Phospholipid biosynthesis in human platelets

The acylation of lyso-platelet-activating factor

Margaret L. MCKEAN and Melvin J. SILVER

Cardeza Foundation, Department of Medicine and the Department of Pharmacology, Thomas Jefferson University, Philadelphia, PA 19107, U.S.A.

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Acyl-CoA:1-alkyl-*sn*-glycero-3-phosphocholine acyltransferase of human platelets is membrane-bound, has a pH optimum of 7.5, is insensitive to 1 mM-Mg²⁺, is inhibited by 1 mM-Ca²⁺, and is stimulated slightly by 1 mM-EDTA. Maximal formation of 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine is observed at 150 μ M-1-alkyl-*sn*-glycero-3-phosphocholine and 20 μ M unsaturated fatty acyl-CoA. The transfer of unsaturated fatty acyl groups to 1-alkyl-*sn*-glycero-3-phosphocholine is 3–14 times slower than to 1-acyl-*sn*-glycero-3-phosphocholine. The CoA esters of linoleate and arachidonate, two unsaturated fatty acyl groups commonly found in platelet phospholipids, are the preferred fatty acyl group donors.

PAF is a biologically active phospholipid that is a potent inducer of platelet aggregation and secretion of granule contents. This activity is independent of that of ADP and the products of arachidonic acid metabolism (Henson, 1977; Cazenave *et al.*, 1979; McManus *et al.*, 1981; Marcus *et al.*, 1981; Chesney *et al.*, 1982; Lotner *et al.*, 1980). In addition, PAF causes hypotension and mediates immediate hypersensitivity reactions, including associated bronchoconstriction and inflammation (Blank *et al.*, 1979; Henson & Pinckard, 1977; Benveniste *et al.*, 1977; Vargaftig *et al.*, 1980). It is a unique phospholipid whose structure has been shown to be 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (Benveniste *et al.*, 1979; Hanahan *et al.*, 1980). PAF is formed in response to specific stimuli by various types of white blood cells (Mencia-Huerta & Benveniste, 1979, 1981; Lynch *et al.*, 1980; Clark *et al.*, 1980). Human and rabbit platelets produce PAF in response to the divalent cation ionophore A23187 (Alam *et al.*, 1983a). More recently, the production of PAF by human platelets treated with the physiological stimuli thrombin and collagen has been demonstrated

conclusively in this laboratory (Alam *et al.*, 1983b).

A homologue of PAF containing long chain unsaturated fatty acids at C-2 (alkylacyl-GPC) may play an important role in the biosynthesis and metabolism of PAF by platelets and other cells. Rat alveolar macrophages incorporate radioactivity from [alkyl-1,2-³H]lysoPAF into their membrane lipids and this is accompanied by increases in the radioactivity of alkylacyl-GPC (Albert & Snyder, 1983). When these cells are stimulated, radioactive PAF and lysoPAF are released into the medium with concomitant decreases in the radioactivity of alkylacyl-GPC, suggesting that alkylacyl-GPC may be the precursor of PAF. Alkylacyl-GPC is also a metabolite of PAF that is formed when PAF is incubated with human platelets or neutrophils (Alam *et al.*, 1983c; Chilton *et al.*, 1983a).

An important pathway for the insertion of arachidonic acid and other unsaturated fatty acids into phospholipids involves the acylation of lyso-phospholipids with fatty acid groups derived from fatty acyl-CoA esters. Lands & Crawford (1976) have reviewed earlier studies on acyl-CoA transferases from various species and tissues and cells other than platelets. We have previously demonstrated that platelets also contain a similar membrane-bound fatty acyl-CoA transferase which may be important for the incorporation of unsaturated fatty acyl groups into 1-acyl-GPC (McKean *et al.*, 1982). However, this enzyme has a lower affinity for arachidonoyl-CoA compared

Abbreviations used: PAF, 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor); lysoPAF, 1-alkyl-*sn*-glycero-3-phosphocholine; 1-acyl-GPC, 1-acyl-*sn*-glycero-3-phosphocholine(2-lysophosphatidylcholine); alkylacyl-GPC, 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine; diacyl-GPC, 1,2-diacyl-*sn*-glycero-3-phosphocholine (phosphatidylcholine); Nbs₂, 5,5'-dithiobis-(2-nitrobenzoic acid).

with oleoyl-CoA and transfers the CoA esters of oleic, linoleic, and arachidonic acids at similar rates. We also have evidence that human platelets have fatty acyl-CoA transferase activities that selectively transfer arachidonate from arachidonoyl-CoA to other 2-lysophospholipids (M. L. McKean & M. J. Silver, unpublished work). These preliminary findings and our published reports suggest that there are several fatty acyl-CoA transferases in platelets or that there is one enzyme which will accept various 2-lysophospholipids as substrates. In the present paper we describe the properties of a fatty acyl-CoA transferase which inserts unsaturated fatty acyl groups into the 1-alkyl-2-lysophospholipid lysoPAF to form alkyl-acyl-GPC and discuss the role of this enzyme in the biosynthesis and metabolism of PAF.

