [^{14}C]arachidonoyl-CoA, 20, 40 or 80 μM other acyl-CoA and 0.1 mg of platelet membrane protein in 0.5 ml of 0.1 mTris–HCl (pH 8.5). After incubation at 25 °C for 2 min, reactions were terminated by adding chloroform–methanol (1 : 2). Products extracted with acidified chloroform–methanol were separated on Silica gel 60 plates (Merck) with chloroform–methanol–acetic acid–water (25 : 15 : 4 : 2) as a solvent.

Radioactivities (^{3}H and ^{14}C) in phosphatidylcholine and phosphatidylinositol were determined by the sample channel ratio method as described previously.[31]

Semi-purified diets (Nihon Clea Co., Ltd.) supplemented either with perilla seed oil or with safflower seed oil were fed to spontaneously hypertensive rats[39] for 8 weeks. The proportions of saturated, oleic (18 : 1n-9) linoleic (18 : 2n-6) and α-linolenic (18 : 3n-3) acids were 10.6, 12.3, 12.8 and 64% for the perilla oil diet, and 11.3, 10.4, 78.0 and 0.05% for the safflower oil diet, respectively. Platelets were isolated according to the method of Billah *et al.*[20] and lipids were extracted with chloroform–methanol. Phosphatidylinositol and phosphatidylcholine were separated by two-dimensional TLC.[39] Fatty acids of the isolated phospholipids were analyzed as methyl esters by gas chromatography.

Results

When maximum velocities for the respective acyl-CoAs were determined under the optimized conditions, the acylations of 1-acyl-GPC were most rapid with 20 : 5n-3, 20 : 3n-6 and 20 : 2n-6, followed by 18 : 1n-9, 18 : 2n-6, 18 : 3n-3, 20 : 3n-3 and 20 : 4n-6 (Table I, the column for V_{max}). Acyl-CoAs with 22 carbons were relatively poor substrates. On the other

NII-Electronic Library Service

TABLE I. Kinetic Parameters for Acyl-CoAs in the Acylations of 1-Acyl-GPC and 1-Acyl-GPI

Acyl-CoA	1-Acyl-GPC acyltransferase					1-Acyl-GPI acyltransferase				
	V_{max}	EqC μM	CE	IC_{50} μM	$K_m^{20:4}/K_m$	V_{max}	EqC μM	CE	IC_{50} μM	$K_m^{20:4}/K_m$
16:1n-7	11.3	>250	0	—	0.08	2.8	∞	—	>500	—
18:1n-9	25.7	>250	0	—	—	3.3	>250	0	>500	—
18:2n-6	23.4	45	0.44	—	0.49	8.0	59	0.25	>500	0.29
18:3n-3	25.7	43	0.46	—	0.48	4.6	>250	0	>500	0.12
18:3n-6	9.8	24	0.86	250	2.06	6.8	100	0.15	>500	0.16
20:2n-6	36.0	240	0.08	—	0.13	3.3	68	0.22	130	0.48
20:3n-3	24.4	>250	0	—	0.07	1.7	>250	0	220	0.24
20:3n-6	39.4	42	0.47	—	0.33	5.6	29	0.51	>500	0.75
20:4n-6	26.8	20	1.00	—	1.06	9.5	13	1.20	>500	0.72
20:5n-3	40.1	28	0.70	—	0.48	6.4	67	0.22	>500	0.27
22:1n-9	0	∞	—	320	—	0.5	>250	0	170	—
22:2n-6	2.1	∞	—	>500	—	0	>250	0	390	—
22:3n-3	4.9	∞	—	>500	—	1.1	>250	0	>500	—
22:4n-6	5.1	>250	0	32	0.43	0.6	63	0.24	97	2.58
22:6n-3	4.6	∞	—	37	—	0	>250	0	430	—

V_{max} values (nmol/min/mg of protein) for various acyl-CoAs were determined in the presence of a single acyl-CoA and a single acceptor under optimized conditions. EqC, CE, concentrations required to give 50% inhibition (IC_{50}) and relative K_m values (the ratio of the K_m for arachidonoyl-CoA/the K_m for given acyl-CoAs) were defined and determined as described in the text. Fatty acids are abbreviated by the carbon chain length: the number of double bonds. The position of the first double bond number from the methyl terminus is designated as n-9, n-7, n-6 or n-3. —: not detectable.

