Discovery of an Arachidonoyl Coenzyme A Synthetase in Human Platelets*

David B. Wilson, Stephen M. Prescott, and Philip W. Majerus

From the Division of Hematology-Oncology, Departments of Internal Medicine and Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri 63110

Platelets contain small amounts of a variety of free fatty acids but essentially no free arachidonate. When free fatty acids are incubated with platelets, there is preferential incorporation of arachidonic acid and 8,11,14-eicosatrienoic acid compared to other fatty acids. We now explain these findings by the discovery that platelets contain two long chain acyl-CoA synthetases. One shows activity with a range of different fatty acids, similar to long chain acyl-CoA synthetases studied previously. A crude platelet membrane preparation contains this enzyme that catalyzes the formation of 0.75 nmol of oleoyl-CoA/min/10^9 platelets. The other enzyme is specific for the prostaglandin precursors arachidonic acid and 8,11,14-eicosatrienoic acid. Based on the ability of fatty acids to inhibit arachidonate and 8,11,14-eicosatrienoate activation, we conclude that other fatty acids including linoleic, 5,8,11-eicosatrienoic, and oleic acids are not substrates for the enzyme. Platelet membranes catalyze formation of 2.9 nmol of arachidonoyl-CoA/min/10^9 platelets and 2.5 nmol of 8,11,14-eicosatrienoyl-CoA/min/10^9 platelets. Arachidonoyl-CoA synthetase has optimal activity at pH 8 and requires ATP (K_m = 0.5 mm), Mg^2+ (K_m = 2.5 mm), CoA (K_m = 0.13 mm), and arachidonic acid (K_m = 0.03 mm). We propose that the arachidonate-specific acyl-CoA synthetase may control the level of free arachidonic acid in platelets, limiting prostaglandin synthesis by the unstimulated cell and capturing free arachidonate from extracellular sources.

When platelets are stimulated by thrombin, arachidonic acid is rapidly liberated from membrane phospholipids. The level of free arachidonate in the cell dramatically increases, and prostaglandin and thromboxane synthesis is initiated (for review see Ref. 1). In unstimulated platelets free arachidonate is present in only trace quantities compared to other long chain fatty acids (2,3). Low free arachidonate levels prevent active prostaglandin production by the unstimulated cells. These levels are maintained when arachidonic acid is added to platelets; exogenous radiolabeled arachidonic acid is efficiently taken up from solutions containing albumin and esterified into platelet phospholipids without the production of oxygenated arachidonic acid metabolites (4). Another cyclooxygenase substrate, 8,11,14-eicosatrienoic acid, is also readily incorporated into cellular phospholipid (2). In contrast, fatty acids which are not prostaglandin precursors, such as palmitic, stearic, oleic, and linoleic acids, are esterified into platelet phospholipids at a relatively low rate. As a result, a significant pool of these four free fatty acids exists as such in the cell (2,5). Although the preferential incorporation of arachidonic acid and 8,11,14-eicosatrienoic acid into platelet phospholipid has been documented, the mechanism for this process has remained unidentified.

The first step in the incorporation of fatty acids into phospholipids involves the formation of an acyl-CoA ester from a fatty acid, CoA, and ATP, and is catalyzed by long chain acyl-CoA synthetase. This enzyme has been examined in a number of tissues and purified from rat liver microsomes and mitochondria (6-9). Most acyl-CoA synthetases examined, including the rat liver enzymes, exhibit a broad specificity with respect to fatty acid substrate. Some reports, however, have argued that a given cell type may contain multiple long chain acyl-CoA synthetases, each exhibiting a different range of specificity (10-14). For example, the fungus Candida lipolytica has been shown to have two distinct long chain acyl-CoA synthetases which differ in chain length specificity and function (12-14). To date, though, no mammalian cell has been shown to possess a highly specific long chain acyl-CoA synthetase.

