Properties of acyl-coenzyme A:1-acylglycerophosphate acyltransferase and lipases in porcine erythrocyte membranes

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Abstract Acyl-CoA:1-acylglycerophosphate acyltransferase activity was found in porcine erythrocyte membranes. However, the membrane preparations did not catalyze the acylation of either 2-acylglycerophosphate or 2-acylglycerophosphocholine. The 1-acylglycerophosphate acyltransferase and the known acyl-CoA:1-acylglycerophosphocholine acyltransferase systems differ in their specificities for acyl-CoAs and in their stabilities to detergents. Diacylglycerol lipase and monoacylglycerol lipase activities were also detected in porcine erythrocytes. These two activities appear to be catalyzed by different enzymes inasmuch as diacylglycerol lipase but not monoacylglycerol lipase was completely inhibited by divalent cations. The diacylglycerol lipase was relatively specific for the 1-position yielding 2-acylglycerol. The monoacylglycerol lipase hydrolyzed 1-acylglycerol and 2-acylglycerol at comparable rates. Phosphatidic acid was dephosphorylated to form 1,2-diacylglycerol but the acyl groups of phosphatidate were not hydrolyzed significantly by the erythrocyte membranes. Thus, the origin of 1-acylglycerophosphate, a substrate for the newly described enzyme, acyl-CoA:1-acylglycerophosphate acyltransferase, in mature erythrocyte could not be ascribed to action of diacylglycerol lipase, glycerophosphate acyltransferase, or phosphatidate-specific phospholipase A. 1-Acylglycerophosphate may be supplied extracellularly or the 1-acylglycerophosphate acyltransferase activity in erythrocytes may be a remnant of de novo phosphatidate synthesizing system of reticulocytes—**Mizuno, M., Y. Sugiura, and H. Okuyama.** Properties of acyl-coenzyme A:1-acylglycerophosphate acyltransferase and lipases in porcine erythrocyte membranes. *J. Lipid Res.* 1984. **25:** 843–850.

Supplementary key words 1-acylglycerophosphate • diacylglycerol • monoacylglycerol

In mature erythrocytes, no intracellular organelles are apparent and the phospholipid de novo synthetic pathway, generally localized in the mitochondrial and microsomal fractions of other types of cells, does not exist (1–3). Erythrocyte membrane phospholipids are renewed mainly by exchange with plasma phospholipids (4–7) and acylation of lysophospholipids on the cytoplasmic side of the membrane (8–13). Because of their relative simplicity, mature erythrocytes provide a useful system for examining the roles of lipid metabolizing enzymes in modulating plasma membrane function and in maintaining membrane lipid integrity. In the process of studies on acyl-CoA:1-acylglycerophosphocholine (1-acyl-GPC) acyltransferase in mature porcine erythrocytes, we discovered very high acyl-CoA:1-acylglycerophosphate acyltransferase activity in erythrocyte membranes. It was surprizing to detect 1-acylglycerophosphate acyltransferase activity because this enzyme is usually associated with phosphatidate synthesis from glycerophosphate or dihydroxyacetonephosphate (14–16). The current dogma is that the phospholipid de novo synthetic pathway is absent in mature erythrocytes. Therefore, we have tried to determine if there is a metabolic link between 1-acylglycerophosphate acyltransferase and known lipid metabolizing systems, such as the formation of diacylglycerol from inositol phospholipids (17, 18), the phosphorylation and dephosphorylation of inositol phospholipids (19–21), the phosphatidate-diacylglycerol cycle (22–25), the methylation of phosphatidylethanolamine to form phosphatidylcholine (26, 27), acyl hydrolysis and transesterification of monoacyl-GPC (28), and the acylation with acyl-CoA of monoacyl-GPC (10, 11). Perhaps related to the present work is the observation of Hokin and Hokin (29) that monoolein is converted to lysophosphatidic acid and phosphatidic acid by human erythrocyte membranes. Lipase activities for diacylglycerol and monoacylglycerol have also been reported in rat erythrocyte membranes (30), but the positional specificities of these enzymes have not been fully elucidated. In this study, we report some properties of 1-acyl-GP acyltransferase, diacylglycerol lipase, and monoacylglycerol lipase in porcine erythrocyte membranes emphasizing their positional specificities.

Abbreviations: -GPC, -*sn*-glycerol-3-phosphocholine; -GP, -*sn*-glycerol-3-phosphate.

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MATERIALS AND METHODS

Radiolabeled acyl-CoAs, unlabeled acyl-CoAs, and isomers of monoacyl-GPC were prepared as described elsewhere (31). Polyunsaturated fatty acids were products of Serdary Research Laboratories. [1-^{14}C]Oleic and [9,10-^{3}H]palmitic acids were purchased from New England Nuclear Corp. Isomers of monoacyl-GP and [2-^{3}H-glycerol]diacyl-GP were prepared as described previously (32). 1-Palmitoyl-glycerophosphate (Serdary) was used as a standard. ^{3}H-labeled diacylglycerol and monoacylglycerol were prepared by partial hydrolysis of trioleoyl[2-^{3}H]glycerol (The Radiochemical Centre, Amersham, diluted with unlabeled trioleoylglycerol to 6,000 cpm/nmol) with *Rhizopus* lipase (Seikagaku Kogyo, Tokyo) (33). ^{3}H-Labeled 2-oleoylglycerol was converted to the 1-acyl isomer by incubation at 37°C for 2 hr in methanol–0.1 M Tris-HCl (pH 8.0) (1:1). 1,2-Dioleoylglycerol,[2] 1-oleoylglycerol,[2] and 2-oleoylglycerol were separated on 0.4 M boric acid–Silica Gel G thin-layer chromatoplates developed in chloroform–acetone–methanol 95:5:2 (v/v/v). These glycerides were extracted, dissolved in methanol, and the concentration of each solution was determined by gas–liquid chromatography with margaric acid as an internal standard.

