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COA-INDEPENDENT TRANSFER OF ARACHIDONIC ACID FROM 1,2-DIACYL-SN-GLYCERO-3-PHOSPHOCHOLINE TO 1-O-ALKYL-SN-GLYCERO-3-PHOSPHOCHOLINE (LYSO PLATELET-ACTIVATING FACTOR) BY MACROPHAGE MICROSOMES

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SUMMARY: Macrophage microsomes catalyzed the transfer of arachidonic acid (20:4) from 1,2-diacyl-glycerophosphocholine (GPC) to 1-alkyl-GPC (lyso platelet-activating factor). This enzyme reaction did not require the presence of cofactors such as Co A. Free arachidonic acid or linoleic acid-labeled phospholipids failed to act as the acyl donor. These results suggest that the reaction is a CoA-independent direct transfer of arachidonic acid. This arachidonoyl transacylation system may play a very important role in the metabolism of lyso platelet-activating factor and also in the elimination or release of arachidonic acid from diacyl-GPC.

In the preceding studies, we found that high levels of alkylacyl-GPC (glycerophosphocholine) as well as alkenylacyl-GPE (glycerophosphoethanolamine) were included in various types of leukocyte such as macrophages (1,2), polymorphonuclear leukocytes (1) and lymphocytes (1,3) and also in platelets (4). The occurrence of high levels of alkylacyl-GPC in inflamatory cells is of particular importance, since this unique ether phospholipids is now considered to be a precursor of potent biologically acitive lipid; platelet-activating factor (PAF) (5). Very interestingly, alkylacyl-GPC contains ca. 5 times higher amounts of 20:4 at the 2-position (38.6%) than corresponding diacyl-GPC (7.8%) in rabbit alveolar macrophages (2). Indeed, 20:4 is highly concentrated in alkyl or alkenyl ether-containing phospholipids in this cell. Furthermore, we found that 20:4 is selectively mobilized from diacyl-GPC to alkylacyl-GPC and alkenylacyl-GPE

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Abbreviations; 20:4, arachidonic acid; 18:2, linoleic acid; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; CGP, choline glycerophospholipid; PAF, platelet-activating factor

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fraction (6). This mobilization may lead to a high abundance of 1-alkyl-2-arachidonoyl-GPC and 1-alkenyl-2-arachidonoyl-GPE. Recent research strongly suggests that 1-alkyl-2-arachidonoyl-GPC might act as the precursor for both PAF and 20:4 metabolites (6-8). In addition, 1-alkyl-GPC (lyso PAF) and 1-alkyl-2-acetyl-GPC (PAF) were rapidly converted to 1-alkyl-2-arachidonoyl-GPC after incubation with several types of leukocytes or platelets (5,9-12). Precise investigation of the metabolical regulation of alkylacyl-GPC would thus seem to be of great importance.

In this paper, the mechanism of the synthesis of 1-alkyl-2-arachidonoyl-GPC using alveolar macrophage microsomes is studied. We found that 20:4 is selectively transferred from diacyl-GPC to 1-alkyl-GPC (lyso PAF) in the CoA-independent manner to form 1-alkyl-2-arachidonoyl-GPC.

MATERIALS AND METHODS

All chemicals were of reagent grade. 1-Stearoyl-2-[^{14}C]arachidonoyl-sn-glycero-3-phosphocholine (60mCi/mmol), 1-palmitoyl-2-[^{14}C]linoleoyl-sn-glycero-3-phosphocholine (56mCi/mmol), 1-stearoyl-2-[^{14}C]-arachidonoyl-sn-glycero-3-phosphoethanolamine (60mCi/mmol), 1-stearoyl-2-[^{14}C]-arachidonoyl--sn-glycero-3-phosphoinositol (58mCi/mmol) and [^{3}H]arachidonic acid (20:4) (110 Ci/mmol) were purchased from Amersham (England). [^{3}H]20:4 was diluted with non-labeled 20:4 as 55mCi/mmol. 1-Alkyl-GPC (prepared by the hydrogenation of lyso choline plasmalogen derived from bovine heart) was a kind gift from Dr. Y. Masuzawa (Teikyo University, Kanagawa).