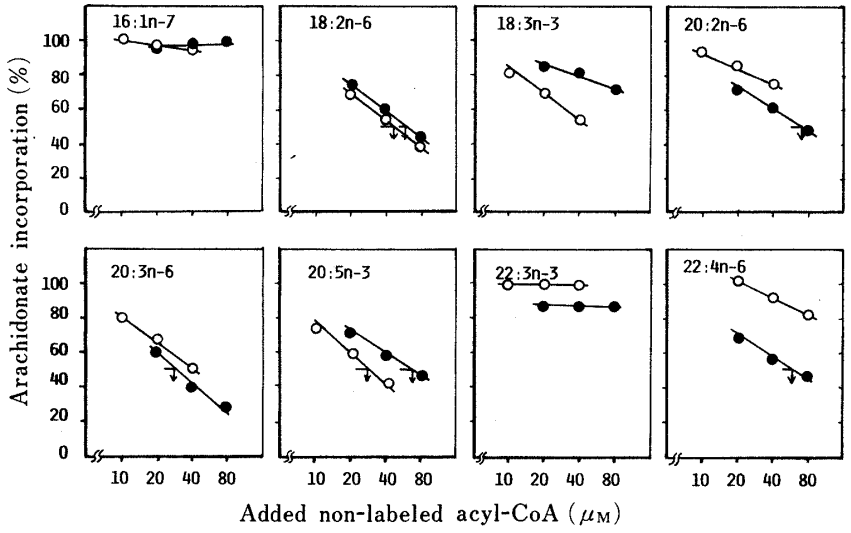

Fig. 1. Determined of CE of Acyl-CoAs in the Acylations of 1-Acyl-GPC and 1-Acyl-GPI in Porcine Platelet Membranes

The assay mixture consisted of ^{3}H-labeled acceptor (1-acyl-GPC or 1-acyl-GPI), ^{14}C-labeled arachidonoyl-CoA, and various concentrations of non-labeled acyl-CoA at a fixed concentration of membrane protein. The incorporation of [^{14}C]arachidonate expressed as percent of the acylation of [^{3}H]1-acyl-GPC (—○—) or [^{3}H]1-acyl-GPI (—●—) was plotted as a function of the exponential of the concentrations of added other acyl-CoAs. EqC was defined as the concentration of other acyl-CoA that yielded 50% of the total acylation of arachidonate incorporation. Typical examples are shown.

hand, the acylations of 1-acyl-GPI were much lower with all the acyl-CoAs examined than in the acylations of 1-acyl-GPC. The CoA esters of 20:4n-6, 18:2n-6, 18:3n-3 and 20:5n-3 were relatively good substrates in the acylation of 1-acyl-GPI but again 22-carbon acyl-CoAs showed very low activities as substrates when maximum velocities were compared.

NII-Electronic Library Service

As a next step, we planned to estimate relative effectiveness of various acyl-CoAs as competitive inhibitors of arachidonoyl-CoA esterification. However, typical enzyme kinetics could not be used in the acyltransferase systems since the enzymes are membrane-bound and substrates bind to membrane proteins and phospholipids non-specifically,[40] which made it difficult to determine absolute concentrations of available substrates, particularly at lower concentrations. Instead, we employed double label experiments using ^{14}C-labeled arachidonoyl-CoA and ^{3}H-labeled acceptors in the presence of various concentrations of non-labeled acyl-CoA and a fixed amount of membrane protein as an enzyme source. Arachidonate incorporation was measured in terms of the amount of ^{14}C-label in diacylphospholipid, total acylation by ^{3}H-label in diacylphospholipid and the incorporation of nonlabeled added acyl-CoA obtained as the difference between ^{3}H and ^{14}C (Fig. 1). Some acyl-CoAs such as 16:1n-7 and 22:3n-3 had no effect on arachidonate incorporation into phosphatidylcholine and phosphatidylinositol, while others such as 18:2n-6, 20:3n-6 and 20:5n-3 were competitively incorporated into phospholipids, resulting in the decrease in arachidonate incorporation.