Membranes were prepared from porcine erythrocytes essentially as described by Dodge, Mitchell, and Hanahan (34). Briefly, fresh, citrated blood was added to an equal volume of 0.109 M potassium phosphate (pH 7.4) and centrifuged at 4,000 *g* for 10 min. The erythrocytes were washed two more times. The washed erythrocytes were hemolyzed in 20 volumes of 7.2 mM potassium phosphate (pH 7.4). The membrane fraction obtained by centrifugation at 20,000 *g* for 30 min was washed three times with 0.109 M sodium phosphate (pH 7.4). The membranes were then suspended in 0.25 M sucrose–20 mM Tris-HCL–5 mM EDTA (pH 7.4) using a glass homogenizer. The suspension was centrifuged at 150,000 *g* for 60 min. The resulting pellets were suspended in the same buffer, frozen in liquid nitrogen, and stored at −80°C. Another kind of membrane preparation was obtained as follows. Washed erythrocytes (15 ml of packed cells) were suspended in 0.25 M sucrose–20 mM Tris-HCL–5 mM EDTA (pH 7.4) (85 ml) and sonicated 10 times—each time for 0.5 min—keeping the temperature below 4°C. The suspension was centrifuged at 150,000 *g* for 30 min and the resulting pellets were washed twice with 7.2 mM potassium phosphate (pH 7.4). The membrane preparation was suspended in 0.25 M sucrose–20 mM Tris-HCl–5 mM EDTA (pH 7.4) and stored as described above. Approximately 6 mg of membrane protein was obtained from 15 ml of packed cells. Rat liver microsomes and membrane preparations from *Mycobacterium butyricum* were prepared as described previously (35, 36).

Typical assay mixtures for 1-acyl-GP and 1-acyl-GPC acyltransferase systems contained, in 1 ml: 0.08 M Tris-HCl (pH 7.4), 1 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), 100 μM 1-acyl-GP or 150 μM 1-acyl-GPC, 20 μM acyl-CoA, and 0.2 mg of membrane protein. Incubations were performed at 22°C and were started by adding acyl-CoA. The activities were followed spectrophotometrically at 413 nm (a molecular extinction coefficient of 13,600 $1 \cdot M^{-1} \cdot cm^{-1}$ was used). Control values without added acceptor were subtracted to give net acyltransfer rates. When labeled acyl-CoAs were used for determining 1-acyl-GP and 2-acyl-GP acyltransferase activities, the substrate concentrations were 75 μM 1-acyl-GP, 75 μM 2-acyl-GP, 15 μM [^{14}C]oleoyl-CoA, and 15 μM [^{3}H]palmitoyl-CoA. The labeled products were extracted with chloroform–methanol after acidification with HCl, neutralized with chloroform–methanol–3% NH_4OH 6:5:1, and separated on Na_2CO_3-Silica Gel H thin-layer plates developed in chloroform–methanol–acetic acid–acetone–water 25:8:4:3:2 (by vol). Labeled diacyl-GP was isolated and the amount of radioactivity at the 1- and 2-positions were determined by phospholipase A_2 (*Crotalus adamanteus* venom) hydrolysis in the presence of egg yolk phosphatidylcholine (36) or by *Rhizopus* lipase hydrolysis. The incubation mixture for the hydrolysis of diacyl-GP by *Rhizopus* lipase contained 0.25 μmol of diacyl-GP, 0.2 ml of diethyl ether, 0.5 ml of 0.1 M boric acid (pH adjusted to 5.8 with NaOH), and 0.5 ml of lipase solution (1 mg of protein, 6,000 units, dissolved in 0.5 ml of 0.2 M sodium acetate (pH 5.8) and 0.5 ml of glycerol). Hydrolysis was complete in 10 min at 37°C.

Typical reaction mixtures for diacylglycerol lipase and monoacylglycerol lipase assays contained, in 0.25 ml: 1 mM dioleoylglycerol (6,000 cpm/nmol) or 1 mM monooleoylglycerol, 0.2 mg of membrane protein, and 50 mM HEPES (pH 7.4). Both substrates were added as 0.25 μmol/25 μl of methanol solution. Reactions at 37°C were started by adding enzyme protein and terminated by adding chloroform–methanol 1:2. Products extracted according to the method of Bligh and Dyer (37) were separated on 0.4 M boric acid–Silica Gel G thin-layer plates developed in chloroform–methanol–acetone 95:1:5 (v/v/v). Radioactivity in regions corresponding to lipid standards was measured by liquid scintillation spectrometry (Searle, Mark II). For the determination of monoacylglycerol lipase activity, radioactivity in the water phase was measured. Phosphorus and protein were determined by the methods of Eibl and Lands (38) and Lowry et al. (39), respectively.

[2] 1-Oleoyl-*sn*-glycerol and 1,2-dioleoyl-*sn*-glycerol are not separated from 3-oleoyl-*sn*-glycerol and 2,3-dioleoyl-*sn*-glycerol, respectively.

RESULTS

Characterization of acyltransferase systems in erythrocyte membranes

Acyl-CoA:1-acyl-GPC acyltransferase is relatively stable and membrane preparations with relatively constant specific activity were obtained after hypotonic lysis of porcine erythrocytes. However, the same membrane preparations showed variable amounts of 1-acyl-GP acyltransferase activity and in some preparations the activity was absent. This loss of activity was probably due to the long time required for fractionation after hypotonic hemolysis. Membrane preparations obtained after sonication exhibited relatively constant and higher specific activity.

