High Affinity Esterification of Eicosanoid Precursor Fatty Acids by Platelets

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ABSTRACT We have examined the relative rates of uptake of several fatty acids into washed, human platelets by measuring incorporation into cellular phospholipids. In the presence of 15 μ M fatty acid-free albumin and with radioactive fatty acid concentrations of 5–500 nM, esterification into phospholipid was linear with time and platelet concentration and saturable with respect to fatty acid concentration. Two distinct classes of uptake rate were observed. Arachidonate and 5,8,11,14,17-eicosapentaenoate exhibited high affinity, relatively rapid incorporation into platelet phospholipids at pH 6.5: apparent Michaelis constant (K_m) = 30 nM, apparent maximum velocity (V_{max}) = 28 pmol/min per 10^9 platelets. Two other eicosanoid precursors, 5,8,11-eicosatrienoate and 8,11,14-eicosatrienoate, exhibited the same V_{max} , but K_m of 85 and 60 nM, respectively. Under the same conditions, stearate, oleate, and linoleate were incorporated into phospholipids much less efficiently ($V_{max} \sim 8$ pmol/ 10^9 cells per min, apparent $K_m \geq 170$ nM). Qualitatively similar results were found at pH 7.4. Uptake of radiolabeled, rapid-uptake fatty acids was not diminished by the presence of excess, unlabeled, slow-uptake fatty acids. Thus, the specificity of this esterification system resembles that of the arachidonate-specific, long-chain acyl-CoA synthetase present in platelets. It may represent the expression in vivo of the synthetase, although the apparent affinity of the synthetase for fatty acid is much less. This esterification system probably represents the physiologic mechanism for platelet arachidonate uptake, whereby arachidonate is collected from plasma, despite the fact that its concentration is

considerably lower than that of other plasma fatty acids.

INTRODUCTION

When human platelets are incubated with low levels of radiolabeled arachidonic acid (micromolar or less) in plasma or in buffer containing albumin, the label is readily esterified into platelet phospholipids (1, 2). This occurs despite the huge excess in plasma of other fatty acids (3) and the presence in platelets of the arachidonate metabolizing enzymes, cyclooxygenase and 12-lipoxygenase. Another cyclooxygenase substrate, 8,11,14-eicosatrienoic acid (ETA),¹ is also readily esterified (1). In contrast, tracer levels of prevalent plasma fatty acids, palmitate, stearate, oleate, and linoleate are esterified more slowly. Platelets, therefore, contain substantial amounts of these free fatty acids, but virtually no free arachidonate (4).

Wilson et al. (5) have recently described a long-chain acyl-CoA synthetase in isolated platelet membranes that is specific for arachidonate and other eicosanoid (6) precursors (cyclooxygenase or lipoxygenase substrates). This activity is not inhibited by the nonprecursors, stearate, oleate, or linoleate, and therefore is a reasonable candidate for the esterification activity observed in intact platelets. In experiments designed to assess the physiological role of this enzyme, we have found that platelets in albumin-containing buffer exhibit remarkable affinity for uptake and esterification into phospholipids of arachidonate, 8,11,14-ETA, 5,8,11-ETA, and 5,8,11,14,17-eicosapen-

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¹ Abbreviations used in this paper are: EPA, eicosapentaenoic acid; ETA, eicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; TLC, thin-layer chromatography.

taenoic acid (EPA), but not of stearate, oleate, or linoleate.

We report here the behavior of this high affinity system at low fatty acid concentrations (5–500 nM) and compare the substrate specificity of the system in intact platelets to that of the arachidonoyl-CoA synthetase in isolated membranes.

METHODS

Materials. 5,8,11-ETA and [1-¹⁴C]5,8,11-ETA (17 μ Ci/ μ mol) were synthesized as described (7). [¹⁴C]Stearic acid (47 μ Ci/ μ mol), [9,10-³H]oleic acid (10 Ci/mmol), and 1-¹⁴C-labeled linoleic acid, arachidonic acid, and 8,11,14-ETA and EPA (all 55 μ Ci/ μ mol) were purchased from New England Nuclear (Boston, MA). Unlabeled EPA and essentially fatty acid-free bovine albumin were from Sigma Chemical Co. (St. Louis, MO); other fatty acids were from Nu-Check Prep., Inc. (Elysian, MN).