Parts of this work were presented at the April 1982 meeting of the Federation of American Societies for Experimental Biology, New Orleans, and at the August 1983 meeting of the American Society of Pharmacology and Experimental Therapeutics, Philadelphia.

Experimental procedures

Materials

Unsaturated fatty acyl chlorides were obtained from Nuchek, Elysian, MN, U.S.A. 1-Acyl-GPC (prepared from egg phosphatidylcholine with snake venom phospholipase A₂), and stearoyl-CoA were purchased from Avanti Biochemicals, Birmingham, AL, U.S.A. Phosphatidylcholine gave one spot on t.l.c. [chloroform/methanol/acetic acid/water (100:65:14:6, by vol.)]. Dithiothreitol was purchased from Sigma and Nbs₂ from Aldrich. CoA, lithium salt (Chromatopure) was obtained from P-L Biochemicals. Di[1-¹⁴C]-palmitoyl phosphatidylcholine and [alkyl-1,2-³H]-PAF were purchased from New England Nuclear. Authentic alkylacyl-GPC for t.l.c. standard was generously given by Dr. Robert L. Wykle. Dr. David Ahern generously provided [alkyl-1,2-³H]-lysoPAF. Polypropylene tubes and pipettes were used throughout. The general experimental conditions were the same as we described elsewhere (McKean *et al.*, 1982).

Purity of lysoPAF

LysoPAF was purchased from Avanti Biochemicals, Birmingham, AL, U.S.A. Since it was prepared from beef heart phosphatidylcholine, we had to determine whether there was contamination with 1-acyl-GPC. A sample of the lysoPAF used in the spectrophotometric assay was subjected to alkaline hydrolysis followed by extraction (Dawson, 1967). Any contaminating 1-acyl-GPC would

be converted to glycerol 3-phosphate by this procedure and lysoPAF would remain unchanged. The organic and aqueous phases obtained were then analysed for phosphorus. Essentially all of the original lipid phosphorus was found in the organic phase. No phosphorus was detected in the aqueous phase (limits of detection were 2.5 nmol of phosphorus, or 0.56% of the starting material). It was calculated that the lysoPAF used for these experiments was >99% lysoPAF with respect to possible contamination with 1-acyl-GPC.

Preparation of platelet microsomes

Special precautions were taken to remove contaminating white blood cells from the human platelet concentrates used to prepare platelet microsomes. Pooled platelet concentrates were centrifuged at 250g for 15 min at 22°C to sediment red and white blood cells. The supernatant platelet concentrate was carefully removed without disturbing the layer which contained the red and white blood cells and centrifuged at 250g for 15 min at 22°C. The platelet concentrate was again carefully removed without disturbing the pellet. A sample of the platelet concentrate diluted 1:10–1:20 with 0.154M-NaCl solution was placed on a haemocytometer slide and examined for white blood cell contamination by phase microscopy. No white blood cells were observed in six of 14 preparations of pooled platelet concentrates. White blood cell contamination in the remaining pooled platelet concentrates averaged 0.03% of the platelet count (range 0.02–0.09%).

Platelet microsomes were prepared as described previously from platelet concentrates (treated as described above to remove contaminating white cells) which were washed free of plasma, suspended in buffer, and stored at –80°C (McKean *et al.*, 1982). The microsomes were then washed to remove dithiothreitol and resuspended in 85 mM-phosphate buffer, pH 7.5. In some experiments, dithiothreitol was removed by dialysis against the same buffer.

Protein was determined by our modification of the method of Lowry *et al.* (1951) described previously (McKean *et al.*, 1982) with bovine serum albumin as standard.

Preparation of fatty acyl esters of CoASH

Unsaturated fatty acyl-CoA esters were synthesized according to the procedure of Bishop & Hajra (1980) and purified by the method of Seubert (1960). The products, dissolved in 10 mM-citrate/phosphate buffer, pH 5.9, were characterized as described previously (McKean *et al.*, 1982). To prevent oxidation, sodium ascorbate, 2.5–5.0 mM final concentration, was added to the CoA ester solutions (McKean *et al.*, 1982).