The 1-acyl-GP acyltransferase in erythrocyte membranes was saturated with 20 μM oleoyl-CoA and 100 μM 1-acyl-GP while 20 μM oleoyl-CoA and 150 μM 1-acyl-GPC saturated the 1-acyl-GPC acyltransferase system under the conditions described in Methods (**Fig. 1, a and b**). Although protein concentrations of 0.05 to 0.3 mg/ml gave proportional amounts of products with 1-acyl-GPC acyltransferase, product formation was linear with added protein over a narrower range with 1-acyl-GP acyltransferase (Fig. 1, c). At lower concentrations of enzyme protein, the 1-acyl-GP acyltransferase was inactivated possibly by the detergent action of substrates. This behavior is similar to that seen with the liver 1-acyl-GP acyltransferase system (14). Under optimal conditions for the 1-acyl-GP acyltransferase system, CoA esters of unsaturated 16- and 18-carbon fatty acids were the best substrates, followed by those of saturated 14-, 16-, and 18-carbon acids, and the unsaturated 20-carbon acids (**Fig. 2**). In contrast, the acylation of 1-acyl-GPC was most rapid with 16-, 18-, and 20-carbon acyl-CoAs with one to five double bonds; saturated acyl-CoAs and 22-carbon acyl-CoAs with four to six double bonds were relatively poor substrates. The CoA esters of 22-carbon chains containing one to three double bonds were virtually unreactive in the acylations of both 1-acyl-GP and 1-acyl-GPC.

These specificities for various acyl-CoAs were similar but not identical to those of the liver microsomal acyltransferase systems (40). Since acyltransferase systems are membrane-bound and cannot be easily resolved physically, we used detergent treatments to differentiate acyltransferase activities. As shown in **Fig. 3, a and b,** concentration-dependent inactivations of acyltransferase systems were observed upon addition of sodium deoxycholate or Triton X-100 to incubation mixtures. The 1-acyl-GPC acyltransferase activity was relatively more stable to deoxycholate than the 1-acyl-GP acyltransferase. Conversely, 1-acyl-GP acyltransferase activity was relatively more stable to Triton X-100. These results indicate that separate enzymes are involved in the acylations of 1-acyl-GP and 1-acyl-GPC. Oleoyl-CoA:1-acyl-GP acyltransferase activity of 1.6 nmol/min per mg of protein was detected in the erythrocyte membranes prepared from outdated human blood, suggesting that this enzyme may be widely distributed in mammalian erythrocytes.

To test for the presence of acyl-CoA:2-acyl-GP acyltransferase activity, 1-acyl-GP, 2-acyl-GP, [^{3}H]palmitoyl-CoA, and [^{14}C]oleoyl-CoA were incubated with eryth-

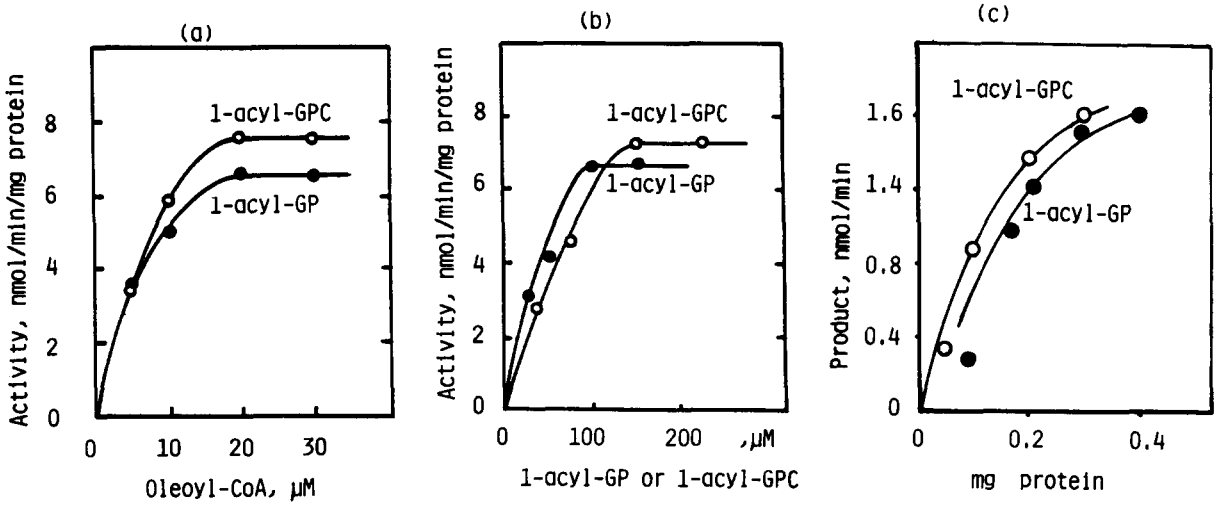

Fig. 1. Characterization of 1-acyl-GP and 1-acyl-GPC acyltransferase systems in erythrocyte membranes. The standard incubation mixture contained, in 1 ml: 0.08 M Tris-HCl (pH 7.4), 1 mM 5,5'-dithiobis-(2-nitrobenzoic acid), 100 μM 1-acyl-GP or 150 μM 1-acyl-GPC, 20 μM oleoyl-CoA, and 0.2 mg of membrane protein. In experiments (a), (b), and (c), the amounts of oleoyl-CoA, 1-acyl-GP, or 1-acyl-GPC and enzyme protein were varied as indicated. Enzyme activity was measured spectrophotometrically as described in the text. The points represent averages of two separate determinations. Control values without acceptor (0.38–0.66 nmol/min per mg of protein) have been subtracted.