We now report that human platelets have two distinct long chain acyl-CoA synthetase activities, one of which is highly specific for the prostaglandin precursors, arachidonic acid and 8,11,14-eicosatrienoic acid. This highly specific acyl-CoA synthetase activity can explain the low free arachidonate levels in unstimulated platelets and the efficient incorporation of arachidonic acid into platelet phospholipid. A second platelet long chain acyl-CoA synthetase exhibiting lower activity and broader specificity permits incorporation of other fatty acids into cellular lipid.

MATERIALS AND METHODS

Materials—Palmitic, stearic, oleic, linoleic, 8,11,14-eicosatrienoic, and arachidonic acids were obtained from Nu Chek Prep, Elysian, MN. 5,8,11-Eicosatrienoic and 5,8,11,14,17-eicosapentaenonic acids were gifts from Dr. Philip Needleman. [9,10-^3H]Palmitic (20 Ci/ mmol), [9,10-^3H]oleic (5 Ci/mmol), 8,11,14-[1-^14C]eicosatrienoic (55 mCi/mmol) and [1-14C]arachidonic acids (55 mCi/mmol) were purchased from New England Nuclear. ATP (dissodium salt), GTP (disodium salt), and N-ethylmaleimide were from Sigma. Coenzyme A (lithium salt) was purchased from P-L Biochemicals. Silica Gel G plates were obtained from Merck, Darmstadt, FRG, and were activated by heating at 110 °C for 1 h prior to use. Bovine thrombin was generously provided by Dr. Craig Jackson.

Preparation of Platelet Membrane Fraction—Washed human platelets were prepared as described elsewhere (15) and suspended at a concentration of 3 x 10^10/ml in a standard phosphate buffer consisting of 26.2 mM sodium phosphate and 6.8 mM potassium phosphate, pH 6.5, 318 mM sodium chloride, and 5.6 mM glucose. The suspension was incubated at room temperature while it was sonicated (100 watts,
3 bursts, 36 s each). The sonicated platelets were then centrifuged at 48,000 g for 1 h at 4 °C, and the supernatant was discarded. The pellet, enriched in platelet membranes and depleted of cytosolic components, was resuspended at a concentration of 5 × 10^7/ml in standard phosphate buffer and stored at −15 °C.

**Preparation of Rat Liver Microsomes**—Three frozen livers from 150-g rats were homogenized in 90 ml of chilled 0.25 M sucrose by 3 strokes of a Teflon pestle tissue homogenizer. The homogenate was centrifuged at 23,000 × g for 20 min at 4 °C to remove nuclei and cellular debris. Centrifugation of the resulting supernatant at 81,000 g for 1 h at 4 °C produced a pellet of microsomes which was suspended in 1 ml of standard phosphate buffer and stored at −15 °C.

**Assay of Long Chain Acyl-CoA Synthetase Activity**—The isotope assay, modified from that described previously (16), relies on the extraction of unreacted fatty acids and the insolubility of long chain acyl-CoA esters in heptane. The standard reaction mixture contained 15 μM of Tris-HCl, pH 8.0, 3 μM of MgCl₂, 1 μM of ATP, 100 nmol of CoA, 150 nmol of 2-mercaptoethanol, 0.3 μM of Triton X-100, 40 μl of 0.5 mM solution of radiolabeled fatty acid (0.5 μCi/μmol) in 50 mM NaHCO₃, and a source of enzyme in a total volume of 0.15 ml. The reaction was initiated by the addition of enzyme. After incubation at 37 °C for 10 min, the reaction was terminated by the addition of 2.25 ml of isopropanol, heptane, 2 M sulfuric acid (40/10/1, by volume). A 1.5-ml portion of heptane and 1 ml of water were then added and the mixture was vortexed vigorously. The upper phase was discarded and the aqueous phase was extracted twice with 2 ml of heptane containing 4 mg/ml of carrier palmitic acid. A 1-ml sample of the aqueous phase was counted in 4 ml of 3α70 scintillation fluid (Research Products, Inc., Elk Grove Village, IL).

