Platelet-activating factor (PAF) acetylhydrolase, which inactivates PAF by removing the acetyl group at the sn-2 position, is distributed widely in plasma and tissues. In a previous study, we demonstrated that the PAF acetylhydrolase activity present in the soluble fraction of bovine brain cortex could be separated chromatographically into three peaks (tentatively designated isoforms Ia, Ib, and II) (Hattori, M., Arai, H., and Inoue, K. (1993) J. Biol. Chem. 268, 18748–18753). In this study, these three isoforms were also detected in kidney and liver cytosols, although their relative activity ratios in these tissues differed. In particular, isoform II was responsible for the majority of the bovine liver PAF acetylhydrolase activity. We purified isoform II from bovine liver cytosol to near homogeneity and demonstrated that it is a single 40-kDa polypeptide. This enzyme was inactivated by diisopropyl fluorophosphate and 5,5'-dithiobis(2-nitrobenzoic acid), suggesting that both serine and cysteine residues are required for the enzyme activity, and [3H]diisopropyl fluorophosphate labeled only the 40-kDa polypeptide, confirming the enzyme's identity. Isoform II showed a comparatively broader substrate specificity than isoform Ia. Isoform Ia hydrolyzed propionyl and butyroyl moieties at the sn-2 position approximately half as effectively as it did PAF, whereas isoform Ib hardly hydrolyzed these substrates.

Taken together with previous data, the current findings indicate that tissue cytosol contains at least two types of PAF acetylhydrolase with respect to polypeptide composition, substrate specificity, and tissue distribution and suggest that these two enzymes may share distinct physiological functions in tissues.

Platelet-activating factor (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine, PAF) is a potent inflammatory mediator that causes microvascular leakage, vasodilation, smooth muscle contraction, endothelial adhesion, and neutrophil, macrophage, and eosinophil activation (for review, see Refs. 1–5). PAF acetylhydrolase was purified from human erythrocytes (18). This enzyme has a molecular mass of 25 kDa and behaves as a homodimer. We found that at least three isoforms (tentatively designated Ia, Ib, and II) of PAF acetylhydrolase existed in bovine brain and that isoform Ib, which was the most abundant, was a heterotrimeric enzyme composed of 29-, 30- and 45-kDa subunits (19). Of these subunits, the 29-kDa one served as the catalytic center, as only this subunit was labeled by [3H]diisopropyl fluorophosphate (DFP), a potent inhibitor of the enzyme (19, 20). The amino acid sequence deduced from its cDNA is unique and not homologous with those of any other known phospholipases and lipases.

In the current study, we purified another isoform (isoform II) of PAF acetylhydrolase from bovine liver and showed that intracellular PAF acetylhydrolases Ib and II are distinct proteins with different substrate specificities and tissue distributions.

**EXPERIMENTAL PROCEDURES**

Materials—1-O-Hexaeryl-2-[3H]acyetyl-sn-glycero-3-phosphocholine ([3H]acyetyl-PAF), 1-palmitoyl-2-[14C]palmitoyl-sn-glycero-3-phosphocholine, 1-[14C]palmitoyl-sn-glycero-3-phospho-choline, 1-[3H]palmitoyl-sn-glycero-3-phosphocholine, 1-[3,4-3H]hexadecyl-lyso-PAF and [1,3,4-3H]diisopropyl fluorophosphate ([3H]DFP) were purchased from DuPont NEN. Unlabeled PAF and lyso-PAF were from Bachem Feinchemikalien AG, Bubendorf, Switzerland. 1-Palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine and 1-palmitoyl-sn-glycero-3-phosphocholine were purchased from Serdary Research Laboratories Inc., Ontario, Canada. DEAE-Sepharose CL-6B, Q-Sepharose Fast Flow, and Mono Q HR5/5 were from Pharmacia Biotech Inc., Bio-Gels A-1.5m and A-0.5m were from Bio-Rad, butyl-Toyopearl 650 M was from Tosoh Co., Ltd., Tokyo, Japan, and hydroxylapatite was from Toagosei Chemical Industry Co., Ltd., Tokyo, Japan. CHAPS was purchased from Dojin Laboratories, Kumamoto, Japan. All the other materials used were from Wako Pure Chemical, Osaka, Japan.