Fatty acyl-CoA transferase assay

Enzyme activity was measured by the spectrophotometric assay of Lands & Hart (1965). This assay was used previously to measure the rate of reacylation of 1-acyl-GPC (McKean *et al.*, 1982). Briefly, in the spectrophotometric assay changes in absorbance were recorded at room temperature using a Unicam SP.1750 dual-beam spectrophotometer as described by Baker & Thompson (1973). The assay mixture in the sample cuvette consisted of 0.8 ml of 85 mM-phosphate buffer, pH 7.5, 0.1 ml of 3.3 mM-Nbs₂, 0.1 ml of 1.5 mM-lysoPAF in distilled water, and 1–4 μ l of fatty acyl-CoA (18–21 μ M final concentration). In experiments in which the concentration of lysoPAF was varied, 0.1 ml of 100–2500 μ M-lysoPAF was added to the cuvette. The mixture in the reference cuvette was similar except that 0.1 ml of water was substituted for the lysoPAF solution. Correction for acyl-CoA hydrolase is intrinsic in this assay. The microsomal preparation (35–50 μ l, 100–115 μ g of protein) was added to each cuvette to initiate the reaction. Initial rates were calculated from the continuous record of enzyme activity. No reaction was observed in the absence of enzyme or when either substrate was incubated alone in the presence of enzyme.

Characterization and quantification of the product

Samples were incubated at room temperature until a plateau on the reaction progress curve was achieved. Control samples to measure time 0 enzyme activity were also prepared. The incubations were terminated by the addition of chloroform/methanol (1:2, v/v) and the samples were extracted (Bligh & Dyer, 1959). The pooled chloroform layers were evaporated to dryness and applied to t.l.c. plates which were developed in chloroform/methanol/acetic acid/water (100:65:14:6, by vol.). After brief exposure to iodine vapour, the phosphatidylcholine zone, in which alkylacyl-GPC cochromatographs, was scraped off and eluted with 10 ml of methanol. After evaporation to dryness under N₂, the samples were subjected to alkaline hydrolysis and extraction (Dawson, 1967). The organic layer containing lysoPAF was evaporated to dryness under N₂ and the residue was acetylated with acetic anhydride, using *NN*-dimethyl-4-aminopyridine as catalyst (Gupta *et al.*, 1977). After evaporation to dryness under N₂, the samples were applied to t.l.c. plates which were developed as before. Following brief visualization with iodine vapour, the zones cochromatographing with authentic PAF standard were scraped off and eluted with 10 ml of methanol. The samples were evaporated to dryness under N₂ and dissolved in

Tris/Tyrodes albumin buffer, pH 7.4 (Alam *et al.*, 1983a).

Rabbit blood was collected by cardiac puncture into 1/10 volume of 3.8% sodium citrate. Platelet-rich plasma was obtained following centrifugation at 220g for 10 min. The platelets were washed by a modification of the method of Minkes *et al.* (1977). Briefly, platelet-rich plasma was chilled at 4°C for 20 min and enough 0.1 M-EDTA (pH 7.4) was added to achieve 5 mM. The platelets were sedimented by centrifugation at 1800g for 6 min at 4°C and 10 ml of Tris/saline buffer, pH 7.4 (Alam *et al.*, 1983a), warmed to 37°C, was added. The platelets were incubated at 37°C for at least 10 min and gently resuspended. They were washed two additional times by centrifugation at 800g for 6 min at room temperature and resuspended as described above in Tris/saline buffer. After the final centrifugation, the platelets were suspended in Tris/Tyrodes albumin buffer and the platelet count was adjusted to 3.5×10^8 /ml.

The amount of PAF contained in the derivatized samples was determined by bioassay using aggregation of washed rabbit platelets. The platelet suspension (0.5 ml) was stirred at 1000 rev./min at 37°C in an aggregometer (Chrono-Log, Havertown, PA, U.S.A.). The extent of aggregation produced by the derivatized samples at 1 min was compared with the extent of aggregation produced by known amounts of standard semisynthetic PAF, both dissolved in Tris/Tyrode's albumin buffer. The amount of alkylacyl-GPC in the sample prior to derivatization was calculated using the bioassay data corrected for recovery of the lipid sample during the various derivatization steps. Di-[1-¹⁴C]palmitoyl phosphatidylcholine was used to monitor recovery of alkylacyl-GPC during the extraction and t.l.c. of the original sample for the spectrophotometric assay. [³H]PAF and [³H]-lysoPAF were used as recovery markers for the alkaline hydrolysis and acetylation steps.