**Identification of Reaction Product**—The product of the arachidonoyl-CoA synthetase was identified by the procedure of Mishina et al. (17). The aqueous extract from a sonicated reaction was subjected to thin layer chromatography on Silica Gel G with isopropanol/pyridine/acetic acid/water (60/15/1/25, by volume). About 80% of the radioactivity co-chromatographed with authentic arachidonoyl-CoA (R₂ = 0.29). The aqueous extract from the reaction mixture was also fractionated with neutral hydroxylamine, and the hydroxylamine derivative chromatographed on Silica Gel G with chloroform/methanol/water (95/5/0.5, by volume). The radioactive product derivative co-chromatographed with authentic arachidonoyl hydroxamate (R₂ = 0.19).

**Assay of Long Chain Acyl-CoA Hydrolase Activity**—Long chain acyl-CoA hydrolase activity in platelets was measured as the disappearance of heptane-insoluble radioactivity from a reaction mixture containing radiolabeled acyl-CoA ester. The reaction mixture consisted of 15 μM of Tris-HCl, pH 8.0, 3 μM of MgCl₂, 150 nmol of 2-mercaptoethanol, 0.3 μM of Triton X-100, 40 μl of 0.5 mM solution of radiolabeled acyl-CoA ester in a total volume of 0.15 ml. The reaction was initiated by the addition of platelet membranes. After incubation at 37 °C for varying lengths of time, the reaction was terminated and the mixture was separated into aqueous and heptane phases by about 95%. A 10-min incubation with N-ethylmaleimide (10 mM) reduced activity by 95%. Both 2 mM EDTA and 20 mM 2-mercaptoethanol decreased activity by about 25%. Finally, a 10-fold increase in the amount of Triton X-100 from the assay system decreased acyl-CoA synthetase activity by about 10–30% in various experiments in both frozen-sonicated platelets and platelet membranes. The platelet membrane fraction was used in all subsequent experiments. The effect of various compounds on arachidonoyl-CoA synthetase activity was investigated. Replacement of ATP with GTP in the incubation mixture resulted in a complete loss of activity. A 10-min incubation with N-ethylmaleimide (10 mM) reduced activity by 95%. Both 2 mM EDTA and 20 mM 2-mercaptoethanol decreased activity by about 25%. Finally, a 10-fold increase in the amount of Triton X-100 in the assay system had a mild inhibitory effect on the reaction.

**TABLE I**

| Activity | Frozen sonicated platelets | Platelet membranes |
|----------|---------------------------|-------------------|
| Complete system | 0.59 g/10 mM | 2.0                  |
| ATP | 0.45 | 0 |
| CoA | 0.13 | 0 |
| Mg²⁺ | 0.19 | 0.05 |
| Triton X-100 | 0.53 | 1.5 |

**RESULTS**

Assay of Long Chain Acyl-CoA Synthetase in Platelet Membrane Fraction—The assay used to test the enzymatic conversion of heptane-extractable radiolabeled fatty acid into water-soluble acyl-CoA esters. In preliminary experiments we found that crude sonicates and a membrane fraction of human platelets catalyzed the incorporation of arachidonate into arachidonoyl-CoA. The reaction was linear with time for up to 30 min with platelet extract up to an amount equivalent to 0.6 × 10^7 platelets/ml. The product was shown to be arachidonoyl-CoA by co-chromatography with arachidonoyl-CoA and by its conversion to arachidonoyl hydroxamate and co-chromatography with this compound as described under "Materials and Methods."
Arachidonoyl-CoA synthetase activity in platelet membranes followed Michaelis-Menten kinetics with respect to fatty acid, CoA, ATP, and Mg\(^{2+}\). The apparent Michaelis constant obtained for each of the substrates is listed in Table II.

**Long Chain Acyl-CoA Hydrolase Activity**—Acyl-CoA hydrolase activity in platelet membranes was investigated to determine whether the product of the acyl-CoA synthetase reaction was being rapidly degraded. Hydrolase activity was measured as the disappearance of heptane-insoluble radiolabel from a reaction mixture containing \([^{14}C]\)arachidonoyl-CoA or \([^{3}H]\)palmitoyl-CoA. Hydrolysis was found to be linear over 30 min (20% of substrate hydrolyzed). The activity varied in different experiments but was less than 0.13 nmol of acyl-CoA hydrolyzed/min/10\(^{8}\) platelets. The hydrolysis reaction was therefore insignificant compared to the acyl-CoA synthetase reaction.