Platelets were obtained from normal human volunteers who had abstained from aspirin for 2 wk or who had taken 1,300 mg of aspirin in the 24 h preceding phlebotomy. The cells were washed as described previously (8). Platelets were resuspended to 10^9 /ml in "pH 6.5 phosphate buffer" (26.2 mM sodium and 6.8 mM potassium phosphate, pH 6.5; 118 mM NaCl, 5.6 mM glucose) and kept at 23°C for up to 4 h before use. Incubations with fatty acids were done either in pH 6.5 phosphate buffer or in "pH 7.4 Tris buffer" (15.4 mM Tris-Cl, pH 7.4; 140 mM NaCl, 5.6 mM glucose).

Fatty acid uptake. Fatty acid uptake was assayed as radioactivity appearing in platelet phospholipids after incubation with radioactive fatty acids. The standard assay was performed at 37°C in 1.5 ml (total volume) of pH 6.5 phosphate buffer. The platelet concentration was 5×10^7 /ml, the radioactive fatty acid concentration was from 5 to 500 nM. Fatty acid-free albumin was present at 1 mg/ml (15 μ M). In some experiments, 1 μ M unlabeled fatty acids was included in reaction mixtures.

For each experiment, platelets (10^9 /ml in pH 6.5 phosphate buffer) and the remainder of each reaction mixture were warmed to 37°C. Each reaction was started by adding platelets and vortexing the mixture gently, and was stopped by 1-min centrifugation in a microfuge (Eppendorf). Some reactions were stopped directly by extraction with chloroform/methanol, in the case of arachidonate uptake. Since similar results were obtained with both methods, the centrifugation method was used thereafter. The supernatant labeling medium was aspirated and the cell pellet was resuspended in 150 μ l of H₂O, plus 1 ml of CHCl₃/CH₃OH/concentrated HCl (50:50:0.3, vol/vol/vol). After the pellet was completely dispersed, the phases were split with 0.3 ml of 1 N HCl and 5 mM EGTA. The CHCl₃ phase was dried under N₂, then analyzed by thin-layer chromatography (TLC) on activated silica gel 60 plates (E. Merck, Darmstadt, West Germany) in a solvent system of ethyl ether, *n*-hexane/glacial acid (70:30:1) (9). In this system, phospholipids remain at the origin and free fatty acid migrates with $R_f = 0.59$. These regions were scraped and radioactivity was determined in 3a70 fluor (Research Products International Corp., Mt. Prospect, IL) in a Beckman LS7000 scintillation spectrometer (Beckman Instruments, Inc., Palo Alto, CA).

[³H]12-hydroxyeicosatetraenoic acid (HETE) production. 100 nmol of [³H]arachidonate, 10^5 cpm/nmol, ([5,6,8,9,11,12,14,15-³H]arachidonate, 110 Ci/mmol, Amersham Corp. [Arlington Heights, IL], diluted with unlabeled fatty

acid) was incubated with 20×10^9 aspirin-treated platelets in 20 ml of pH 7.4 Tris buffer at 37°C, for 15 min, in the presence of 2 U/ml bovine thrombin and 1 mM Ca⁺⁺. The reaction mixture was acidified with HCl and extracted twice with 2 vol of ethyl ether. The organic phases were pooled and dried. HETE was isolated by TLC in the solvent system described above, then scraped and eluted with methanol. 25% of the radioactivity added to the platelets was recovered in this TLC fraction.

[¹⁴C]15-HETE was prepared from [¹⁴C]arachidonate according to the method of Crawford et al. (10). Long-chain acyl-CoA synthetase was assayed as described by Wilson et al. (5).