From the data shown in Fig. 1, the concentrations of nonlabeled acyl-CoA which exhibit 50% of arachidonate incorporation could be calculated and these concentrations were defined as equivalent concentrations (EqC). At this concentration of a given acyl-CoA, arachidonate and the given acyl-CoA can be incorporated equally. The competitive effectiveness (CE) was defined as the ratio of the concentration of arachidonoyl-CoA used to the EqC of the given acyl-CoA; the larger the CE value is, the more effective the given acyl-CoA is as a competitive inhibitor. As shown in Table I (the columns for EqC and CE), some acyl-CoAs such as 18:2n-6, 18:3n-3, 20:3n-6 and 20:5n-3 in the acylation of 1-acyl-GPC, and also 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:5n-3 in the acylation of 1-acyl-GPI showed relatively higher V_{max} values and also higher CE values. However, 20:2n-6 in the acylation of 1-acyl-GPC and 18:3n-3 in the acylation of 1-acyl-GPI had relatively higher V_{max} but lower CE values. On the other hand, 18:3n-6 in the acylation of 1-acyl-GPC and 22:4n-6 in the acylation of 1-acyl-GPI had relatively lower V_{max} but higher CE values. The absence of parallelism between the

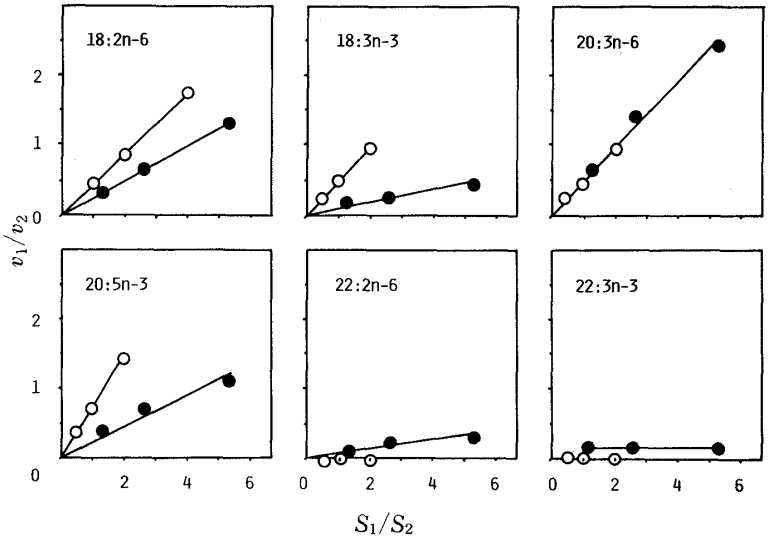

Fig. 2. Determination of Relative K_m Values

The incubation system was the same as described in Fig. 1. The ratio of the rates of incorporation of a given acyl-CoA (v_1) and arachidonoyl-CoA (v_2) was plotted against the ratio of the concentrations of the given acyl-CoA (S_1) and arachidonoyl-CoA [$S_2 = 20\,\mu$M for 1-acyl-GPC (—○—) or $S_2 = 15\,\mu$M for 1-acyl-GPI (—●—)]. The relative K_m values (K_m for arachidonoyl-CoA/K_m for the given acyl-CoA) shown in Table I were estimated from the slopes and the maximum velocities given in Table I as described in the text. Typical examples are presented.

NII-Electronic Library Service

V_{max} values and CE values is interpreted kinetically as being due to the variations of the relative K_m values,[31] as will be described below.