Results

Properties of platelet acyl-CoA:1-alkyl-sn-glycero-3-phosphocholine acyltransferase

This enzyme activity was localized in the high speed microsomal fraction prepared from sonicated platelets. Activity was low or undetectable in other platelet subcellular fractions. Maximal fatty acyl group transfer was observed at pH 7.5 with oleoyl-CoA (Fig. 1). Linoleoyl-CoA gave similar results. These findings are similar to the pH optimum we found for fatty acyl group transfer to 1-acyl-GPC (McKean *et al.*, 1982). Initial velocity was linear up to at least 160 μ g of microsomal protein, and 100–115 μ g of protein was routinely used for further studies.

Fig. 1. *pH Optimum of acyl-CoA:1-alkyl-sn-glycero-3-phosphocholine acyltransferase*

The spectrophotometric assay is described under 'Experimental procedures'. Oleoyl-CoA was the fatty acyl donor in these experiments and 100 μ M-lysoPAF was used as acceptor. Data shown are mean $\pm$ S.D. for four determinations. The buffers used were 80 mM-citrate/phosphate for pH 5.0 and 5.5, 85 mM-phosphate in the range pH 6.0–8.0, and 87.5 mM-Tris/HCl for pH 8.5 and 9.0.

Table 1. *Effect of divalent cations and EDTA on the rate of acylation of lysoPAF*

The spectrophotometric assay is described under 'Experimental procedures'; 0.1 M-Tris/HCl, pH 7.5, was used instead of phosphate buffer for these experiments. Data are means $\pm$ S.D. obtained on three different microsomal preparations, except for data for 2 mM- Ca^{2+} and - Mg^{2+} which was obtained on two microsomal preparations. Relative initial velocities were calculated in relation to the control for each experiment (100% initial velocity). Linoloyl-CoA was used as the fatty acyl group donor.

Conditions	Relative initial velocity (%)
Control	100
Ca^{2+}	
1 mM	81.3 $\pm$ 5.4
2 mM	40.2 $\pm$ 1.7
Mg^{2+}	
1 mM	98.6 $\pm$ 4.2
2 mM	92.5 $\pm$ 4.0
5 mM	53.8 $\pm$ 9.2
EDTA	
1 mM	112 $\pm$ 5.4

The possible effect of divalent cations or chelating agents on the rate of fatty acyl group transfer was studied (Table 1). In these experiments Tris buffer was substituted for the phosphate buffer because divalent cations were pre-

sent. No effect of initial velocity was observed with 1 mM- MgCl_2 . EDTA at 1 mM caused a slight stimulation in activity compared with the control ($P < 0.05$, Student's unpaired *t* test). Enzyme activity was inhibited by at least 1 mM- CaCl_2 as well as by concentrations of MgCl_2 greater than 1 mM.

Substrate requirements

The assay conditions were optimized with respect to both substrates (acceptor and donors). LysoPAF, the acceptor, was saturating at 150 μ M, whereas the CoA esters (donors) all reached saturation at 20 μ M. When acceptor and donors were at these concentrations, the maximal rate of reaction was observed. Apparent K_m and V_{max} values were obtained for lysoPAF at saturating concentrations of the CoA esters of oleic, linoleic, and arachidonic acids (Table 2). It was not possible to determine accurately the apparent K_m and V_{max} for the CoA esters. The initial rates of reaction recorded when both substrates were at saturation were well within the detection limits of the assay procedure (the absorption coefficient of 2-nitro-4-thiobenzoic acid is 136000 $\text{M}^{-1}\cdot\text{cm}^{-1}$; Ellman, 1959). However, when the CoA ester concentrations were less than saturation, the rate of

Table 2. *Apparent K_m and V_{max} values for lysoPAF with respect to unsaturated fatty acyl donors*

The spectrophotometric assay is described under 'Experimental procedures'. Data are the mean of duplicate determinations. Double bond positions are in parentheses. Saturating concentrations of each fatty acyl-CoA ester (20 μ M) were employed.