RESULTS

Measurement of radiolabeled phospholipid synthesis was an accurate reflection of total fatty acid uptake. Under the conditions described here, measurement of radioactivity at the origin of a neutral-lipid TLC system (phospholipids) accounts quantitatively for uptake of fatty acid, in a manner indicative of a platelet enzymatic function.

(a) Platelet pellet-associated radioactivity. Over 90% of esterified radiolabeled fatty acid in extracted platelet pellets was present in phospholipids, as opposed to di- or triglycerides. Free fatty acid in the pellets amounted to 1% or less of the total fatty acid present in the labeling medium, so long as fatty acid-free albumin was present in large molar excess (not shown). Boiled platelet blanks exhibited no phospholipid synthesis whatsoever, but trapped amounts of free substrate equal to or greater than those in intact platelets. The free fatty acid was <15% of total pellet-associated radioactivity for eicosanoid precursors, and up to 30% for other fatty acids.

(b) Albumin was a necessary component of the reaction mixture. In its absence, from 3 to 25% of the labeled free fatty acid in the reaction medium was associated with the platelet pellet. This percentage varied among individual fatty acids, making local concentrations in the platelet membrane quite variable. It was therefore impossible to compare substrates for esterification in the absence of albumin.

(c) Removal of the unreacted labeling medium by centrifugation and aspiration was important because commercial supplies of radiolabeled fatty acids contain up to 5% impurities that migrate with phospholipids in our TLC system.

(d) The assay under standard conditions (described in Methods) was linear with time for ≥ 15 min at maximal fatty acid concentrations (Fig. 1).

(e) The assay was linear with platelet concentration from 5×10^7 /ml up to the physiological range (Fig. 2). Higher platelet concentrations caused nonlinearity because much of the substrate is consumed very rapidly.

FIGURE 1 Phospholipid synthesis vs. time at maximal (500 nM) substrate concentrations. [^3H]oleate (X), [^{14}C]arachidonate (Δ), or 5,8,11-ETA ($\square$) were incubated at 37° with 5×10^7 platelets/ml.

FIGURE 2 Radioactive phospholipid synthesis as a function of platelet concentration. Different amounts of platelets were incubated for 2 min with 75 nM [^{14}C]arachidonate (Δ) or 50 nM [^{14}C]5,8,11-ETA ($\square$); or for 5 min with 55 nM [^{14}C]linoleate ($\bullet$). Phospholipids were then isolated and counted as described in Methods.

Two classes of uptake behavior were distinguished. Fig. 3 shows the dependence of esterification of radiolabeled fatty acid on concentration of substrate. Arachidonate, EPA, 5,8,11-ETA, and 8,11,14-ETA constituted a "rapid-uptake" class. The "slow-uptake" group comprised stearate, oleate, and linoleate. When the data for Fig. 3 were plotted in double-reciprocal form, the difference in rates was readily seen to be due both to a greater apparent affinity for rapid-uptake fatty acids, and a higher apparent maximum velocity (Table I, A). Qualitatively similar results were seen if pH 7.4 Tris buffer was substituted for pH 6.5 phosphate buffer in the standard assay mixture (Table I, B).

Esterification "efficiency" for various fatty acids can be compared by the parameter maximum velocity/Michaelis constant ($V_{\max}/K_m$; higher $V_{\max}$ or lower K_m corresponds to higher efficiency). According to this criterion, arachidonate was utilized most effectively at either pH 6.5 or 7.4, followed closely by EPA, then 8,11,14- and 5,8,11-ETA. Arachidonate efficiency, moreover, was 10–90-fold greater than that of the slow-uptake fatty acids (Table I). The magnitude of this difference was much greater than observed in previous studies (1, 2), presumably because the earlier

FIGURE 3 Initial rates of radioactive phospholipid synthesis vs. substrate concentration. Two classes of uptake rate were distinguished. Rapid-uptake fatty acids, arachidonate (Δ), EPA ($\blacktriangle$), 5,8,11-ETA ($\square$), and 8,11,14-ETA ($\blacksquare$), were incubated for 5 min in the standard assay conditions (see Methods). Slow-uptake fatty acids, stearate ($\circ$), oleate ($\times$), and linoleate ($\bullet$) were incubated for 10 min.