Synthesis of Phospholipids—The specific activity of labeled lyso-PAF was adjusted to 4,000 dpm/nmol by dilution with unlabeled lyso-PAF. The sn-2 short chain phospholipids were synthesized by the coupling synthesis of a variety of mammalian cells has been examined and is known to be tightly regulated (9). Its inactivation is catalyzed by a specific acetylhydrolase, which removes the acetyl group at the sn-2 position of the glycerol backbone, to produce the biologically inactive lyso-PAF (10, 11). This process can also regulate PAF accumulation in certain types of cell. For example, human macrophages regulate PAF accumulation by altering intracellular PAF acetylhydrolase activity levels (12). It was also demonstrated that 10–12 times more PAF was produced when platelets were pretreated with phenylmethane-sulfonyl fluoride, an inhibitor of intracellular PAF acetylhydrolase, and stimulated with thrombin than after thrombin stimulation alone (13).

PAF acetylhydrolase activity was examined in various animal tissues and shown to be present in most of rat tissues (11, 14–16). It has been proposed that there is a family of distinct enzymes in animal tissues and cells (14, 17). Recently, PAF acetylhydrolase was purified from human erythrocytes (18). This enzyme has a molecular mass of 25 kDa and behaves as a homodimer. We found that at least three isoforms (tentatively designated Ia, Ib, and II) of PAF acetylhydrolase existed in bovine brain and that isoform Ib, which was the most abundant, was a heterotrimeric enzyme composed of 29-, 30- and 45-kDa subunits (19). Of these subunits, the 29-kDa one served as the catalytic center, as only this subunit was labeled by [3H]diisopropyl fluorophosphate (DFP), a potent inhibitor of the enzyme (19, 20). The amino acid sequence deduced from its cDNA is unique and not homologous with those of any other known phospholipases and lipases.

In the current study, we purified another isoform (isoform II) of PAF acetylhydrolase from bovine liver and showed that intracellular PAF acetylhydrolases Ib and II are distinct proteins with different substrate specificities and tissue distributions.
method of Gupta et al. (21). To a suspension of 4 µmol of labeled lyso-PAF in 0.4 ml of chloroform/pyridine (4:1, v/v), 2 mg of 4-(N,N-dimethylamino)pyridine and 40 µmol each of acid anhydride were added and stirred for 18 h. The product was extracted by the method of Bligh and Dyer (22) under mild alkaline and acidic conditions three times each and then purified by silica gel plate, using chloroform/methanol/ammonia solution (65:25:5, v/v) as the mobile phase.

Assays—The specific activity of [3H]acetyl-PAF was adjusted to 3,200 dpm/mmol by dilution with unlabeled PAF. The standard incubation system for assaying PAF acetylhydrolase comprised 50 mM Tris-HCl (pH 7.4), 5 mM EDTA, 5 mM 2-ME, 20 nmol of substrate, and enzyme in a total volume of 0.25 ml. After incubation for 30 min at 37°C, the reaction was stopped by adding 2.5 ml of chloroform/methanol (4:1, v/v) and 0.25 ml of water. The radioactivity of an aliquot (0.6 ml) of each upper phase was measured to determine the amount of acetate liberated. The incubation system for assaying hydrolytic activities with sn-2 short chain phospholipids as the substrates comprised 50 mM Tris-HCl (pH 7.4), 5 mM EDTA, 5 mM 2-ME, 20 nmol of [3H]-acyl-PAF, and the sample in a total volume of 0.25 ml. After incubation for 30 min at 37°C, the reaction was stopped by adding 2.5 ml of chloroform/methanol (4:1, v/v) and 0.25 ml of water. The radioactivity of an aliquot (0.6 ml) of each upper phase was measured to determine the amount of acetate liberated. The incubation system for assaying hydrolytic activities with sn-2 short chain phospholipids as the substrates comprised 50 mM Tris-HCl (pH 7.4), 5 mM EDTA, 5 mM 2-ME, 20 nmol of substrate, and enzyme in a total volume of 0.25 ml. Incubation was carried out for 30 min at 37°C, the products were extracted by the method of Bligh and Dyer (22), and separated by silica gel plate, using chloroform/methanol/ammonia solution, (65:25:5, v/v) as the mobile phase. The bands corresponding to the substrate and lyso-PAF were scraped off and examined for radioactivity. Hydrolytic activities with phosphatidylcholine and lyso-PAF were determined using the same buffer containing 20 nmol of labeled substrate as described above. The labeled free fatty acid was released by each reaction was extracted by the method described previously (19) and radioassayed.