Alveolar macrophages were prepared from pulmonary lavage of rabbits injected with 1ml of Freund's complete adjuvant intravenously 3 weeks prior to the slaughter. Macrophages suspended in 0.25M sucrose-0.1M Tris-HCl buffer (pH7.4) containing 1mM EDTA were then homogenated by Potter glass-teflon homogenizer. The homogenate was centrifuged 10000xg for 20 min twice. The supernatant was collected and centrifuged 105000xg for 60 min. The pellet was further washed once and resuspended in 0.1M Tris-HCl buffer (pH7.4) containing 1mM EDTA. This fraction was used as the microsomes for the enzyme assay. Microsomes were stored at 4°C and used within 24hr after preparation. The protein content was estimated by the method of Lowry et al. (13).

The typical enzyme reaction was performed as follows. The incubation mixture contains labeled phospholipids (25000 dpm), 1-alkyl-GPC (2-160μM at the final concentration) and microsomes (0.02-0.4mg protein) in 250μl of 0.1M Tris-HCl buffer (pH7.4) containing 5mM EGTA. To estimate the incorporation rate of [^{3}H]20:4 into lyso phospholipids, [^{3}H]20:4 (100000dpm) dissolved in 0.2% fatty acid free bovine serum albumin was incubated with 1-alkyl-GPC (20μM) and microsomes (0.2mg protein) in the presence or absence of CoA (20μM), ATP (1mM) and $MgCl_2$ (5mM) in the same buffer. The incubation was carried out for 15-120 min and terminated by adding 940μl of chloroform:methanol (1:2).

Total lipids were extracted by the method of Bligh and Dyer (14). Individual phospholipids were separated by 2-dimensional TLC. Alkenylacyl, alkylacyl and diacyl lipid classes were separated as 1,2-diradyl-3-acetyl-glycerols by TLC (1-4). Each lipid spot was scraped off from the plate to

scintillation counting vials. The radioactivity was estimated with a liquid-scintillation counter (Aloka, Model LSC 903), using the scintillation fluid as described earlier (15).

RESULTS AND DISCUSSION

Macrophage microsomes catalyze the transfer of 20:4 from diacyl-GPC to 1-alkyl-GPC to form 1-alkyl-2-arachidonoyl-GPC. Fig. 1 (a) shows the effect of protein concentration and Fig. 1 (b) shows the effect of the incubation time on the transfer of 20:4 to 1-alkyl-GPC, respectively. The percentage of 20:4 transferred to 1-alkyl-GPC was increased with protein and with time. Fig. 1 (c) shows the effect of the concentration of 1-alkyl-GPC. The optimal activity was observed at 20μM. However, when higher amounts of 1-alkyl-GPC were added to the incubation mixture, the enzyme activity was rather reduced. This inhibitory effect may be due to the detergent effect of 1-alkyl-GPC, since low concentration of Triton X-100 (0.02%) or cholate (0.2%) inhibited the enzyme activity (Fig. 2).

We also found that 1-alkenyl-GPE can be transacylated with 20:4 from diacyl-GPC by macrophage microsomes, similar to the case for 1-alkyl-GPC (data not shown). Very similar results were obtained for human platelet membrane (16). On the other hand, several investigators reported the

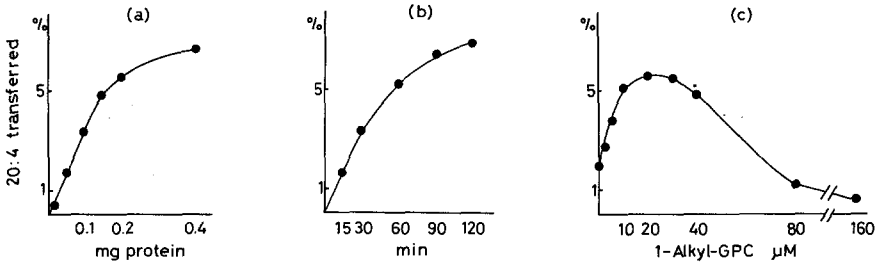

Fig.1. Transfer of arachidonic acid (20:4) from diacyl-GPC to 1-alkyl-GPC. (a) The effect of protein content. The incubation mixture contains 20μM 1-alkyl-GPC, microsomes and 1-stearoyl-2-[^{14}C]arachidonoyl-GPC (25000 dpm) in 5mM EGTA-containing 0.1M Tris-HCl buffer (pH7.4). The incubation was carried out at 37°C for 60 min. (b) The effect of incubation time. The incubation mixture contains 20μM 1-alkyl-GPC, 0.2mg of microsomal protein and 1-stearoyl-2-[^{14}C]arachidonoyl-GPC in the same buffer as in (a). (c) The effect of acceptor concentration. The incubation mixture contains different concentration of 1-alkyl-GPC, 0.2mg of microsomal protein and 1-stearoyl-2-[^{14}C]arachidonoyl-GPC in the same buffer as in (a) and were kept at 37°C for 60 min. The values (means of two determinations) were expressed as the percentages of 20:4 transferred from diacyl-GPC to 1-alkyl-GPC.

