Mobilization of arachidonic acid between diacyl and ether phospholipids in rabbit alveolar macrophages

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The metabolism of 20:4 (arachidonic acid) in alkenylacyl, alkylacyl and diacyl lipid classes in choline glycerophospholipids (CGP) and ethanolamine glycerophospholipids (EGP) in rabbit alveolar macrophages was examined. [^{3}H]20:4 was very rapidly incorporated into diacyl glycerophosphocholine (GPC). After the removal of free 20:4, the radioactivity was gradually lost from diacyl GPC. Concomitantly, the radioactivities in alkylacyl GPC and alkenylacyl glycerophosphoethanolamine (GPE) were increased, indicating that 20:4 was mobilized from diacyl GPC to alkylacyl GPC and alkenylacyl GPE. The mobilization was considered to be a 20:4-specific event. The gradual accumulation of 20:4 in ether phospholipids leads to a high abundance of 20:4 in these lipids. These results suggest metabolic relationships between 20:4 and ether phospholipids, including platelet-activating factor (PAF).

Macrophage *Arachidonic acid* *Ether phospholipid* *Alkylacyl derivative* *Alkenylacyl derivative*

1. INTRODUCTION

In [1] we have demonstrated that high levels of alkylacyl GPC (32.5% of CGP) and alkenylacyl GPE (61.2% of EGP) are observed in rabbit alveolar macrophages. It seems a distinctive feature of various types of leukocytes such as macrophages [1–3], polymorphonuclear leukocytes [2,4] and lymphocytes [2,5], that they contain high amounts of alkylacyl GPC which are utilized for the synthesis of PAF by a deacylation–reacetylation reaction [3].

Interestingly, ether phospholipids contain higher amounts of 20:4 than diacyl analogue in these cells. About 73.6% of 20:4-containing species in CGP and 85.9% of those in EGP possess alkenyl or alkyl ether moieties at their 1-positions in rabbit alveolar macrophages [1]. It will, therefore, be a very important problem to investigate precisely, the metabolism of 20:4 both in ether phospholipids and diacyl phospholipids in order to gain a better understanding of the regulatory mechanism of 20:4 in these cells. Indeed, little is known about the biochemical nature of ether phospholipids in immunological competent cells.

We here found that the mobilization of 20:4 from diacyl GPC to alkylacyl GPC and alkenylacyl GPE occurs in rabbit alveolar macrophages. This observation may be closely related to the high abundance of 20:4 in ether phospholipids.

Abbreviations: 20:4, arachidonic acid; 18:2, linoleic acid; CGP, choline glycerophospholipids; EGP, ethanolamine glycerophospholipids; IGP; inositol glycerophospholipids; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; BSA, bovine serum albumin; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; PAF, platelet-activating factor

2. MATERIALS AND METHODS

Alveolar macrophages were prepared from pulmonary lavage as in [1]. [5,6,8,9,11,12,14,15-^{3}H] 20:4 (110 Ci/mmol) and [1-^{14}C]18:2 (55 mCi/mmol) were purchased from Amersham. [^{3}H]20:4 was diluted with non-radioactive 20:4

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(final specific activity, 55 mCi/mmol). Both [^{3}H]20:4 and [^{14}C]18:2 were purified by thin-layer chromatography (TLC) before use. Macrophages were suspended in 20 mM Hepes-Eagle's minimal essential medium (MEM) (pH 7.2) at 10^6 cells/ml; 0.2 μCi of labeled fatty acid, dissolved in 50 μl of 0.2% bovine serum albumin (BSA)-containing saline, was added to 1 ml of the cell suspension and incubated at 37°C for 7.5 ([^{3}H]20:4) or 60 min ([^{14}C]18:2). Then, the cells were cooled (4°C) and sedimented by centrifugation. After the removal of the medium, cells were washed 3 times with ice-cold (4°C) 0.2% BSA-containing 20 mM Hepes-MEM and resuspended in the same medium at 10^6 cells/ml. Further incubation was carried out at 37°C and terminated by adding chloroform:methanol (1:2).