**Fatty Acid Specificity of Platelet Acyl-CoA Synthetase**

| Substrate    | Apparent \(K_m\) \(\mu M\) |
|--------------|--------------------------|
| ATP          | 500                      |
| Mg\(^{2+}\)  | 2500                     |
| CoA          | 130                      |
| Arachidonic acid | 30                      |

**Activity**—Competition experiments were used to examine the fatty acid specificity of platelet acyl-CoA synthetase activity. Varying amounts of \([^{14}C]\)arachidonic acid were incubated in the standard assay system with different unlabeled fatty acids at a concentration of 100 \(\mu M\). As shown in Fig. 1, platelet membranes catalyzed the synthesis of \([^{14}C]\)arachidonoyl-CoA with a \(V_{\text{max}}\) of 2.9 nmol/min/10\(^8\) platelets. Unlabeled arachidonic acid competitively inhibited the formation of \([^{14}C]\)arachidonoyl-CoA with a \(K_i\) (38 \(\mu M\)) approximately equal to the \(K_m\) of 30 \(\mu M\). A second cyclooxygenase substrate, 8,11,14-eicosatrienoic acid, also competed with \([^{14}C]\)arachidonate for the enzyme, but not as well as unlabeled arachidonate (\(K_i = 88 \mu M\)). By contrast, oleic acid, which is not a prostaglandin precursor, did not inhibit the formation of \([^{14}C]\)arachidonoyl-CoA. This experiment was repeated 3 times with the same results as in Fig. 1. In separate experiments (data not shown) stearic, linoleic, and 5,8,11-eicosaatrienoic acids were found to give the same result as oleic acid. This observation of arachidonoyl-CoA synthetase activity which is not competitively inhibited by olate, linoleate, or stearate could account for the low free arachidonate concentration in resting platelets. The fact that 5,8,11-eicosatrienoic acid was ineffective as an inhibitor while the 8,11,14-isomer was effective suggests that the fatty acid specificity of this acyl-CoA synthetase is similar to that of cyclooxygenase.

8,11,14-[\(^{14}C\)]eicosatrienoic acid activation was examined in the presence of arachidonate, oleate, and 8,11,14-eicosatrienoate. The results, which are shown in Fig. 2, complement those in Fig. 1. 8,11,14-Eicosatrienoyl-CoA synthetase activity (\(V_{\text{max}} = 2.5 \text{ nmol/min/10}^{8}\) platelets) was inhibited by arachi-
Acyl-CoA synthetase activities have at least two long chain acyl-CoA synthetase activities. The activation of arachidonic, 8,11,14-eicosatrienoic, and oleic acids, then the activation of oleoyl-CoA synthesis, we suggest that platelet membranes contain acyl-CoA synthetase activity which can use arachidonic acid or 8,11,14-eicosatrienoic acid as a substrate. The activation of arachidonate is slightly preferred over 8,11,14-eicosatrienoate. Neither arachidonoyl-CoA nor 8,11,14-eicosatrienoyl-CoA formation is inhibited by oleic acid.