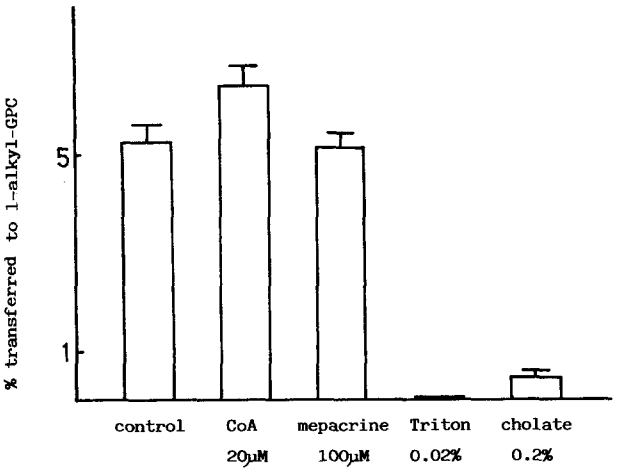

Fig.2. Effects of several agents on the transfer of 20:4 from diacyl-GPC to 1-alkyl-GPC. The incubation mixture contains 20μM 1-alkyl-GPC, 0.2mg of microsomal protein, 1-stearoyl-2-[^{14}C]arachidonoyl-GPC (25000 dpm) and several compounds in the same buffer as in Fig.1 and were kept at 37°C for 60 min. The values (means of three determinations ± S.D.) were expressed as the percentages of 20:4 transferred to 1-alkyl-GPC.

occurrence of CoA-mediated transfer of 20:4 between phospholipids (17-21). So, we next examined the CoA-dependency of the reaction. We observed only a slight increase (20-30%) in the transfer of 20:4 to 1-alkyl-GPC in the presence of CoA (20μM). This result indicates that the reaction mainly depends upon the CoA-independent transfer, with a small part being CoA-dependent if CoA is present.

In order to examine the possibility that liberated free 20:4 is converted to 20:4-CoA and reincorporated into lysophospholipids, we estimated the incorporation rate of [^{3}H]20:4 into choline glycerophospholipid fraction in the presence or absence of several cofactors. As shown in TABLE I, the addition of CoA, ATP and Mg^{++} was absolutely required for the acylation of lysophospholipids with [^{3}H]20:4. This means that microsomes do not contain enough endogenous CoA, ATP or Mg^{++}. In addition, the reaction proceeds even in the presence of 5mM EGTA. Moreover, an inhibitor of phospholipase A_2, mepacrine did not show any inhibitory effect on the transfer of 20:4 up to 100μM (Fig. 2). These results strongly support the conclusion that 20:4 transfer from diacyl-GPC to 1-alkyl-GPC differes from

TABLE I

Incorporation of Free [^{3}H]Arachidonic Acid into Choline Glycerophospholipids (CGP)

	% incorporated into CGP
complete system	44.3 ± 3.5
-CoA	0.7 ± 0.1
-ATP	0.1 ± 0.0
-Mg^{++}	2.2 ± 0.2

Microsomes were incubated in the presence of 20μM 1-alkyl-GPC, [^{3}H]20:4 (100000dpm), 20μM CoA, 1mM ATP and 5mM $MgCl_2$ at 37°C for 60 min. The values (means ± S.D. of three determinations) were expressed as the percentages of the radioactivities incorporated into CGP.

the deacylation-reacylation system mediated by phospholipase A_2 and subsequently by acyltransferase.