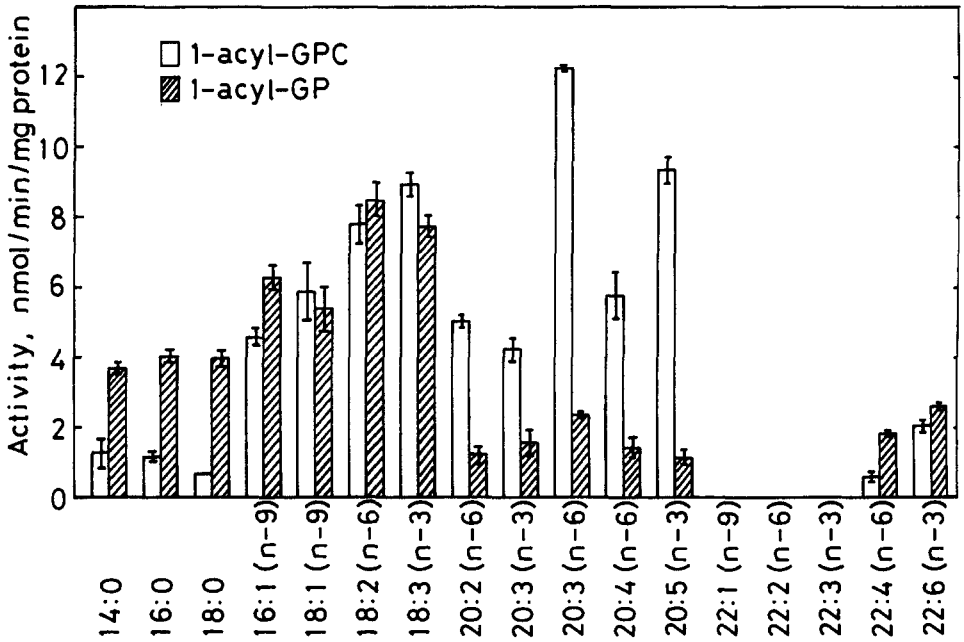

Fig. 2. Specificities for acyl-CoAs of 1-acyl-GP and 1-acyl-GPC acyltransferase systems in erythrocyte membranes. Each incubation mixture contained, in 1 ml: 0.08 M Tris-HCl (pH 7.4), 1 mM 5,5'-dithiobis-(2-nitrobenzoic acid), 100 μM 1-acyl-GP or 150 μM 1-acyl-GPC, 20 μM oleoyl-CoA, and 0.2 mg of enzyme protein. Activities were measured spectrophotometrically. Figures are averages of four separate determinations (±SE).

rocyte membranes, and the amount of radioactivity at the 1- and 2-positions of isolated phosphatidate was determined after hydrolysis by *Rhizopus* lipase or phospholipase A_2. As shown in **Fig. 4,** phosphatidate derived from 1-acyl-GP was mainly acylated with oleate and palmitate. Although approximately 10% of the radioactivity was released by lipase hydrolysis, no radioactivity remained in the 1-acyl-GP fraction after hydrolysis of labeled diacyl-GP with phospholipase A_2 (data not shown). Some parts of the fatty acids released by lipase could well be derived from the 2-position after isomerization to the 1-position. Thus, no significant 2-acyl-GP acyltransferase activity was

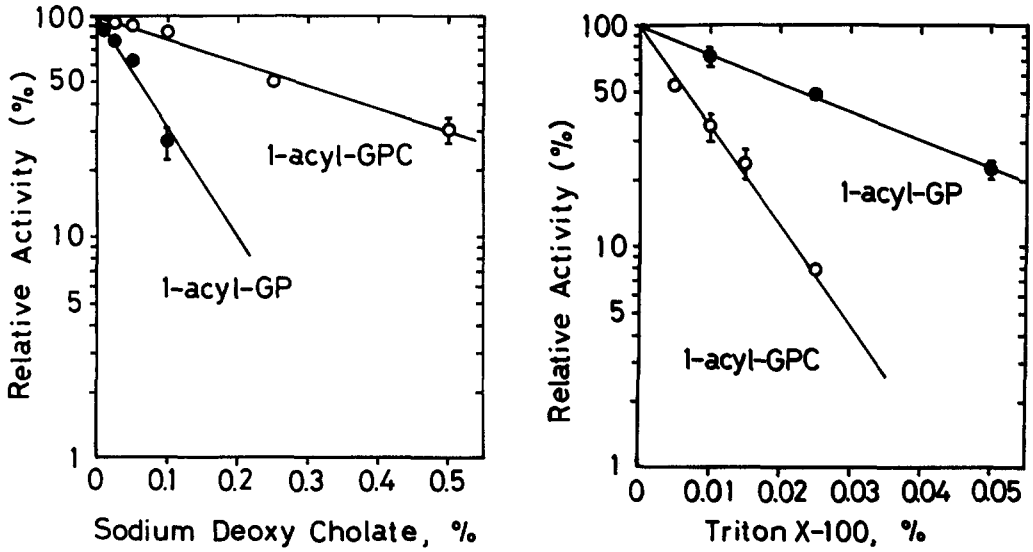

Fig. 3. Effect of detergents on 1-acyl-GP and 1-acyl-GPC acyltransferase activities in erythrocyte membranes. Standard incubation mixtures were used in the presence of the indicated concentrations of detergents. Before starting the reactions by adding acyl-CoAs, the mixtures were preincubated for 10 min after the addition of enzyme protein. Each point represents the average of four separate determinations (±SE).