The absolute K_m values for acyl-CoAs cannot be determined accurately for these enzymes in membrane-bound form for the reasons described above. Instead, we derived a kinetic equation to estimate the relative K_m values (not the absolute K_m values). Assuming a single enzyme to be involved in the incorporation of two given acyl-CoAs, the ratio of velocities (v_1/v_2) is a function of the ratio of substrate concentrations (S_1/S_2), the ratio of V_{max} values and the ratio of K_m values.

$$v_1/v_2 = (V^1_{max}/V^2_{max}) \times (K^2_m/K^1_m) \times (S_1/S_2)$$

Plotting v_1/v_2 *vs.* S_1/S_2 gives straight lines (Fig. 2) and the relative K_m values (K^2_m/K^1_m) could be calculated from the slopes in Fig. 2 and V_{max} values given in Table I. The relative K_m values determined this way are shown in the last column of Table I. For some acyl-CoAs, v_1/v_2 *vs.* S_1/S_2 plots did not give straight lines and the ratios of K_m values could not be calculated (Table I). It should be noted that more than 20-fold differences exist in the relative K_m values of the acyl-CoAs examined. These differences, as well as differences in V_{max} values, are considered to affect the CE values.

Ideally, the total acylation of 1-acyl-GPC and 1-acyl-GPI should be constant in the range of added acyl-CoA concentrations examined. However, a significant inhibition of total acylation was seen with 22:4n-6 or 22:6n-3 in the acylation of 1-acyl-GPC, while a slight inhibition was observed with 20:2n-6 or 22:4n-6 in the acylation of 1-acyl-GPI; the inhibitions by other acyl-CoAs were essentially not significant since the IC_{50} values (concentrations required to cause 50% inhibition of total acylation) were very high (Fig. 3). In contrast, 20:3n-6 stimulated the total acylation of 1-acyl-GPC significantly. This kind of inhibition and stimulation of total acylation should be taken into account in choosing effective competitive inhibitors, although such an inhibition may not occur *in vivo*.

The fatty acids which can effectively compete with arachidonate but do not inhibit the total acylation in the esterification step could be candidates for decreasing arachidonate in platelet phospholipids and thereby decreasing the platelet aggregability. From the data shown in Table I, several fatty acids may be effective for this purpose. However, precursors of

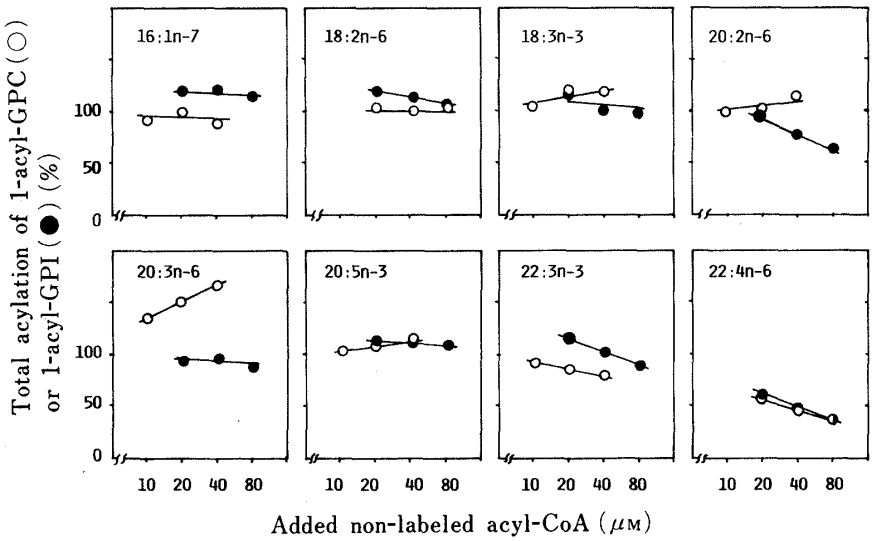

Fig. 3. Total Acylation of 1-Acyl-GPC and 1-Acyl-GPI in the Presence of Arachidonoyl-CoA and Various Concentrations of Other Acyl-CoA

The incubation systems were the same as in Fig. 1. The total amounts of acylation were determined from the ^3H radioactivity in phosphatidylcholine (—○—) and phosphatidylinositol (—●—). Typical examples are presented.