We then checked the donor specificity in the transfer of 20:4 to 1-alkyl-GPC. As shown in Fig. 3, the most effective acyl donor was 1-acyl-2-arachidonoyl-GPC. 1-Acyl-2-arachidonoyl-GPE was also effective to some extent, though the percentage of 20:4 transferred to 1-alkyl-GPC was

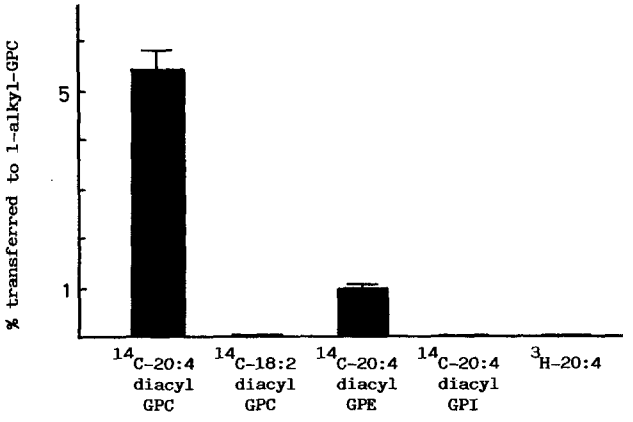

Fig.3. The donor specificity in the transfer of fatty acid to 1-alkyl-GPC. The incubation mixture contains 20μM 1-alkyl-GPC, 0.2mg of microsomal protein and labeled phospholipids or fatty acid (25000 dpm) in the same buffer as in Fig.1. The incubation was carried out at 37°C for 60 min. The values (means of three determinations ± S.D.) were expressed as the percentages of fatty acid transferred to 1-alkyl-GPC.

very low compared with the case for diacyl-GPC. On the other hand, 1-acyl-2-linoleoyl-GPC and 1-acyl-2-arachidonoyl-GPI (glycerophosphoinositol) as well as free 20:4 failed to act as the acyl donor. These results indicate that there is a distinct donor specificity in the CoA-independent transfer of 20:4 to 1-alkyl-GPC. Previously, we found that 20:4 once incorporated into diacyl-GPC was selectively mobilized to alkylacyl-GPC and alkenylacyl-GPE in intact macrophages (6). Other phospholipids except for lyso-bis-phosphatidic acid did not participate significantly in the mobilization of 20:4. Also we found that 18:2 did not mobilize between phospholipids. Thus, the results obtained in the present investigation seem to coincide well with these earlier observations in living cells, suggesting that the selective mobilization of 20:4 found in cells is attributed to the CoA-independent transfer. Although 1-acyl-2-arachidonoyl-GPE also can act as the acyl donor in in vitro enzyme assay, the radioactivity of [^{3}H]20:4 found in this lipid fraction was indeed only small in living cells. This will explain the fact that mobilization of 20:4 occurs between diacyl-GPC and alkylacyl-GPC and alkenylacyl-GPE (6). It seems possible that 20:4 is generally incorporated into ether phospholipids via such a CoA-independent transacylation rather than via an acyl-CoA:lysophospholipids acyltransferase action, since the latter enzyme activity is very low (22,23). Further studies are necessary to compare in detail the activities of transacylase and acyltransferase.

It has been already shown that 1-alkyl-2-acetyl-GPC (PAF) or 1-alkyl-GPC incubated with macrophages, polymorphonuclear leukocytes or platelets is soon metabolized to the long chain fatty acid-containing alkylacyl-GPC (5, 9-12). PAF might be first deacetylated by acetylhydrolase (24) and then acylated. It has been reported that the long chain fatty acid recovered in alkylacyl-GPC was mainly 20:4 (10). This acylation system seems to be very important for the inactivation or elimination of PAF or lyso PAF. Moreover, the reaction in turn provides 1-alkyl-2-arachidonoyl-GPC which is a potential precursor of PAF and 20:4 metabolites.

We have shown that the formation of 1-alkyl-2-arachidonoyl-GPC is due to the CoA-independent direct transfer of 20:4 from diacyl-GPC to 1-alkyl-GPC. This reaction is very similar to the CoA-independent transfer of 20:4 from diacyl-GPC to 1-alkenyl-GPE reported in platelet membrane (16), though the methods used were considerably different. In any case, such a transacylation system might regulate the availability of 20:4 and lyso PAF. Furthermore, it should be noted that there exists a unique pathway for the cleavage of 1-acyl-2-arachidonoyl-GPC in intact macrophages and microsomes. This enzyme also might play an important role in providing 20:4 from diacyl-GPC for prostaglandin and leukotriene synthesis under stimulating conditions.

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