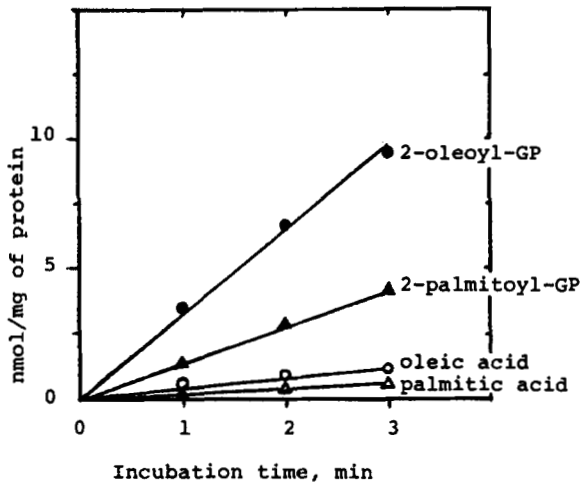

Fig. 4. Acylation of monoacyl-GP isomers in the presence of palmitoyl-CoA and oleoyl-CoA. The incubation mixture consisted of 75 μM 1-acyl-GP, 75 μM 2-acyl-GP, 15 μM [^{3}H]palmitoyl-CoA, 15 μM [^{14}C]oleoyl-CoA, and 0.2 mg of membrane protein in 1 ml of 0.08 M Tris-HCl (pH 7.4). The labeled products were extracted and separated on Na_2CO_3–Silica Gel H thin-layer chromatoplates. Radioactivity at the 1- and 2-positions of diacyl-GP was determined after *Rhizopus* lipase hydrolysis as described in the text. Some parts of fatty acids released by the lipase-treatment may be derived from 2-acyl-GP after isomerization to the 1-position (more details in text). In a parallel experiment, the 2-acyl-GP preparation was actively acylated to form diacyl-GP by an enzyme preparation from *Mycobacterium butyricum* (32) (data not shown).

detected under the conditions examined. Similarly, acyl-CoA:2-acyl-GPC acyltransferase activity was undetectable in the membrane preparations.

Glycerophosphate acyltransferase activity was examined by following the incorporation of [^{3}H]glycerophosphate into lipids. We confirmed previous observations that the acylation of glycerophosphate is not catalyzed by porcine erythrocyte membranes using conditions under which liver microsomes showed an activity of 1 to 2 nmol/min per mg of protein.

Diacylglycerol lipase and monoacylglycerol lipase

Diacylglycerol is produced from polyphosphoinositides in the presence of Ca^{2+} ionophore (17, 18), and monoacylglycerol as well as diacylglycerol is phosphorylated to form monoacyl-GP in human erythrocyte membranes (24, 29). We reasoned that 1-acyl-GP might be produced from diacylglycerol with 1-acyl-glycerol as an intermediate. To test this possibility, 1,2-dioleoylglycerol was incubated with erythrocyte membranes and monoacylglycerol formation was determined. The reaction was linear with time for 10 min and product formation was proportional to added protein at protein concentrations of 0.05 to 0.2 mg/ml. A diacylglycerol concentration of 0.5 mM saturated the enzyme system. The major product was 2-acylglycerol. We also detected a small amount of 1-acylglycerol, but most of this could have been formed non-enzymatically by the isomerization of 2-acylglycerol. This latter interpretation was suggested to us by the pH profile of diacylglycerol hydrolysis; the optimum pH for the formation of 2-acylglycerol was 7.5, but the proportion of the 1-acyl isomer is relatively higher at alkaline pH (pH 8.0–8.5) where the rate of isomerization is higher. Thus, the diacylglycerol lipase hydrolyzed the acyl groups from the 1-position to form 2-acylglycerol as the major product. The maximal activity was 3.0 to 3.5 nmol/min per mg of protein. $CaCl_2$ inhibited diacylglycerol lipase activity with IC_{50} (50% inhibition concentration) of 5 mM (**Fig. 5**). $MgCl_2$ at 20 mM inhibited the diacylglycerol lipase activity completely, but the same concentrations of NaCl and KCl had no effect on the enzyme.

When [^{3}H]monoacylglycerol was incubated with erythrocyte membranes, glycerol and fatty acid were produced. Both 1-acylglycerol and 2-acylglycerol were hydrolyzed at comparable rates. Product formation was linear for 60 min and at protein concentrations of 0.1 to 0.4 mg/ml. A monoacylglycerol concentration of 1 mM saturated the system, and the enzyme showed a broad pH profile with optimum at 7.4. The specific monoacylglycerol lipase activity of the membrane preparation was approximately $^1/_{10}$ of that of diacylglycerol lipase. The monoacylglycerol lipase and diacylglycerol lipase activities also differed in positional specificity and sensitivity to divalent cations; neither $CaCl_2$ nor $MgCl_2$ affected the monoacylglycerol lipase significantly (data not shown).

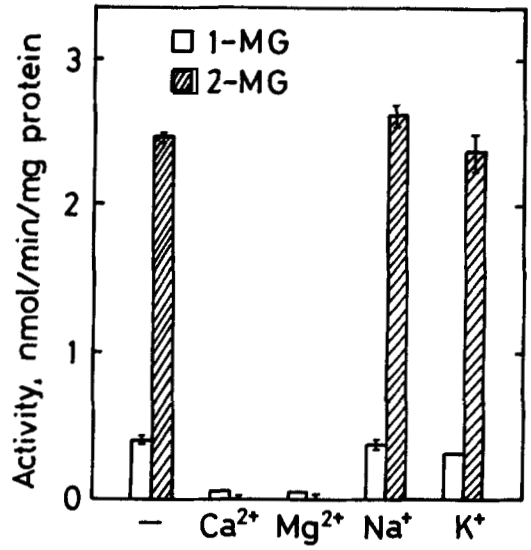

Fig. 5. Effect of metal ions on diacylglycerol lipase. The incubation mixture was the same as that described in the legend to Fig. 4, except that the pH of the buffer was 7.4 and 20 mM metal ions was present. Figures are averages of three separate determinations (±SE). The products, 1-oleoylglycerol (1-MG) and 2-oleoylglycerol (2-MG), were separated on 0.4 M boric acid–Silica Gel G thin-layer plates as described in the text.