Total lipids were extracted as in [6]. Individual phospholipids were separated by 2-dimensional TLC [1,2]. A portion of the total lipids was also subjected to neutral lipid analysis by TLC, using the solvent, petroleum ether:ethyl ether:acetic acid (80:20:1). Alkenylacyl, alkylacyl and diacyl lipid classes of CGP and EGP were separated as 1,2-diradyl-3-acetylglycerol derivatives, using the solvents, petroleum ether:ethyl ether:acetic acid (90:10:1) and then toluene as described in [1,2,5]. The lipid spots were scraped off from the plates into counting vials. The radioactivity was measured in a scintillation counter, using the toluene-Triton X-100 2:4, v/v) scintillation fluid.

3. RESULTS AND DISCUSSION

About 88.9% of the radioactivity of [^{3}H]20:4 incorporated into cells was found in the phospholipid fraction. The radioactivity of the total phospholipids and those among individual neutral lipids were not significantly changed after the removal of unincorporated labeled 20:4 (not shown). Table 1 shows the distribution of [^{3}H]20:4 amon individual phospholipids. At the zero time, the radioactivities were mainly distributed in CGP, EGP, inositol glycerophospholipids (IGP) and lyso-bis-phosphatidic acid and those in other phospholipids were only small. After the exchange of the medium, the radioactivity of CGP decreased with time. On the contrary, that of EGP increased gradually. A small increase was also observed for lyso-bis-phosphatidic acid and IGP. These redistributions of [^{3}H]20:4 indicate that 20:4 once incorporated into cells were then mobilized between these phospholipids. Authors in [7] demonstrated the CoA-mediated transfer of 20:4 from CGP to EGP in mouse lymphocytes and macrophages. Our results support this finding. In order to examine this point in more detail, we separated CGP and EGP into alkenylacyl, alkylacyl and diacyl classes which are known to be different from each other in various biochemical properties [8].

Fig.1 shows the distribution of [^{3}H]20:4 in alkenylacyl, alkylacyl and diacyl GPC and GPE as a function of time after the exchange of the

Table 1

Percent distribution of [^{3}H]20:4 among phospholipids after the removal of free [^{3}H]20:4

Phospholipids	Time (min)				
	0	15	30	60	120
Choline glycerophospholipids	61.5±1.2	56.6±0.3	52.0±0.5	43.0±0.9	33.0±0.7
Ethanolamine glycerophospholipids	10.3±0.5	13.5±0.3	15.9±0.3	20.5±0.4	27.2±0.5
Inositol glycerophospholipids	11.3±0.3	12.2±0.7	12.8±0.7	14.4±0.7	13.9±1.0
Lyso-bis-phosphatidic acid	15.0±0.3	16.0±0.9	16.9±0.3	19.9±0.3	23.1±0.2
Sphingomyelin	0.4±0.2	0.4±0.1	0.4±0.1	0.3±0.1	0.6±0.2
Serine glycerophospholipids	1.2±0.1	1.1±0.7	1.8±0.6	1.5±0.2	1.9±0.4
Cardiolipin	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.1	0.2±0.1
Phosphatidic acid	0.2±0.1	0.1±0.1	0.1±0.0	0.2±0.1	0.1±0.1

Macrophages were prelabeled with [^{3}H]20:4 (0.2 μCi) for 7.5 min. Then the cells were washed 3 times with 0.2% BSA-containing Eagle's MEM and further incubated at 37°C in the same medium. Individual phospholipids were separated by 2-dimensional TLC as described in the text. The values represent the means ± SD taken from 3 determinations

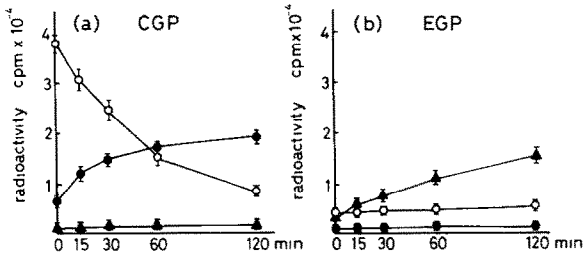

Fig.1. The radioactivities of [^{3}H]20:4 in alkenylacyl, alkylacyl and diacyl GPC (a) and GPE (b) after the removal of free [^{3}H]20:4. Alkenylacyl (▲), alkylacyl (●) and diacyl (○) lipid classes were separated as described in the text. Each point is the mean of 3 determinations ±SD.