TABLE I
Efficiency of the High Affinity Uptake System for Various
Substrates at pH 6.5 and 7.4

	Apparent K_m	Apparent V_{max}	Efficiency	Efficiency vs. arachidonate
	nM	pmol/ min/ 10^9	V_{max}/K_m	%
A pH 6.5				
Stearate	230	11	0.048	9.9
Oleate	170	2.5	0.015	1.9
Linoleate	230	4.8	0.021	2.7
5,8,11-ETA	84	26	0.31	41
8,11,14-ETA	61	26	0.42	56
Arachidonate	34	26	0.76	100
EPA	28	16	0.59	77
B pH 7.4				
Stearate	No saturation observed			
Oleate	375	2.6	0.007	1.1
Linoleate	480	6.7	0.014	2.1
5,8,11-ETA	57	24	0.42	64
8,11,14-ETA	184	46	0.25	38
Arachidonate	77	51	0.66	100
EPA	43	27	0.62	95

The data shown in Fig. 3 were replotted in double-reciprocal form to give apparent K_m (column 1) and V_{max} values (column 2) for each fatty acid at pH 6.5. Data for a pH 7.4 experiment were similarly treated. The ratio, apparent V_{max} /apparent K_m (column 3) is an index of the relative efficiency with which the uptake system can esterify these fatty acids. Column 4 expresses these efficiencies compared with arachidonate (set to 100%).

uptake measurements were done at saturating label concentrations (≥ 500 nM) for long periods of time, thereby obscuring the effect of the high affinity system.

Platelets also contained a low affinity esterification system with relatively high capacity for phospholipid synthesis. In studies using much higher (50–300 μ M) fatty acid concentrations, platelets have been shown to esterify oleate (11) and linoleate (12) into phospholipids with apparent $V_{max} \sim 100$ pmol/min per 10^9 platelets. The affinity for oleate and linoleate esterification observed in these studies was too low to contribute to the rates observed in Fig. 3.

It might be argued that the low rates of uptake observed for radiolabeled, common fatty acids (stearate, oleate, and linoleate) were an artifact of dilution by endogenous pools of these fatty acids. However, at the platelet concentrations of the standard assay, 5×10^7 /ml, the cells could contribute only 50 nM or less to each reaction (4). This could raise the apparent K_m of the slow-uptake fatty acids only slightly, and it could not account for the diminished V_{max} .

Slow-uptake fatty acids cannot compete with rapid-uptake fatty acids for esterification. Table II shows

the results of an experiment in which several unlabeled fatty acids were tested for their ability to inhibit the formation of radiolabeled phospholipid when present in 14-fold excess over radioactive substrate. It is clear that although 5,8,11-ETA and arachidonate could compete for each other's uptake, oleate and linoleate could not inhibit the esterification of these fatty acids when present in 14-fold excess. Unlabeled 5,8,11-ETA was somewhat less effective as a competing fatty acid than arachidonate, presumably because it was less efficiently recognized by the uptake system (Table I).

Similar results were found in every pair-wise combination tested. Thus, EPA and 8,11,14-ETA uptake were inhibited by arachidonate, but not by oleate, and stearate had no effect on phospholipid synthesis from rapid-uptake fatty acids (not shown). We conclude that slow-uptake fatty acids are not recognized by the esterification site for rapid-uptake substrates, although the converse may be true, since arachidonate inhibited linoleate uptake (Table II).