Other enzyme activities examined

Since phosphatidate-specific phospholipase A has been shown to be present in horse platelet membranes, [^{3}H]phosphatidate was incubated with erythrocyte membranes either under the conditions described for the horse platelet membranes (41) or the conditions established for porcine platelet membranes (42); however, no measurable acylhydrolase activity was detected. Instead, phosphatidic acid was dephosphorylated to form diacylglycerol as the major product. The specific activity of phosphatidate phosphatase was 0.6 nmol/min per mg of protein under the conditions used. The diacylglycerol kinase and monoacylglycerol kinase activities were also detected; the specific activities were 0.23 and 0.18 nmol/min per mg of protein, respectively.

DISCUSSION

In mature erythrocytes, there are usually fewer membrane receptors and less adenylate cyclase activity than in reticulocytes (43–45). The arachidonate cascade in erythrocytes has not been fully explored. Recently, Kobayashi and Levine (46) showed that a lipoxygenase capable of forming hydroxylated arachidonate derivatives was present in erythrocytes from several mammals. The precursor arachidonate appears to be released from prelabeled phosphatidylcholine and phosphatidylethanolamine, but not from phosphatidylinositol. However, the presence of phospholipase A_2 acting on diacyl-GPC and diacyl-GPE has been shown in vitro only in ruminant erythrocytes (47). On the other hand, diacylglycerol is formed from inositol phospholipids by stimulation of erythrocytes with Ca^{2+} (17, 18) and this diacylglycerol can be metabolically related to phosphatidic acid cycle involving diacylglycerol kinase and phosphatidate phosphatase as revealed earlier by Hokin and Hokin (23, 29) and others (24). From the positional specificities of diacylglycerol lipase and monoacylglycerol lipase determined in the present experiments, it seems possible that arachidonate is released by monoacylglycerol lipase from inositol phospholipids with diacylglycerol as an intermediate, as is the case with platelets (48).

The finding of acyl-CoA:1-acyl-GP acyltransferase activity in mature erythrocyte membranes was unexpected, but this activity appears to be intrinsic to erythrocyte membranes inasmuch as the washed erythrocyte preparations contained less than 0.01% of white cells and because the glycerophosphate acyltransferase activity, which is known to exist in white cells and platelets, was not detectable in the erythrocyte membrane preparations. The physiological significance of the 1-acyl-GP acyltransferase is not easily explained based on the enzymes known to be present in erythrocytes. Since the major monoacylglycerol formed from diacylglycerol is the 2-acyl isomer, 2-acyl-GP could be supplied if the monoacylglycerol is phosphorylated. However, we found no evidence for the acylation of 2-acyl-GP by erythrocyte membranes (Fig. 4). Although the presence of phosphatidate-specific phospholipase A_2 has been described in horse platelet membranes (41), no such phospholipase activity was detected in porcine erythrocyte membranes. Furthermore, we have shown that the major phospholipase A acting on phosphatidic acid in porcine platelets is of the A_1-type producing 2-acyl-GP (42). Thus, other pathways must supply 1-acyl-GP, the substrate for the acyl-CoA:1-acyl-GP acyltransferase. In this context, we suggest the following possibilities: *i*) although 1-acylglycerol is a minor product of diacylglycerol lipase and this could be formed from 2-acylglycerol by non-enzymatic isomerization, a small amount of 1-acylglycerol may be formed enzymatically and this may be metabolized to 1-acyl-GP by monoacylglycerol kinase; *ii*) 1-acyl-GP may be formed by unknown enzymes such as a phospholipase D capable of hydrolyzing lysophospholipids (49); *iii*) phosphatidate-specific phospholipase A_2 may be present; or *iv*) 1-acyl-GP may be supplied from extracellular sources. Another possible explanation for the presence of 1-acyl-GP acyltransferase activity in mature erythrocytes is that this enzyme is a remnant of the de novo phosphatidate synthesizing system active in reticulocytes or stem cells. A similar interpretation has been presented to account for the existence of the fatty acid synthetase complex in mature erythrocytes; de novo fatty acid synthesis from acetate does not occur in these cells since acetyl-CoA carboxylase is absent (50).

The fatty acid compositions of erythrocyte phospholipids fluctuate in response to changes in dietary supply of fatty acids and in response to abnormal physiological conditions (51–53). Changes in membrane fatty acid compositions are known to accompany changes in morphology and stability of erythrocytes (52). There are some in vivo observations on the turnover of fatty acyl moiety of phospholipids in mature erythrocytes, but enzymatic events underlying these processes have not been fully elucidated (54–57). Further investigations will be necessary to determine enzymatic bases for such changes and for stimulus-activation coupling in erythrocytes.

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REFERENCES

1. Rowe, C. E., A. C. Allison, and J. E. Lovelock. 1960. Synthesis of lipids by different human blood cell types. *Biochim. Biophys. Acta.* **41:** 310–314.