Platelets must be able to incorporate fatty acids that are not prostaglandin precursors into phospholipids. The conversion of [3H]oleic acid into [3H]oleoyl-CoA was therefore measured in the presence of unlabeled fatty acids. As shown in Fig. 3, oleoyl-CoA synthetase activity differed from arachidonoyl-CoA and 8,11,14-eicosatrienoyl-CoA synthetase activities in several respects. The V_max of 0.75 nmol of oleoyl-CoA formed/min/10^9 platelets is lower than observed with the other two. Hence, the differences in the rates of incorporation of arachidonic, 8,11,14-eicosatrienoic, and oleic acids into platelet membrane phospholipid are reflected in the rates of synthesis of the fatty acyl-CoAs. The enzyme catalyzing the activation of [3H]oleic acid (K_m = 41 μM) was not as specific as the enzyme activating the other two fatty acids tested. [3H]Oleoyl-CoA formation was inhibited to varying degrees by arachidonic acid (K_i = 170 μM), 8,11,14-eicosatrienoic acid (K_i = 130 μM), unlabeled oleic acid (K_i = 39 μM), and linoleic acid (K_i = 38 μM, data not shown). If the same enzyme catalyzed the activation of arachidonate, 8,11,14-eicosatrienoic, and oleic acids, then the K_m for each fatty acid as a substrate should be equal to its K_i in inhibiting the activation of one of the other fatty acids. Since the K_m for arachidonate activation (30 μM) is quite different from the K_i of arachidonate (170 μM) in inhibiting oleoyl-CoA synthesis, we suggest that platelet membranes have at least two long chain acyl-CoA synthetase activities. One activity is specific for arachidonate and 8,11,14-eicosatrienoate, while a second activity is responsible for the activation of other fatty acids such as oleic and linoleic acids.

In addition, Fig. 3 rules out the trivial explanation that differences in fatty acid partitioning between hydrophobic and hydrophilic phases in the assay system account for the differences in acyl-CoA synthetase activity for the various substrates. If the high rate of arachidonic acid activation were due to preferential access to the enzyme to arachidonate, then arachidonic acid would inhibit [3H]oleoyl-CoA formation better than unlabeled oleic acid.

Heat Inactivation of Acyl-CoA Synthetase Activities—Samples of platelet membranes were heated at 45 °C for varying times and subsequently assayed for arachidonoyl- and oleoyl-CoA synthetase activities. As shown in Fig. 4, oleoyl-CoA synthetase activity was relatively stable (t_1/2 = 31.5 min) compared to arachidonoyl-CoA synthetase activity (t_1/2 = 8 min). This finding confirms the presence of two different enzymes as inferred from the competition experiments. In the experiment shown arachidonoyl-CoA synthetase was measured in the presence of unlabeled oleate (100 μM) to eliminate any conversion due to the ability of the oleoyl-CoA synthetase to activate arachidonate. This potential problem was significant only at 20 and 30 min of heating where the arachidonate-specific enzyme was largely inactivated while significant oleoyl-CoA synthetase remained. Further evidence for two separate enzymes comes from the finding that in some preparations of microsomes oleoyl-CoA synthetase was lost upon repeated freezing and thawing without significant loss of arachidonoyl-CoA synthetase. We have solubilized both enzymes using Triton X-100 but have been thus far unable to resolve the two activities.

Comparison of Arachidonoyl-CoA Synthetase Activities in Platelets and Rat Liver Microsomes—In order to demonstrate clearly that platelet arachidonoyl-CoA synthetase from platelets differed in fatty acid specificity from previously studied acyl-CoA synthetases we compared the platelet activity to that present in the rat liver microsomes. Varying amounts of unlabeled fatty acids were added to 20 μM [3H]oleoyl-CoA, and the incorporation of [3H]oleoyl-CoA into platelet membrane phospholipids was measured. The percentage of unlabeled fatty acids that could be incorporated into platelet membranes was determined. The incorporation of [3H]oleoyl-CoA into platelet membranes was linear with increasing amounts of unlabeled fatty acids added to the reaction mixture.