Fatty acyl-CoA	K_m (μ M)	V_{max} (nmol/min per mg)
18:1(9)	16.5	3.1
18:2(9,12)	82.5	18.6
20:4(5,8,11,14)	89.7	19.4

Table 3. *Maximal rates of transfer of long chain fatty acyl groups to lysoPAF*

The spectrophotometric assay is described under 'Experimental procedures'. Data are means $\pm$ S.D. obtained on four different microsomal preparations. Double bond positions are in parentheses. Saturating concentrations of each fatty acyl-CoA ester (20 μ M) and lysoPAF (150 μ M) were employed.

Fatty acyl-CoA	Rate (nmol/min per mg)
18:0	Not detectable
18:1(9)	3.05 $\pm$ 0.38
18:2(9,12)	11.9 $\pm$ 1.7
18:3(6,9,12)	10.1 $\pm$ 1.7
18:3(9,12,15)	5.43 $\pm$ 1.0
20:3(8,11,14)	7.35 $\pm$ 0.89
20:4(5,8,11,14)	12.2 $\pm$ 2.5

reaction fell below the limits of detection of the assay at about the middle of the progress curve for the CoA esters.

The fatty acyl group selectivity of the fatty acyl-CoA transferase was determined using maximal rates of transfer at saturation for both substrates, since the apparent K_m values for the CoA esters could not be determined as discussed above. These rates are shown in Table 3. Enzyme activity was undetectable with the saturated CoA ester stearoyl-CoA. Linoleate, arachidonate and 6,9,12-linolenate were transferred at similar rates and are the preferred substrates. The rates of transfer of linoleate and arachidonate were about 4 times that of oleate.

Identity of the product

Experiments were carried out to prove that alkylacyl-GPC is the product formed during the spectrophotometric assay. This assay is non-specific and measures only the liberation of free thiol groups (in this case, the appearance of CoASH).

The identity and the amount of the phospholipid product formed during the spectrophotometric assay was determined by the stepwise conversion of alkylacyl-GPC to PAF. Proof that PAF had indeed been formed by this procedure, and the amount of PAF in the sample, was demonstrated by the ability of minute amounts of the presumed PAF product to induce platelet aggregation. Derivatized samples prepared from time 0 controls produced shape change, but not aggregation when the platelets were stirred for up to 5 min. The amount of alkylacyl-GPC calculated for the derivatized samples was compared with the amount of alkylacyl-GPC calculated to be present in the sample from the absorption coefficient of 2-nitro-4-thiobenzoic acid (see above). It was found that when arachidonoyl-CoA was the fatty acyl group donor, 11.0 ± 0.4 nmol of alkylacyl-GPC was formed, as determined spectrophotometrically ($n = 7$) and 9.1 ± 2.4 nmol of alkylacyl-GPC was calculated to be present in the sample from derivatization to PAF and bioassay ($n = 9$). There was no significant difference in the amounts of alkylacyl-GPC in these samples ($P < 0.05$, Student's unpaired t test).

Discussion

In this paper we have characterized a fatty acyl-CoA transferase in human platelets which inserts long chain unsaturated fatty acyl groups into lyso-PAF. This enzyme, acyl-CoA:1-alkyl-*sn*-glycero-3-phosphocholine acyltransferase, is membrane-bound, has a pH optimum of 7.5, and does not require divalent cation for activity. In fact, Ca^{2+} or

Mg^{2+} may inhibit the activity somewhat. These general properties are similar to those of the fatty acyl-CoA transferase which transfers unsaturated fatty acids to 1-acyl-GPC. The transfer of fatty acids to 1-alkyl phospholipids in other cells and from other animal species has been described (Waku & Nakazawa, 1970; Wykle *et al.*, 1973).

Alkylacyl-GPC is the product obtained when a long chain acyl group is inserted into lysoPAF by fatty acyl-CoA transferase. To prove that this product was indeed formed in our assay system, which simply measures the appearance of free thiol groups, we started with a lysoPAF substrate which was >99% pure. The product of the incubation of this substrate with our platelet microsomes and fatty acyl-CoA ester was shown to be alkylacyl-GPC by replacing the long chain acyl group with acetate and bioassaying the newly formed PAF.