Purification Procedure—Fresh bovine livers were obtained from a local slaughterhouse and processed within 3 h of slaughter. All procedures were carried out at 0–4°C. The livers were homogenized with a Waring blender in five volumes of homogenizing buffer (10 mM Tris-HCl (pH 7.4), 250 mM sucrose, 1 mM EDTA), and the homogenate was centrifuged at 10,000 × g for 30 min to remove the bulk of the solid material. The resulting supernatant was centrifuged at 100,000 × g for 1 h to obtain the soluble fraction (supernatant), which was adjusted to 1× with NaCl, stirred for 15 min, and applied to a butyl-Toyopearl 650M column preequilibrated with 50 mM Tris-HCl (pH 7.4), 1 mM EDTA, and 1 M NaCl. The column was washed with this buffer, and the protein was eluted with a linear gradient of NaCl (1–500 mM) in buffer A. The activity was present among the fractions eluted by 1 M NaCl. The PAF acetylhydrolase activity was eluted as a single peak among the 1–500 mM NaCl fractions. The active fractions from the butyl-Toyopearl column were applied to a Q-Sepharose column equilibrated with 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, and 20% (v/v) glycerol (buffer A). The column was washed with buffer A, and the protein was eluted with a linear gradient of NaCl (0–500 mM) in buffer A. The activity was present among the fractions eluted by about 300 mM NaCl. The active fractions from the Q-Sepharose column were concentrated in an Amicon ultrafiltration cell using a YM-10 membrane to approximately 6 ml. This concentrated fraction was applied to a Bio-Gel A-1.5m gel filtration column preequilibrated with 10 mM Tris-HCl (pH 7.4). 700 mM NaCl, 5 mM 2-ME, 20% (v/v) glycerol, and 0.5% (v/v) CHAPS (buffer B). The activity was eluted as a single peak in a fraction corresponding to molecular mass of approximately 40 kDa. The active fractions from the Bio-Gel A-1.5m column were applied to a hydroxyapatite column preequilibrated with 10 mM Tris-HCl (pH 7.4), 5 mM 2-ME, 20% (v/v) glycerol, and 0.5% (v/v) CHAPS (buffer C). The protein was eluted with a linear gradient from buffer C alone to buffer C containing 150 mM K2HPO4. The activity was present among the fractions eluted by about 50 mM K2HPO4. The active fractions from the hydroxylapatite column were dialyzed against buffer C and then applied to an FPLC Mono Q HR 5/5 column preequilibrated with buffer C and the protein was eluted with a linear gradient of NaCl (0–500 mM) in buffer C. The activity was present among the fractions eluted by 250 mM NaCl.
Bovine Liver PAF Acetylhydrolase II

RESULTS

Isoform Distribution among Brain, Kidney, and Liver—The soluble fractions obtained from bovine brain, kidney, and liver exhibited significant levels of PAF acetylhydrolase activity; the respective specific activities of these fractions were approximately 0.8, 1.2, and 1.4 nmol/min/mg, none of these activities were affected by EDTA. This level of activity was not detected in any of these tissues when phosphatidylcholine with two long acyl chains was used as a substrate.