2. Percy, A. K., E. Schmel, B. J. Earles, and W. J. Lennarz. 1973. Phospholipid biosynthesis in the membranes of immature and mature red blood cells. *Biochemistry.* **12:** 2456–2461.
3. Ballas, S. K., and E. R. Burka. 1974. Pathways of de novo phospholipid synthesis in reticulocytes. *Biochim. Biophys. Acta.* **337:** 239–247.
4. Lovelock, J. E., A. T. James, and C. E. Rowe. 1960. The lipids of whole blood. *Biochem. J.* **74:** 137–140.
5. Sakagami, T., O. Minari, and T. Orii. 1965. Interaction of individual phospholipids between rat plasma and erythrocytes in vitro. *Biochim. Biophys. Acta.* **98:** 356–364.
6. Mulder, E., and L. L. M. Van Deenen. 1965. Metabolism of red cell lipids. III. Pathways for phospholipid renewal. *Biochim. Biophys. Acta.* **106:** 348–356.
7. Smith, N. B., and D. Rubinstein. 1981. Exchange of phosphatidylcholine between rabbit erythrocytes and plasma in vivo. *Lipids.* **16:** 937–939.
8. Oliveira, M. M., and M. Vaughan. 1964. Incorporation of fatty acids into phospholipids of erythrocyte membranes. *J. Lipid Res.* **5:** 156–162.
9. Mulder, E., and L. L. M. Van Deenen. 1965. Metabolism of red cell lipids. I. Incorporation in vitro of fatty acids into phospholipids from mature erythrocytes. *Biochim. Biophys. Acta.* **106:** 106–117.
10. Robertson, A. F., and W. E. M. Lands. 1964. Metabolism of phospholipids in normal and spherocytic human erythrocytes. *J. Lipid Res.* **5:** 88–93.
11. Waku, K., and W. E. M. Lands. 1968. Control of lecithin biosynthesis in erythrocyte membranes. *J. Lipid Res.* **9:** 12–18.
12. Renooij, W., L. M. G. Van Golde, R. F. A. Zwaal, B. Roelofsen, and L. L. M. Van Deenen. 1974. Preferential incorporation of fatty acids at the inside of human erythrocyte membranes. *Biochim. Biophys. Acta.* **363:** 287–292.
13. Dise, C. A., D. B. P. Goodman, and H. Rasmussen. 1980. Definition of the pathway for membrane phospholipid fatty acid turnover in human erythrocytes. *J. Lipid Res.* **21:** 292–300.
14. Okuyama, H., H. Eibl, and W. E. M. Lands. 1971. Acyl coenzyme A:2-acyl-*sn*-glycerol 3-phosphate acyltransferase activity in rat liver microsomes. *Biochim. Biophys. Acta.* **248:** 263–273.
15. Okuyama, H., and S. J. Wakil. 1973. Positional specificities of acyl coenzyme A:monoacylglycerophosphate acyltransferases in *Escherichia coli*. *J. Biol. Chem.* **248:** 5197–5205.
16. Hajra, A. K., and C. Burke. 1978. Biosynthesis of phosphatidic acid in rat brain via acyl dihydroxyacetone phosphate. *J. Neurochem.* **31:** 125–134.
17. Allan, D., and R. Michell. 1977. Calcium ion-dependent diacylglycerol accumulation in erythrocytes is associated with microvesiculation but not with efflux of potassium ions. *Biochem. J.* **166:** 495–499.
18. Allan, D., and R. H. Michell. 1978. A calcium-activated polyphosphoinositide phosphodiesterase in the plasma membrane of human and rabbit erythrocytes. *Biochim. Biophys. Acta.* **508:** 277–286.
19. Schneider, R. P., and L. B. Kirshner. 1970. Di- and triphosphoinositide metabolism in swine erythrocyte membranes. *Biochim. Biophys. Acta.* **202:** 283–294.
20. Buckley, J. T., and J. N. Hawthorne. 1972. Erythrocyte membrane polyphosphoinositide metabolism and the regulation of calcium binding. *J. Biol. Chem.* **247:** 7218–7223.
21. Roach, P. D., and F. B. Palmer. 1981. Human erythrocyte cytosol phosphatidylinositol bisphosphate phosphatase. *Biochim. Biophys. Acta.* **661:** 323–333.
22. Hokin, L. E., M. R. Hokin, and D. Mathison. 1963. Phosphatidic acid phosphatase in the erythrocyte membrane. *Biochim. Biophys. Acta.* **67:** 485–497.
23. Hokin, L. E., and M. R. Hokin. 1964. The incorporation of ^{32}P from [γ-^{32}P]adenosine triphosphate into polyphosphoinositides and phosphatidic acid in erythrocyte membranes. *Biochim. Biophys. Acta.* **84:** 563–575.
24. Allan, D., P. Thomas, and S. Gatt. 1980. 1,2-Diacylglycerol kinase of human erythrocyte membranes. *Biochem. J.* **191:** 669–672.
25. Allan, D., R. Watts, and R. H. Michell. 1976. Production of 1,2-diacylglycerol and phosphatidate in human erythrocytes treated with calcium ions and ionophore A23187. *Biochem. J.* **156:** 225–232.
26. McLeod, M. E., and R. Bressler. 1967. Some aspects of phospholipid metabolism in the red cell. *Biochim. Biophys. Acta.* **144:** 391–396.
27. Hirata, F., and J. Axelrod. 1978. Enzymatic synthesis and rapid translocation of phosphatidylcholine by two methyltransferases in erythrocyte membranes. *Proc. Natl. Acad. Sci. USA.* **75:** 2348–2352.
28. Mulder, E., J. W. O. Van den Berg, and L. L. M. Van Deenen. 1965. Metabolism of red-cell lipids. II. Conversion of lysophosphoglycerides. *Biochim. Biophys. Acta.* **106:** 118–127.
29. Hokin, L. E., and M. R. Hokin. 1963. Diglyceride kinase and other pathways for phosphatidic acid synthesis in the erythrocyte membranes. *Biochim. Biophys. Acta.* **67:** 470–484.
30. Michell, R. H., R. Coleman, and J. B. Finean. 1973. Hydrolysis of 1,2-diglyceride by membrane-associated lipase activity during phospholipase C treatment of membranes. *Biochim. Biophys. Acta.* **318:** 306–312.
31. Okuyama, H., and M. Inoue. 1982. Preparation and analysis of radiolabeled phosphatidylcholine and phosphatidylethanolamine containing ^{3}H- and ^{14}C-labeled polyunsaturated fatty acids. *Methods Enzymol.* **86:** 370–376.
32. Okuyama, H., Y. Kameyama, M. Fujikawa, K. Yamada, and H. Ikezawa. 1977. Mechanism of diacylglycerophosphate synthesis in *Mycobacteria*. *J. Biol. Chem.* **252:** 6682–6686.
33. Ishihara, H., H. Okuyama, H. Ikezawa, and S. Tejima. 1975. Studies on lipase from *Mucor javanicus*. I. Purification and properties. *Biochim. Biophys. Acta.* **388:** 413–422.
34. Dodge, J. T., C. Mitchell, and D. J. Hanahan. 1963. The preparation and chemical characteristics of hemoglobin-free ghosts of human erythrocytes. *Arch. Biochem. Biophys.* **100:** 119–130.
35. Yamada, K., and H. Okuyama. 1978. Selectivity of diacylglycerophosphate synthesis in subcellular fractions of rat liver. *Arch. Biochem. Biophys.* **190:** 409–420.
36. Okuyama, H., K. Yamada, H. Ikezawa, and S. J. Wakil. 1976. Factors affecting the acyl selectivities of acyltransferases in *Escherichia coli*. *J. Biol. Chem.* **251:** 2487–2492.
37. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 912–917.
38. Eibl, H., and W. E. M. Lands. 1969. A new, sensitive determination of phosphate. *Anal. Biochem.* **30:** 51–57.
39. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
40. Lands, W. E. M., M. Inoue, Y. Sugiura, and H. Okuyama.