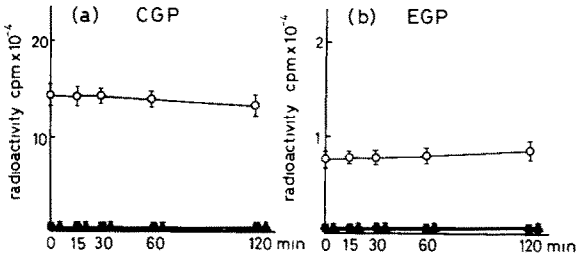

Fig.2. The radioactivities of [^{14}C]18:2 in alkenylacyl, alkylacyl and diacyl GPC (a) and GPE (b) after the removal of free [^{14}C]18:2. Alkenylacyl (▲), alkylacyl (●) and diacyl (○) lipid classes were separated as described in the text. Each point is the mean of 3 determinations ±SD.

medium. Diacyl GPC was most rapidly labeled with [^{3}H]20:4 after 7.5 min incorporation. However, the radioactivity was lost from this lipid class soon after the removal of free 20:4, indicating that the turnover of 20:4 is very rapid in diacyl GPC. In contrast, the radioactivities of alkylacyl GPC and alkenylacyl GPE were markedly increased during the incubation. The radioactivity of diacyl GPE was, however, almost unaffected. Although the radioactivities of alkenylacyl GPC and alkylacyl GPE were also increased to some extent, these were very low compared with the radioactivities in other lipid classes. Therefore, it can be considered that 20:4 was mainly transferred from diacyl GPC to alkylacyl GPC and alkenylacyl GPE. This may be closely related to the fact that both alkylacyl GPC and alkenylacyl GPE contain high amounts of 20:4 in rabbit alveolar macrophages [1]. It is very likely that ether phospholipids act as the storage of 20:4. Indeed, it has already been reported that 20:4 was retained in ether phospholipids in rat testes during the essential fatty acid deficiency [9].

We did not find any marked change of the radioactivity for 20:4 (fig.2). This suggests that the mobilization is a 20:4-specific phenomenon. Some years ago, it was shown [10] that 20:4 is mobilized from CGP or IGP to alkenylacyl GPE in thrombin-treated platelets. Our present result is compatible with their observation. We also detected the selective mobilization of 20:4 from diacyl GPC to alkylacyl GPC. This leads to high levels of alkyl-arachidonyl GPC (12.5% of CGP) in rabbit alveolar macrophages [1]. The selective acylation of alkyl GPC with 20:4 has been also demonstrated in human neutrophils [11]. It is very noticeable that both alkyl GPC and 20:4 can act as the substrates for the potent lipid mediators, PAF or prostaglandins (PGs), thromboxanes (TXs) and leukotrienes (LTs). The biosynthesis of these lipid mediators might be, therefore, a coupled event. The transesterification rate of 20:4 to alkyl GPC (lyso PAF) or alkenyl GPE might affect their availabilities for lipid mediator synthesis. Furthermore, the modes of actions of these lipids may be closely related to each other. In fact it has been shown that 20:4 metabolite, 5-L-hydroxyeicosatetraenoic acid (5-L-HETE) potentiates the degranulating action of PAF in human neutrophils [12]. However, it remains to be determined which lipid class is most responsible for 20:4 generation for the synthesis of cyclooxygenase or lipoxygenase products under stimulated conditions.

It is possible that acyltransferase is involved in the transesterification of 20:4 as pointed out in [7]. The acyltransferase activities to alkenyl GPE [13–15] and to alkyl GPC [14–16] have been already demonstrated using the microsomes from several tissues. Although their activities are shown to be considerably lower in comparison with those to 1-acyl compounds, they are highly specific for unsaturated fatty acids including both 20:4 and 18:2 [14,16,17]. Further studies are necessary to clarify the mechanism and the significance of the selective mobilization of 20:4 between diacyl and ether-containing phospholipids.

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