Next, these soluble fractions were subjected to DEAE-Sepharose followed by hydroxylapatite column chromatography (Fig. 1). The kidney and liver PAF acetylhydrolase activities separated into three peaks, as did brain PAF acetylhydrolase activity. Activity was present in two peaks among the fractions eluted by about 150 and 300 mM NaCl from the DEAE column (corresponding to peaks I and II, respectively, of brain PAF acetylhydrolase), although peak I of the liver fraction was very small. The peak I fractions of liver and kidney were both separated into two peaks by hydroxylapatite chromatography (corresponding to peaks la and Ib of brain PAF acetylhydrolase). The above results indicate that bovine kidney and liver possess the same set of isoforms as bovine brain. In contrast to the brain, abundant peak II activity was present in the soluble fractions of kidney and liver. Remarkably, peak II was responsible for most of the PAF acetylhydrolase activity in the liver. No other peak showing activity was observed in the soluble fractions of the kidney or liver during the sequential chromatography.

Purification of PAF Acetylhydrolase Isoform II from Bovine Liver—As isoform II was the most abundant PAF acetylhydrolase isoform in the liver soluble fraction, we employed this tissue as a starting material for subsequent purification of the enzyme. First the liver soluble fraction was subjected to butyl-Toyopearl hydrophobic column chromatography. The PAF acetylhydrolase activity was adsorbed to the column with a buffer containing 1 mM NaCl and then eluted with buffer containing 1 mM EDTA. The elution profile of this fraction contrasted markedly with that of isoform Ib in the brain (19), which is not adsorbed to butyl-Toyopearl under the conditions. The activity was eluted as a single peak by the subsequent chromatography using Q-Sepharose, gel filtration, hydroxylapatite and Mono Q FPLC columns. When the enzyme was chromatographed on a Bio-Gel A-1.5m gel filtration column using a conventional buffer, the activity was eluted as a broad peak with extremely low recovery. In contrast, the activity was eluted as a sharp peak at the region corresponding to a molecular mass of approximately 40 kDa when a detergent (CHAPS) was included in the running buffer (data not shown). Moreover, the recovery was over 80% of the starting material under the latter conditions. Therefore, we used a buffer containing CHAPS for purification after Q-Sepharose chromatography. A typical elution profile of the activity and the SDS-PAGE pattern of the fraction after the final step, Mono Q FPLC chromatography, are shown in Fig. 2. A single major 40-kDa protein comigrated with PAF acetylhydrolase activity. In fact, this band was found to comigrate with the activity during every chromatographic procedure after the gel filtration column step. The purification procedure is summarized in Table I. The overall purification was about 4,000-fold, and the recovery was 3%.

Inhibitor Sensitivity—Various compounds were tested for their effects on the activity of the purified PAF acetylhydrolase II. Among the agents tested, DFP (1 mM), an active serine-blocking reagent, and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) (1 mM), a sulfhydryl-blocking agent, almost abolished the activity of the purified PAF acetylhydrolase II (data not shown). These data indicate strongly that both serine and

**Fig. 2.** Elution profile of PAF acetylhydrolase II on a Mono Q FPLC column. The active fractions from the hydroxylapatite column were pooled (about 25 ml), dialyzed against 500 ml of buffer C, and then applied to a Mono Q HR 5/5 FPLC column equilibrated with buffer C. After washing the column with buffer C, elution was carried out with an 18-ml linear gradient of NaCl (0–500 mM) in buffer C. Fractions (0.3 ml) were collected for PAF acetylhydrolase activity, and subjected to SDS-PAGE, after which the gel was stained with Coomassie Brilliant Blue.

**Table I.** Purification of PAF Acetylhydrolase Isoform II from Bovine Liver. | Step | Overall | Recovery | Activity | Purification Fold |
|------|---------|----------|----------|-------------|
| Initial | 1 | 100 % | 1 | 1 |
| DEAE-Sepharose | 2 | 20 | 8.5 | 20 |
| Q-Sepharose | 3 | 40 | 4.5 | 40 |
| Hydroxylapatite | 4 | 80 | 1.5 | 80 |
| Mono Q FPLC | 5 | 84 | 0.6 | 84 |