Measurement of platelet arachidonate uptake from plasma. To assess the physiological role of the high affinity system, we assayed [14 C]arachidonoyl-phospholipid synthesis by platelets in plasma. Blood was collected from eight healthy volunteers after an overnight fast. Washed platelets and platelet-poor plasma were prepared from each blood sample. Radiolabeled arachidonate, 10–1,000 nM, was added to samples of plasma and allowed to equilibrate for 1 h. Platelets (5×10^7 /ml) were then added back to each plasma sample and esterification was measured as described in Methods for standard assays. At 1 μ M [14 C]arachidonate, the rate of uptake of label was 7.7 ± 2.3 pmol/min per 10^9 platelets. Since we (unpublished observation) and others (13) find that fasting

TABLE II
Inhibition of High Affinity Fatty Acid Uptake by Excess
Unlabeled Fatty Acids

Radiolabeled fatty acid (70 nM)	Uptake				
	Unlabeled fatty acid (1 μ M)				
	None	Oleate	Linoleate	5,8,11-ETA	Arachidonate
pmol radioactive phospholipid/ 10^9 platelets					
Oleate	9	3	6	3	6
Linoleate	17	9	9	7	6
5,8,11-ETA	134	115	131	27	17
Arachidonate	270	217	243	104	24

Standard assay mixtures containing 70 nM radiolabeled fatty acid were incubated for 10 min at 37°C with or without competing unlabeled fatty acid (1 μ M), then extracted to determine radioactive phospholipid synthesis, as described in Methods.

plasma arachidonate is $\sim 10 \mu\text{M}$, the actual arachidonoyl phospholipid synthesis was ~ 11 -fold higher than the rate of label uptake, or 85 pmol/min per 10^9 platelets. This value is close to the maximal rate observed in assays with pH 7.4 Tris buffer of 51 pmol/min per 10^9 cells (Table I). In Tyrode's buffer, similarly, we found that V_{max} for esterification was 50 pmol/min per 10^9 platelets in the presence of 10 mM EDTA, and 100 pmol/min per 10^9 platelets without EDTA (not shown). Thus, the high affinity esterification system can largely account for the arachidonate uptake observed in platelet-rich plasma.

High affinity fatty acid esterification by intact platelets was similar to long-chain acyl-CoA synthesis by platelet membranes. Our present results correspond closely with the fatty acid specificity previously reported for arachidonoyl-CoA synthetase. An exception is that radiolabeled 5,8,11-ETA was a rapid-uptake substrate for intact platelets, while unlabeled 5,8,11-ETA was previously reported to be unable to inhibit synthesis of [^{14}C]arachidonoyl-CoA in vitro (5). To resolve this discrepancy, we reexamined 5,8,11-ETA as a substrate for arachidonoyl CoA synthetase (Table III). Using freshly synthesized ^{14}C -labeled and unlabeled 5,8,11-ETA, we found that this fatty acid was recognized about one-third as well as arachidonate or, EPA as a substrate for esterification and as an inhibitor of this process. Our previous conclusion (5) is therefore incorrect, perhaps because the unlabeled 5,8,11-ETA used in earlier inhibition studies was chemically degraded. Table IV lists the similarities between the arachidonoyl-CoA synthetase and high affinity uptake by intact platelets.

12-HETE and 15-HETE were not substrates for the high affinity esterification system. Neutrophils (14) and macrophages (15) can incorporate products of

their own lipoxygenase pathways into cellular phospholipids. We assayed esterification of radiolabeled 12-HETE (a platelet product) and 15-HETE (a macrophage product) to see if intact platelets might have a similar capacity at submicromolar levels of these metabolites. In 10-min standard assays we were unable to detect any radiolabeled phospholipid synthesis from these compounds (the limit of detection was ~ 5 – 10% of the linoleate uptake rate). We conclude that if platelets do form hydroxylated acyl-phospholipids, either the substrate must be endogenous or the exogenous concentration must be very high.

DISCUSSION

We have demonstrated that intact platelets contain a phospholipid synthesizing system with very high affinity for exogenous arachidonate and other C_{20} polyunsaturated fatty acids. C_{18} saturated and unsaturated fatty acids are used much less efficiently by platelets and cannot compete with arachidonate for uptake. In this regard, the intact cell system is identical to the arachidonate-specific, acyl-CoA synthetase described by Wilson et al. (5). The enzyme, studied in isolated platelet membranes, has different apparent kinetic parameters, although the catalytic efficiencies for arachidonate (i.e., 0.86 vs. $1.5 \text{ mmol} \cdot \text{min}^{-1} \cdot \text{M}^{-1}$ per 10^9 platelets, respectively) and other eicosanoid precursors are approximately the same, as shown in Table IV.