**Fig. 3.** Inhibition of oleoyl-CoA synthetase activity by arachidonic, 8,11,14-eicosatrienoic, and oleic acids. The conversion of [3H]oleic acid into [3H]oleoyl-CoA was assayed as described under "Materials and Methods." The reaction mixture contained 15 μmol of Tris-HCl, pH 8.0, 3 μmol of MgCl₂, 1 μmol of ATP, 100 nmol of Triton X-100, varying amounts of sodium [3H]oleate (0.5 μCi/μmol), membranes from 10⁸ platelets, plus 15 nmol of the following unlabeled inhibitor fatty acids in a total volume of 0.15 ml: ○, no inhibitor; ▲, arachidonic acid; ◆, 8,11,14-eicosatrienoic acid; and △, oleic acid.
arachidonic acid in the standard assay mixture containing either platelet membranes or rat liver microsomes as a source of enzyme. The results are shown in Fig. 5. As in the previous experiments, arachidonoyl-CoA synthetase was inhibited most efficiently by unlabeled arachidonate and moderately inhibited by 8,11,14-eicosatrienoic acid. Eicosapentaenoic acid, a poor cyclooxygenase substrate, was a slight inhibitor. Oleic, linoleic, stearic, and 5,8,11-eicosatrienoic acids did not inhibit platelet arachidonoyl-CoA synthetase activity. In contrast, liver microsome acyl-CoA synthetase activity was diminished by the presence of linoleate, oleate, stearate, 8,11,14-eicosatrienoate, and arachidonate.

To estimate the relative inhibition of arachidonoyl-CoA synthetase activity by the various fatty acids, the data in Fig. 5 were plotted as 1/V versus inhibitor concentration (Dixon plot). The slope of this plot is given by $K_{inhibitor}^{-1}[S]/V_{max}$. Since $K_m$ and $V_{max}$ are constant, the slope is inversely proportional to $K_i$. Comparing the slopes for the platelet enzyme, the following relative $K_i$ values were obtained: arachidonate = 1.0, 8,11,14-eicosatrienoate = 3.9, eicosapentaenoate = 9.2, linoleate, 5,8,11-eicosatrienoate, stearate, and oleate did not inhibit. These results are consistent with the $K_i$ values obtained from the Lineweaver-Burk plot in Fig. 1. The relative $K_i$ values calculated for the rat liver microsomal enzyme were: linoleate = 1.0, oleate = 1.1, 8,11,14-eicosatrienoate = 2.2, arachidonate = 3.0, stearate = 3.4, eicosapentaenoate = 5.7.

The rat liver microsomal enzyme and platelet enzyme were also compared at different pH values. The arachidonoyl-CoA synthetase activity from platelet membranes and rat liver microsomes exhibited early nearly identical pH profiles, similar to that obtained by Bar-Tana et al. (8). Optimal activity was at pH 8.0. The standard acyl-CoA synthetase assay was used in these measurements except that pH was varied with Tris-maleate (pH 5.5-8.5) and glycine-NaOH (pH 8.75-9.50) buffers.

**Long Chain Acyl-CoA Levels During Thrombin Stimulation—**Long chain acyl-CoA levels in thrombin-stimulated platelets were determined by the modified phosphotransacetylase assay as described under "Materials and Methods." Although the level of free fatty acids in platelets is known to increase during the first 15 min of thrombin stimulation (3), no significant change in the level of long chain acyl-CoA esters was observed over this period. The level remained at about 50 pmol of long chain acyl-CoA/10³ platelets. Acyl-CoA transferase activity in platelets may be high enough that no transient accumulation of acyl-CoA occurs during thrombin stimulation.

**Effect of Ca²⁺ on Arachidonoyl-CoA Synthetase Activity—**The effect of increasing Ca²⁺ concentrations on arachidonoyl-CoA synthetase activity was examined since, if arachidonoyl-CoA synthetase were sensitive to Ca²⁺ concentration, the rise in intracellular Ca²⁺ concentration associated with the platelet release reaction might provide a means to regulate the enzyme. With the concentration of magnesium set at 3 mM, calcium ion was varied from 0-5 mM. Roughly linear inhibition from 0-20% was observed over this range. This is relatively insignificant inhibition, since most Mg²⁺-requiring enzymes are inhibited somewhat by Ca²⁺.