NII-Electronic Library Service

TABLE II. Predicted and Observed Compositions of Major Unsaturated Fatty Acids in Platelet Phospholipids

	18:2 n-6	18:3 n-3	20:3 n-6	20:4 n-6	20:5 n-3	Total n-6	Total n-3	18:2 n-6	18:3 n-3	20:3 n-6	20:4 n-6	20:5 n-3	Total n-6	Total n-3
	(A) Perilla oil diet							(B) Safflower oil diet						
Fatty acids of diet (%)	12.8	64.0	nd	0.2	nd	13.0	64.0	78.0	0.05	nd	0.2	nd	78.2	0.05
Fatty acids of plasma lipid (%)	10.3	1.0	tr	15.6	14.1	25.9	19.2	15.1	tr	tr	35.2	1.5	50.3	1.6
Fatty acids of platelet PC (%)	7.6	1.1	0.5	4.0	3.2	12.1	4.9	6.3	0.2	0.6	10.9	0.2	19.5	0.6
Fatty acids of platelet PI (%)	0.8	tr	1.2	26.3	5.9	28.7	6.9	1.2	tr	0.3	34.5	tr	38.4	0.2
S_1/S_2	0.66	0.06	—	1	0.90	—	—	0.43	0.01>	0.01>	1	0.04	—	—
Predicted (v_1/v_2 in PC)	0.34	0.04	0.01>	1	0.72	—	—	0.22	0.01>	0.01>	1	0.03	—	—
Observed ratio in PC	1.90	0.28	0.13	1	0.80	—	—	0.57	0.02	0.06	1	0.02	—	—
Predicted (v_1/v_2 in PI)	0.18	0.01	0.01>	1	0.23	—	—	0.12	0.01>	0.01>	1	0.01	—	—
Observed ratio in PI	0.03	0.01>	0.04	1	0.22	—	—	0.03	0.01>	0.01	1	0.01<	—	—

Platelets were isolated from rats fed either the perilla oil diet or the safflower oil diet and the fatty acid compositions of lipids were analyzed by gas chromatography. The relative concentrations of acyl donors (S_1/S_2) were calculated from the proportions of fatty acids in the total lipid, where S_2 represents the proportion of 20:4n-6. The v_1/v_2 values were calculated by applying the S_1/S_2 values to Fig. 2. Phosphatidylcholine (PC) and phosphatidylinositol (PI) of platelets from rats fed perilla oil diet (A) or safflower oil diet (B) were examined. The abbreviations nd and tr denote not detectable and trace amount, respectively.

arachidonate such as 18:2n-6, 18:3n-6 and 20:3n-6 may be excluded from the candidates, since the administration of such precursors would increase arachidonate in the long run and be practically of little use. Other n-6 fatty acids such as 22:4n-6 may also be excluded since the retroconversion system would work to produce arachidonate.[41,42] Based on these criteria, only 18:3n-3 and 20:5n-3 remain as such candidates among the fatty acids examined, 20:5n-3 being expected to be a better competitive inhibitor. The administration of 18:3n-3 would produce 20:5n-3 after desaturation and elongation but 18:3n-3 itself is expected to be a weaker competitive inhibitor of arachidonate incorporation into phosphatidylcholine and phosphatidylinositol.

To test the validity of the above considerations, semi-purified diets supplemented with vegetable oil rich in 18:2n-6 or 18:3n-3 were fed to rats and the fatty acid compositions of platelet phosphatidylcholine and phosphatidylinositol were determined (Table II). Dietary 18:2n-6 and 18:3n-3 were significantly desaturated and elongated in rats and were esterified to phospholipids in platelets. The major unsaturated fatty acid was 20:4n-6 in phosphatidylinositol, whereas in phosphatidylcholine 18:2n-6 and 20:4n-6 (as well as 20:5n-3 in the case of perilla oil-diet) were found in comparable amounts.