It is likely that the present results reflect the in vivo expression of the arachidonoyl-CoA synthetase. The apparent K_m of the synthetase in vitro may be 200-fold higher because of nonspecific binding of fatty acid substrate to membranes and membrane proteins in the assay mixture that are not normally exposed in intact platelets. The small V_{max} of esterification we measured in vivo, 150-fold lower than the V_{max} of the synthetase, may be a function of less-than-optimal reaction conditions in intact cells (for example, CoA concentrations may be suboptimal intracellularly).

Another possible explanation for the disparity between in vivo and in vitro measurements is that high affinity arachidonate uptake reflects the presence of a specific carrier for arachidonate entry into platelets, distinct from the arachidonoyl-CoA synthetase, but with similar fatty acid specificity. This seems unlikely, since long-chain fatty acids can diffuse readily into and out of platelet membranes (12). It is also unlikely that the rate-limiting step in arachidonoyl-phospholipid synthesis occurs at a step beyond formation of arachidonoyl-CoA, since total acyl-CoA levels in platelets are always exceedingly low ($\sim 0.05 \text{ nmol}/10^9$ platelets; 5).

Platelet esterification of arachidonate is relatively independent of albumin concentration. At fatty acid

TABLE III

Apparent K_m and K_i Values for Arachidonoyl-CoA Synthetase Assayed with Arachidonate, 5,8,11-ETA, and EPA as Substrates

Radiolabeled substrate	Apparent K_m	Inhibitor	Apparent K_i
	μM		μM
[^{14}C]Arachidonate	14	—	—
[^{14}C]EPA	12	—	—
[^{14}C]5,8,11-ETA	27	—	—
[^{14}C]Arachidonate	—	Arachidonate	9
[^{14}C]Arachidonate	—	EPA	8
[^{14}C]Arachidonate	—	5,8,11-ETA	34

Platelet membranes were incubated with varying concentrations of radiolabeled fatty acids (6– $150 \mu\text{M}$) and long-chain acyl CoA formation was assayed (5). K_m values were determined by Lineweaver-Burk plots. For inhibition studies, unlabeled inhibitors ($170 \mu\text{M}$) were added to reaction mixtures containing [^{14}C]arachidonate.

TABLE IV
Comparison of Fatty Acid Esterification by Intact Platelets to Long-chain
Acyl-CoA Synthesis by Platelet Membranes

	Intact platelets	Isolated membranes
Arachidonate esterification	$V_{\max} \cong 77 \text{ pmol/min/}10^9 \text{ platelets}$ $K_m \cong 51 \text{ nM}$	$V_{\max} \cong 12 \text{ nmol/min/}10^9 \text{ platelets}$ $K_m \cong 14 \text{ }\mu\text{M}$
Catalytic efficiency, $V_{\max}/K_m$ ($\text{mmol} \cdot \text{min}^{-1} \cdot \text{M}^{-1}/10^9 \text{ platelets}$)	1.5	0.86
Relative catalytic efficiency for:		
Arachidonate	100	100
EPA	100	110
5,8,11-ETA	33	50
8,11,14-ETA	38	50

Esterification in vivo, assayed in pH 7.4 Tris buffer as described in Methods, is compared with a representative assay of arachidonoyl-CoA synthetase using four eicosanoid precursors as substrates. The two precursors exhibit similar catalytic efficiencies for these substrates. Furthermore, neither process is inhibited by nonprecursors (stearate, oleate, or linoleate).