**DISCUSSION**

The uptake of free fatty acids by platelets has been examined previously, and a preferential incorporation of free arachidonic acid and 8,11,14-eicosatrienoic acids into membrane phospholipids has been observed. Bills et al. (4) found that when [¹⁴C]arachidonate was added to platelet-rich plasma, radiolabel was rapidly incorporated into platelet phospholipids without the accumulation of radioactively free fatty acid, production of oxygenated [¹⁴C]arachidonic acid metabolites, or appreciable β-oxidation of the added fatty acid. In a separate report (2) the same authors noted that exogenous 8,11,14-eicosatrienoic acid was incorporated into platelet phospholipid better than oleic or linoleic acid, but not as well as arachidonic acid. Spector et al. (5) observed that radiolabeled palmitate, stearate, oleate, and linoleate were taken up by platelets, but tended to accumulate in the cell membrane as free fatty acids. The authors concluded that the most important factor in regulating the amount of free palmitate, stearate, oleate, and linoleate in platelets was the concentration of free fatty acid in the surrounding medium. Similarly, Nordøy et al. (22) found a highly significant correlation between the amount of stearic acid in plasma and platelets. A significant correlation was also noted for oleic acid, but no correlation was found for polyunsaturated fatty acids such as arachidonate. Our results indicate that the specific long chain acyl-CoA synthetase activity in platelets may be responsible for the selective incorporation of arachidonic acid and 8,11,14-eicosatrienoic acid into cellular phospholipids. The specific acyl-CoA synthetase activity (2.9 nmol of arachidonoyl-CoA formed/min/10⁸ platelets and 2.5 nmol of 8,11,14-eicosatrienoyl-CoA formed/min/10⁸ platelets) was greater than that of a second platelet long chain acyl-CoA synthetase that uses oleic acid as a substrate.
stimulation, leaving open the possibility that acyl-CoA synthetase may permit platelets to incorporate fatty acids such as oleate, linoleate, stearate, and palmitate into phospholipids and neutral lipids.

Platelet arachidonate-specific acyl-CoA synthetase activity has some properties in common with the extensively investigated acyl-CoA synthetase from rat liver microsomes. Both enzymes require ATP, CoA, Mg++, and fatty acid for activity. Moreover, the apparent M values for ATP, CoA, and Mg++ are similar for the two enzymes (8). The platelet arachidonate-specific and the rat liver enzymes also have the same pH activity profile, with an optimum at pH 8. Despite these similarities, the two enzymes differ in fatty acid specificity as shown by competition experiments. Unlike the platelet activity, arachidonoyl-CoA synthetase by rat liver microsomes is inhibited competitively by a number of fatty acids including linoleate, oleate, stearate, and 8,11,14-eicosatrienoate.

There are no previous reports of a highly specific long chain acyl-CoA synthetase in a mammalian cell. Most of the tissue preparations examined including rat liver (6, 8, 21, 23) and adipose tissue (16), exhibit acyl-CoA synthetase activities with broad fatty acid specificity. Some reports have argued that multiple long chain acyl-CoA synthetases with differing fatty acid specificities may exist in a given cell (11). On the basis of differences in CoA requirement, Pande and Mead (10) proposed that rat liver microsomes contain two different activation systems—one for long chain saturated fatty acids and the other for unsaturated fatty acids. However, subsequent work by Bar-Tana et al. (8) and Normann and co-workers (23) indicated that only one acyl-CoA synthetase was present in rat liver microsomes. The fungus Candida lipolytica exhibits two distinct acyl-CoA synthetases which differ in fatty acid specificity and function (12, 13). Acyl-CoA synthetase I is responsible for the production of acyl-CoA ester to be used in the synthesis of cellular lipids. Acyl-CoA synthetase II exhibits a broader substrate specificity with respect to fatty acid and provides acyl-CoA for β-oxidation.