Assuming that the relative concentrations of polyunsaturated fatty acids available for incorporation into phospholipids in platelets are the same as those in the plasma total lipids under steady-state conditions, S_1/S_2 values were calculated from the fatty acid composition (%) of the total plasma lipids shown in Table II. By applying the S_1/S_2 values to Fig. 2, the predicted proportions of other fatty acids (v_1/v_2 values) were estimated (predicted values shown in Table II). As can be seen in Table II, the predicted values were grossly correlated with the observed proportions of fatty acids in phosphatidylcholine and phosphatidylinositol in the case of relative proportions of eicosanoid precursors (20:4n-6 *vs.* 20:5n-3). On the

NII-Electronic Library Service

other hand, considerable discrepancies were observed in the cases of 18:2n-6 and 18:3n-3. Docosahexaenoate (22:6n-3) and 20:3n-6 were minor components of these phospholipids.

The two kinds of diets contained similar amounts of saturated and monoenoic fatty acids, the major difference being in the proportions of 18:2n-6 and 18:3n-3. Despite a large difference in the proportions of 18:2n-6 and 18:3n-3 in the diets, the difference in the n-3/n-6 ratios of platelet phospholipids was relatively small. Arachidonate (20:4n-6) was highly conserved in phosphatidylinositol, while the fatty acids of phosphatidylcholine were relatively more variable than in phosphatidylinositol (Table II).

Discussion

It has been established that V_{max} value alone does not predict the behavior of a given fatty acid, but K_m value as well as the availability of substrates must also be considered in the esterification step to form phospholipids.[5,31,43] Due to the membrane-bound nature of acyltransferase systems, the micellar nature of substrates and non-specific binding of substrates to membrane proteins and phospholipids, techniques used in typical enzyme kinetics could not be applied directly to acyltransferase systems, but more indirect methods as used here have been applied to obtain relative K_m values for acyl-CoAs under competitive conditions.[5,31,43]

Fatty acid patterns of tissue phospholipids show very little species specificity among higher animals, but a high degree of organ specificity has been recognized. Moreover, fatty acid patterns are quite different among different phospholipid classes. These facts might indicate the presence of a high degree of organ specificity rather than species specificity in the properties of acyltransferase systems as partly described previously.[44,45] As expected, the values of competitive effectiveness determined for acyltransferases were quite different in liver and platelets (Fig. 4). Thus, it is not easy to predict the fate of a given fatty acid when ingested. However, it is practically of no use to perform feeding experiments for many kinds of fatty acids in order to find good competitive inhibitors of arachidonate metabolism. The experiments as shown here might be useful as the first screening for such fatty acids. Relatively good correlations observed between the predicted values for the 20:5n-3/20:4n-6 ratio and the compositions of these acids in platelet lipids (Table II) proved the usefulness of this method and supported the idea that these fatty acids were incorporated into phospholipids mainly through the acyl-CoA:lysophospholipid acyltransferase systems. Eighteen-carbon unsaturated fatty acids and docosahexaenoate are known to be incorporated *via* the *de novo* synthetic pathway in liver.[4,5] This is probably the reason why kinetic parameters determined

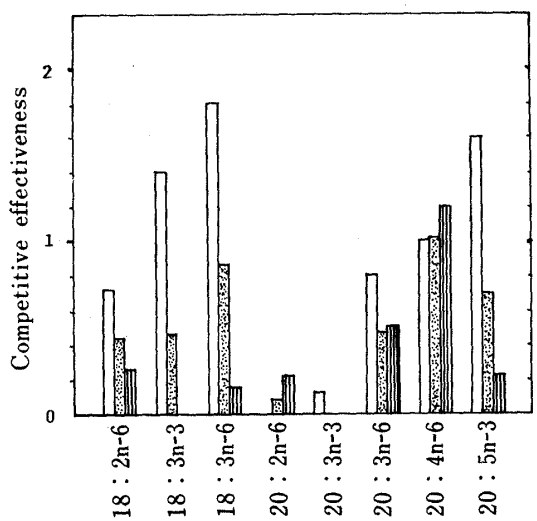

Fig. 4. Comparison of CE of Some Acyl-CoAs in the Acylations of 1-Acyl-GPC and 1-Acyl-GPI

CE of some acyl-CoAs in platelet acyltransferase systems were compared with that in the rat liver acyltransferase system determined previously.[24] □, rat liver 1-acyl-GPC ATf; ▨, platelet 1-acyl-GPC ATf; ▥, platelet 1-acyl-GPI ATf.