concentrations $<0.1 \text{ }\mu\text{M}$ in albumin-free solution, uptake into phospholipids has apparent $V_{\max}$ and K_m values similar to those obtained in our standard assay using $15 \text{ }\mu\text{M}$ albumin (data not shown). Furthermore, arachidonate esterification in plasma ($550 \text{ }\mu\text{M}$ albumin) also gives uptake rates comparable to our standard assay. We cannot explain this apparent albumin independence. It has proven to be very difficult even to quantitate binding of long-chain fatty acids to albumin (16); the problem seems even more complex in the presence of platelet membranes and an active platelet esterification system. Albumin molecules have one fatty acid binding site with a dissociation constant (K_D) approaching 10^{-8} M , and another with $K_D \sim 10^{-7} \text{ M}$ (16). Thus, according to the relationship, $K_D = [(\text{free fatty acid})(\text{free albumin})]/(\text{bound fatty acid})$, with $15 \text{ }\mu\text{M}$ total albumin in our assay mixtures, 50 nM total arachidonate corresponds to $\sim 40 \text{ pM}$; and 500 nM arachidonate, to $\sim 340 \text{ pM}$ free fatty acid. Because of this albumin binding, free fatty acid levels are also exceedingly low in plasma, although total unesterified fatty acid approaches 1 mM in fasting subjects (3, 17). We and others (3, 13) found that unesterified plasma arachidonate is $\sim 10 \text{ }\mu\text{M}$ or 1% of the total. Since plasma albumin is $\sim 550 \text{ }\mu\text{M}$, free fatty acid concentrations will be in the micromolar, rather than millimolar range, and free arachidonate will constitute only a small percentage of this amount.

Arachidonate turnover, as well as accumulation, is unique among platelet fatty acids. Chambaz et al. (18) have shown that unstimulated platelets, prelabeled with ^{14}C -fatty acid, release labeled arachidonate from cellular phospholipids much more rapidly than oleate or linoleate. Esterified arachidonate may turn over as fast at $5\text{--}6 \text{ nmol/h}$ per 10^9 platelets ($80\text{--}100 \text{ pmol/}$

$\text{min/}10^9$ cells; 2); this turnover may be related to phospholipid remodeling. The reason that high affinity arachidonate esterification may be necessary in platelets is that to the extent that free arachidonate is generated in the process of altering the fatty acid composition of phospholipids, it must be collected by a specific esterification system. Otherwise this released arachidonate would be replaced by the more prevalent platelet and plasma free fatty acids.

Exogenous arachidonate at submicromolar concentrations apparently enters phospholipids not by *de novo* synthesis (acylation of glycerol phosphate) but via transacylation of lysophospholipids (Lands pathway). We have shown that phosphatidic acid in unstimulated platelets turns over with $t_{1/2} \sim 2\text{--}4 \text{ min}$ ($80\text{--}300 \text{ pmol/min}$ per 10^9 platelets; 19). Given this turnover rate, phosphatidate is never labeled with exogenous radioactive arachidonate enough to account for its accumulation in phospholipids (unpublished observation). The determinants of specificity for acyl-transferase reactions are not known; presumably, the acyl-CoA synthetase cannot account for preferential transacylase incorporation of arachidonate. McKean et al. (20) have shown that platelets contain a lysophosphatidylcholine acyl transferase activity that prefers unsaturated acyl-CoA, but this activity lacks the absolute arachidonate specificity of the acyl CoA synthetase (20).

The demand for acyl CoA in platelets, for *de novo* synthesis of phospholipids alone, is $160\text{--}600 \text{ pmol/min}$ per 10^9 platelets (two times the phosphatidate synthetic rate). It is therefore clear that the high affinity uptake described here accounts for only a small portion of total fatty acid esterification. Nevertheless, an uptake system for arachidonate with very high affinity

but low capacity can serve two important roles. First, it can efficiently scavenge arachidonate from plasma despite low free arachidonate levels and high concentrations of other fatty acids. Second, it can keep free arachidonate levels within unstimulated platelets so low that cyclooxygenase and lipoxygenase have negligible reaction rates. Upon stimulation, platelets release enough arachidonate to overwhelm this system completely (5–20 nmol/10⁹ platelets in <1 min [1, 21]), thereby allowing production of thromboxane and 12-HETE.

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