The kinetics for the formation of alkylacyl-GPC and diacyl-GPC are somewhat different. Saturating concentrations for lysophospholipid and fatty acyl-CoA ester substrates are different in each case. Maximal rate of formation of alkylacyl-GPC is achieved at $150 \mu\text{M}$ -lysoPAF and $20 \mu\text{M}$ -fatty acyl-CoA, whereas the maximal rate of diacyl-GPC formation is achieved at $100 \mu\text{M}$ -1-acyl-GPC and 60 – $65 \mu\text{M}$ fatty acyl-CoA (McKean *et al.*, 1982). Of the unsaturated fatty acyl groups which are commonly found in platelet phospholipids, the CoA esters of linoleic and arachidonic acids are preferred as substrate for the acylation of lysoPAF. This is in contrast to the acylation of 1-acyl-GPC, where there is no preference for the CoA esters of oleic, linoleic, or arachidonic acids as suggested by their apparent V_{max} values. The rate of fatty acyl group transfer to lysoPAF is 3–14 times slower than to 1-acyl-GPC. The maximal initial rate of transfer and apparent V_{max} values can be compared, since rates of fatty acyl transfer determined using saturating conditions for both substrates approximate to the apparent V_{max} values.

The preference of acyl CoA:1-alkyl-*sn*-glycero-3-phosphocholine acyltransferase for the CoA esters of both linoleic and arachidonic acids suggests that endogenous human platelet alkylacyl-GPC should be equally enriched in these fatty acids. On the other hand, human platelets contain fatty acyl-CoA synthetase that is specific for arachidonic acid (Wilson *et al.*, 1982), and free arachidonic acid is undetectable in the platelet free fatty acid pool that contains other unsaturated fatty acids (Bills *et al.*, 1977). Therefore, the small pool of long chain CoA esters in platelets (Wilson *et al.*, 1982) may contain more arachidonoyl-CoA relative to other unsaturated fatty acyl-CoA esters. Since arachidonoyl-CoA is a preferred substrate in our system *in vitro*, endogenous platelet alkylacyl-GPC could contain large amounts of arachidonic

acid. Indeed, it has recently been shown that arachidonic acid makes up about 44% of the fatty acid at C-2 of alkylacyl-GPC in human platelets (Mueller *et al.*, 1983). An alkylacyl-GPC pool also exists in the endogenous choline-containing phospholipids of macrophages and neutrophils from rabbits, guinea pigs and humans (Sugiura *et al.*, 1982; Mueller *et al.*, 1982; Chilton *et al.*, 1983a). In human neutrophils, alkylacyl-GPC contains more arachidonic acid than does diacyl-GPC. A possible explanation for this enrichment comes from the finding that exogenous lysoPAF is selectively acylated by arachidonic acid in these cells (Chilton *et al.*, 1983b).

Alkylacyl-GPC could contribute to the pool of arachidonic acid which is liberated in stimulated platelets and converted to the endoperoxides and thromboxane A₂. Findings reported for rabbit neutrophils support this hypothesis. Even though the diacyl-GPC and alkylacyl-GPC of these cells contain small amounts of endogenous arachidonic acid (Chilton *et al.*, 1983a), radioactive arachidonic acid is readily incorporated into both of these phospholipids, and is released equally well from both following treatment of the labelled neutrophils with the divalent cation ionophore A23187 (Swendsen *et al.*, 1983).

Acyl-CoA:1-alkyl-*sn*-glycero-3-phosphocholine acyltransferase may play a central role in the biosynthesis and metabolism of PAF (Fig. 2). Its product, alkylacyl-GPC, may be both a precursor and a metabolite of PAF in platelets. It has recently been reported that human platelets contain alkylacyl-GPC (Natarajan *et al.*, 1983; Mueller *et al.*, 1983). It may be that alkylacyl-GPC is a biologically inert homologue of PAF which can be stored in the membranes of unstimulated platelets. It is a likely precursor of PAF which can

be converted to PAF by a deacylation-reacylation reaction as suggested by others (Albert & Snyder, 1983; Chilton *et al.*, 1983a) and as indicated in Fig. 2.

PAF, when incubated with platelets, is rapidly converted to lysoPAF (Alam *et al.*, 1983c). Lyso-PAF differs from 1-acyl-GPC only in the nature of the linkage of C-1 of the glycerol backbone and should have detergent properties similar to those of 1-acyl-GPC (Weltzein, 1979). The formation of alkylacyl-GPC by the reacylation of lysoPAF, catalysed by acyl-CoA:1-alkyl-*sn*-glycero-3-phosphocholine acyltransferase, may prevent the accumulation of this potential detergent in platelets. In support of this suggestion a metabolite of PAF which probably contains long chain fatty acyl groups at C-2 has been recovered from incubations of PAF with human platelets (Alam *et al.*, 1983c).

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Fig. 2. Schematic diagram indicating the role of alkylacyl-GPC as a precursor in the formation of PAF and as end product of the metabolism of PAF by human platelets

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