NII-Electronic Library Service

in vitro for the acyl-CoA : lysophospholipid acyltransferase systems did not fit well with the *in vivo* phenomena. In order to elucidate the overall mechanism by which various fatty acids are esterified to different phospholipids in platelets, many other enzymes such as acyl-CoA synthetase,[46,47] acyltransferase systems involved in *de novo* synthesis of phospholipids in different organs and phospholipases must also be examined.

Based on these results, 20 : 5n-3 and 18 : 3n-3 can be chosen among the fatty acids examined, (except for some n-6 fatty acids) as good competitive inhibitors of arachidonate esterification. Feeding 20 : 5n-3 has been shown by many workers to be effective in decreasing platelet aggregability[9,13,29,48-50] and we have recently reported beneficial effects of feeding 18 : 3n-3 to rats.[39,51,52]

Acknowledgment We are grateful to Miss Atsuko Moriuchi for her excellent technical assistance.

References

1) A. J. Marcus, H. L. Ullman, and L. B. Safier, *J. Lipid Res.*, **10**, 108 (1969).
2) A. Imai, K. Yano, Y. Kameyama, and Y. Nozawa, *Biochem. Biophys. Res. Commun.*, **103**, 1092 (1981).
3) V. G. Mahadevappa and B. J. Holub, *Biochim. Biophys. Acta*, **713**, 73 (1982).
4) B. Akesson, J. Elovson, and J. Arvidson, *Biochim. Biophys. Acta*, **210**, 15 (1970).
5) H. Okuyama, K. Yamada, and H. Ikezawa, *J. Biol. Chem.*, **250**, 1710 (1975).
6) M. Inoue, S. Murase, and H. Okuyama, *Arch. Biochem. Biophys.*, **231**, 29 (1984).
7) N. Iritani, Y. Ikeda, and H. Kajitani, *Biochim. Biophys. Acta*, **793**, 416 (1984).
8) Y. Kameyama, Y. Yoshioka, A. Imai, and Y. Nozawa, *Biochim. Biophys. Acta*, **752**, 244 (1983).
9) J. Dyerberg and H. O. Bang, *Lancet*, i, 152 (1978).
10) W. E. M. Lands, P. R. Tellier, L. H. Rome, and J. Y. Vanderhoek, *Adv. Biosci.*, **9**, 15 (1973).
11) A. Raz, M. S. Minkes, and P. Needleman, *Biochim. Biophys. Acta*, **488**, 305 (1977).
12) B. R. Culp, B. G. Titus, and W. E. M. Lands, *Prostagl. Med.*, **3**, 269 (1979).
13) P. Needleman, A. Wyche, L LeDuc, S. K. Sankarappe, B. A. Jakschik, and H. Sprecher, *Progr. Lipid Res.*, **20**, 415 (1981).
14) T. K. Bills, J. B. Smith, and M. J. Silver, *J. Clin. Invest.*, **60**, 1 (1971).
15) R. Kannagi, K. Koizumi, and T. Masuda, *J. Biol. Chem.*, **256**, 1177 (1981).
16) S. M. Prescott and P. W. Majerus, *J. Biol. Chem.*, **258**, 764 (1983).
17) R. L. Bell, D. A. Kennerly, N. Stanford, and P. W. Majerus, *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 3238 (1979).
18) S. Rittenhouse-Simmons, *J. Biol. Chem.*, **255**, 2259 (1980).
19) M. Inoue and H. Okuyama, *J. Biol. Chem.*, **259**, 5083 (1984).
20) M. M. Billah, E. G. Lapetina, and P. Cuatrecasas, *J. Biol. Chem.*, **256**, 5399 (1981).
21) E. G. Lapetina, M. M. Billah, and P. Cuatrecasas, *Nature* (London), **292**, 367 (1981).
22) E. J. Neufeld and P. W. Majerus, *J. Biol. Chem.*, **258**, 2461 (1983).
23) E. G. Lapetina, M. M. Billah, and P. Cuatrecasas, *J. Biol. Chem.*, **256**, 5037 (1981).
24) S. M. Prescott and P. W. Majerus, *J. Biol. Chem.*, **256**, 579 (1981).
25) R. W. Walenga, E. E. Opas, and M. B. Feinsterin, *J. Biol. Chem.*, **256**, 12523 (1983).
26) M. J. Broekman, J. W. Ward, and A. J. Marcus, *J. Clin. Invest.*, **66**, 275 (1980).
27) M. J. Broekman, *J. Lipid Res.*, **27**, 884 (1986).
28) H. Takamura, H. Narita, H. J. Park, K. Tanaka, T. Matuura, and M. Kito, *J. Biol. Chem.*, **262**, 2262 (1987).
29) W. E. M. Lands, "Fish and Human Health," Academic Press, 1986.
30) R. J. Apitz-Castro, M. A. Mas, M. R. Cruz, and M. K. Jain, *Biochem. Biophys. Res. Commun.*, **91**, 63 (1979).
31) W. E. M. Lands, M. Inoue, Y. Sugiura, and H. Okuyama, *J. Biol. Chem.*, **257**, 14968 (1982).
32) H. Okuyama, W. E. M. Lands, W. W. Cristie, and F. D. Gunstone, *J. Biol. Chem.*, **244**, 6514 (1969).
33) W. Seubert, *Biochem. Prep.*, **7**, 80 (1960).
34) S. Murase and H. Okuyama, *J. Biol. Chem.*, **260**, 262 (1985).
35) H. Eibl and W. E. M. Lands, *Anal. Biochem.*, **30**, 51 (1969).
36) H. Okuyama and S. J. Wakil, *J. Biol. Chem.*, **248**, 5197 (1973).
37) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
38) E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.*, **37**, 911 (1959).
39) N. Yamamoto, M. Saitoh, A. Moriuchi, M. Nomura, and H. Okuyama, *J. Lipid Res.*, **28**, 144 (1987).
40) R. F. Barden and W. W. Cleland, *J. Biol. Chem.*, **244**, 3677 (1969).
41) C. J. Mann, T. L. Kanduce, P. H. Figard, and A. A. Spector, *Arch. Biochem. Biophys.*, **244**, 813 (1986).