We propose that arachidonate-specific acyl-CoA synthetase controls the level of free arachidonic acid in platelets by diverting arachidonic acid taken up from plasma away from cyclooxygenase and into membrane phospholipids. In this way the level of free arachidonate in the platelet is kept low and prostaglandin production is limited. We have determined that acyl-CoA levels in platelets do not change during thrombin stimulation, leaving open the possibility that acyl-CoA synthetase activity is modulated during the release reaction or alternatively that acyl transferase activities are so great that no acyl-CoA accumulates even transiently. The arachidonate-specific acyl-CoA synthetase may only be important in prostaglandin-producing cells. Most studies to date have used liver or adipose tissue as a source of enzyme. The acyl-CoA esters synthesized in these two tissues are mainly used for synthesis of triglycerides, not the regulation of arachidonate levels in the cell. Preliminary results indicate that the microsomal fraction of sheep seminal vesicles, another prostaglandin-producing tissue, exhibits arachidonate-specific acyl-CoA synthetase activity. Further research may reveal the presence of the activity in other prostaglandin-producing cells.

Acknowledgments—We wish to thank Teresa Brose for assistance in the completion of this study. Helpful discussions with Dr. Douglas Tollefsen are also gratefully acknowledged.

REFERENCES

1. Rittenhouse-Simmons, S., and Deykin, D. (1981) in Platelets in Biology and Pathology (Gorden, J. L., ed) Vol. 2, pp 349-371, Elsevier/North Holland, New York
2. Bills, T. K., Smith, J. B., and Silver, M. J. (1977) J. Clin. Invest. 60, 1-6
3. Broekman, M. J., Ward, J. W., and Marcus, A. J. (1961) J. Biol. Chem. 236, 8271-8274
4. Bills, T. K., Smith, J. B., and Silver, M. J. (1976) Biochim. Biophys. Acta 424, 303-314
5. Spector, A. A., Hoak, J. C., Warner, E. D., and Fry, G. L. (1970) J. Clin. Invest. 49, 1489-1496
6. Tanaka, T., Hosaka, K., Hoshinaru, M., and Numa, S. (1979) Eur. J. Biochem. 98, 165-172
7. Philipp, D. P., and Parsons, P. (1979) J. Biol. Chem. 254, 10776-10784
8. Bar-Tana, J., Rose, G., and Shapiro, B. (1971) Biochim. J. 122, 353-362
9. Bar-Tana, J., and Rose, G. (1973) Biochem. J. 131, 443-449
10. Pande, S. V., and Mead, J. F. (1968) J. Biol. Chem. 243, 352-361
11. Ass, M. (1975) Biochim. Biophys. Acta 386, 32-47
12. Kamiryo, T., Mishina, M., Tashiro, S., and Numa, S. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 4947-4950
13. Mishina, M., Kamiryo, T., Tashiro, S., and Numa, S. (1978) Eur. J. Biochem. 82, 347-354
14. Hosaka, K., Mishina, M., Tanaka, T., Kamiryo, T., and Numa, S. (1979) Eur. J. Biochem. 93, 197-203
15. Baenziger, N. L., and Majerus, P. W. (1974) Methods Enzymol. 31, 149-155
16. Banis, R. J., and Tove, S. B. (1974) Biochim. Biophys. Acta 348, 210-220
17. Stadtman, E. R. (1965) Methods Enzymol. 1, 596-599
18. Jacobs, R. A., and Majerus, P. W. (1973) J. Biol. Chem. 248, 8392-8401
19. Fett, R. C., Lands, W. E. M., Christie, W. W., and Holman, R. T. (1968) J. Biol. Chem. 243, 2241-2246
20. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1961) J. Biol. Chem. 236, 285-275
21. Philipp, D. P., and Parsons, P. (1979) J. Biol. Chem. 254, 10785-10790
22. Nordey, A., Bjerge, J. M., and Strom, E. (1973) Acta Med. Scand. 193, 59-64
23. Normann, P. T., Thomasen, M. S., Christiansen, E. N., and Flatmark, T. (1981) Biochim. Biophys. Acta 664, 416-427
Discovery of an arachidonoyl coenzyme A synthetase in human platelets.
D B Wilson, S M Prescott and P W Majerus

J. Biol. Chem. 1982, 257:3510-3515.

Access the most updated version of this article at http://www.jbc.org/content/257/7/3510

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/257/7/3510.full.html#ref-list-1