1982. Selective incorporation of polyunsaturated fatty acids into phosphatidylcholine by rat liver microsomes. *J. Biol. Chem.* **257:** 14968–14972.
41. Billah, M. M., E. G. Lapetina, and P. Cuatrecasas. 1981. Phospholipase A_2 activity specific for phosphatidic acid. A possible mechanism for the production of arachidonic acid in platelets. *J. Biol. Chem.* **256:** 5399–5403.
42. Inoue, M., and H. Okuyama. 1984. Phospholipase A_1 acting on phosphatidic acid in porcine platelet membranes. *J. Biol. Chem.* **259:** 5083–5087.
43. Bilezikian, J. P., A. M. Spiegel, D. E. Gammon, and G. D. Aurbach. 1977. The role of guanyl nucleotides in the expression of catecholamine-responsive adenylate cyclase during maturation of the rat reticulocyte. *Mol. Pharmacol.* **13:** 786–795.
44. Mantione, C. R., and I. Hanin. 1980. Further characterization of human red cell membrane cholinergic receptors. *Mol. Pharmacol.* **18:** 28–32.
45. Dons, R. F., L. M. Corash, and P. Gorden. 1981. The insulin receptor is an age-dependent integral component of the human erythrocyte membrane. *J. Biol. Chem.* **256:** 2982–2987.
46. Kobayashi, T., and L. Levine. 1983. Arachidonic acid metabolism by erythrocytes. *J. Biol. Chem.* **258:** 9116–9121.
47. Zwaal, R. F. A., R. Fluckiger, S. Moser, and P. Zahler. 1974. Lecithinase activities at the external surface of ruminant erythrocyte membranes. *Biochim. Biophys. Acta.* **373:** 416–424.
48. Prescott, S., and P. W. Majerus. 1983. Characterization of 1,2-diacylglycerol hydrolysis in human platelets. Demonstration of an arachidonoyl-monoacylglycerol intermediate. *J. Biol. Chem.* **258:** 764–769.
49. Yamashita, M., H. Homma, K. Inoue, and S. Nojima. 1983. The metabolism of platelet-activating factor in platelets and plasma of various animals. *J. Toxicol. Sci.* **8:** 177–188.
50. Pittman, J. G., and D. B. Martin. 1966. Fatty acid biosynthesis in human erythrocytes: evidence in mature erythrocytes for an incomplete long chain fatty acid synthesizing system. *J. Clin. Invest.* **45:** 165–172.
51. Salvioli, G. 1978. Changes of red cell and lipoprotein lipid composition after polyunsaturated phosphatidylcholine infusion in patients with liver cirrhosis. *In* International Conference on Atherosclerosis. L. A. Carson et al., editors. Raven Press, New York.
52. Rao, G. A., K. Siler, and E. C. Larkin. 1979. Diet-induced alterations in the discoid shape and phospholipid fatty acid compositions of rat erythrocytes. *Lipids.* **14:** 30–38.
53. Kaya, K., and T. Miura. 1982. Selective changes in fatty acid composition of phosphatidylserine in rat erythrocyte membrane induced by nitrate. *Biochim. Biophys. Acta.* **688:** 305–315.
54. Shohet, S. B. 1971. The apparent transfer of fatty acid from phosphatidylcholine to phosphatidylethanolamine in human erythrocytes. *J. Lipid Res.* **12:** 139–142.
55. Marinetti, G. V., and K. Cattieu. 1982. Asymmetric metabolism of phosphatidylethanolamine in the human red cell membranes. *J. Biol. Chem.* **257:** 245–248.
56. Dise, C. A., D. B. P. Goodman, and H. Rasmussen. 1980. Selective stimulation of erythrocyte membrane phospholipid fatty acid turnover associated with decreased cell volume. *J. Biol. Chem.* **255:** 5201–5207.
57. Dise, C. A., W. C. Lake, D. B. P. Goodman, and H. Rasmussen. 1976. Calcium-dependent stimulation of erythrocyte membrane phospholipid fatty acid incorporation by the ionophore A23187. *J. Biol. Chem.* **251:** 4162–4164.