NII-Electronic Library Service

42) H. S. Hansen, B. Jensen, and P. von Wettstein-Knowles, *Biochim. Biophys. Acta*, **878**, 284 (1986).
43) H. Okuyama and W. E. M. Lands, *J. Biol. Chem.*, **247**, 1414 (1972).
44) H. Okuyama, K. Yamada, T. Miyagawa, M. Suzuki, R. Prasad, and W. E. M. Lands, *Arch. Biochem. Biophys.*, **221**, 99 (1983).
45) R. Takenaka, M. Inoue, T. Hori, and H. Okuyama, *Biochim. Biophys. Acta*, **754**, 28 (1983).
46) D. B. Wilson, S. M. Prescott, and P. W. Majerus, *J. Biol. Chem.*, **257**, 3510 (1982).
47) M. Laposata, E. L. Reich, and P. W. Majerus, *J. Biol. Chem.*, **260**, 11016 (1985).
48) Y. Nagakawa, H. Orimo, M. Harasaki, I. Morita, K. Yashiro, and S. Murota, *Atherosclerosis*, **47**, 71 (1983).
49) T. Terano, A. Hirai, T. Hamazaki, S. Kobayasi, T. Fujita, Y. Tamura, and A. Kumagai, *Atherosclerosis*, **46**, 321 (1983).
50) I. Morita, N. Komeshima, R. Takahashi, H. Orimo, and S. Murota, *Prostagl. Leukotri. Med.*, **14**, 123 (1984).
51) I. Watanabe, M. Kato, H. Aonuma, A. Hashimoto, Y. Naito, A. Moriuchi, and H. Okuyama, *Adv. Biosci.*, **62**, 563 (1987).
52) T. Hori, A. Moriuchi, H. Okuyama, T. Sobajima, K. Tamiya-Koizumi, and K. Kojima, *Chem. Pharm. Bull.*, **35**, 3925 (1987).

NII-Electronic Library Service