**Table II.** Inhibitor Sensitivity of PAF Acetylhydrolase II. | Inhibitor | Concentration | % Inhibition | K_i (mM) |
|------------|-------------|---------------|-----------|
| DTNB | 1 mM | 90 | 1.2 |
| DFP | 1 mM | 98 | 0.5 |
| Acetylthiocholine | 1 mM | 50 | 10 |
| Acetylthiocholine chloride | 1 mM | 50 | 5 |
| Choline | 1 mM | 0 | 0.2 |

**Table III.** Amino Acid Sequences of Peptides from 40-kDa PAF Acetylhydrolase II. | Peptide | Sequence |
|--------|----------|
| N-terminal | Ser-Glu-Glu-Glu |
| C-terminal | Glu-Glu-Glu-Glu |

Labeling of PAF Acetylhydrolase with [3H]DFP—Partial purification of PAF acetylhydrolase II from liver, kidney, and brain was carried out by sequential column chromatographies using butyl-Toyopearl, DEAE-Sepharose CL-6B, and Bio-Gel A-0.5m. Each partially purified PAF acetylhydrolase II (corresponding to an activity of 5 nmol/min) was incubated with [3H]DFP (5 µCi, 0.58 nmol) in 50 µl of buffer B for 30 min at 25 °C. Then, 25 µl of Laemmli sampling buffer (0.19 M Tris-HCl (pH 7.4), 30% (v/v) glycerol, 6% (v/v) SDS, 15% (v/v) 2-ME, 0.04 mg/ml bromophenol blue) were added, followed by incubation for 10 min at 90 °C. Then, SDS-polyacrylamide gel electrophoresis (PAGE) was carried out, using an 11% acrylamide slab gel as described by Laemmli, after which the gel was washed for 15 min with 30% (v/v) methanol containing 7.5% (v/v) acetic acid, impregnated with ENHANCE (DuPont, NEN), dried under vacuum, and then autoradiographed using Kodak XRP-5 film for 3 days at ~70 °C with an intensifying screen.

Amino Acid Sequences of Peptides from 40-kDa PAF Acetylhydrolase—Approximately 0.2 mg of purified liver enzyme was reduced with 1 mg of dithiothreitol for 2 h at room temperature and then S-alkylated with 0.6% (w/v) 4-vinylpyridine for 2 h at room temperature. This reaction mixture was applied to a reverse-phase high performance liquid chromatography (HPLC) system with a 4.6-mm × 250-mm Vydac 304-1251 C4 column pre-equilibrated with 20% (v/v) acetonitrile containing 0.1% (v/v) trifluoroacetic acid, and the proteins were eluted with a linear gradient of acetonitrile (20–85% v/v) containing 0.1% (v/v) trifluoroacetic acid. The HPLC-purified 40-kDa polypeptide was dialyzed against lysyl endopeptidase digestion buffer (0.5 M Tris-HCl (pH 8.5) and 4 mM urea). Then, 1 µg of lysyl endopeptidase was added to the sample, and the reaction mixture was incubated for 18 h at 37 °C, followed by separation by reverse-phase HPLC on a 4.6 mm × 250-mm Vydac 304-1251 C4 column using a linear gradient of acetonitrile (5–70% v/v) containing 0.1% (v/v) trifluoroacetic acid. The amino acid sequences of the peptide fragments were determined using an Applied Biosystems model 477A automated sequenator.
cysteine residues are required for enzyme activity of PAF acetylhydrolase II. Involvement of free sulfhydryl groups in the catalysis was also supported by the fact that the addition of 10 mM 2-ME reversed the inhibitory effect of DTNB (data not shown).

Sodium fluoride has been reported to inhibit partially rat lung cytosolic PAF acetyl hydrolase (23), and Stafforini et al. (17) have shown that PAF acetylhydrolase in human erythrocytes is very sensitive to sodium fluoride. We tested the effect of sodium fluoride on purified PAF acetylhydrolase II, which we found was quite resistant to this agent over a wide range of concentrations (data not shown), in agreement with the observation of Stafforini et al. (17) that PAF acetylhydrolase activity in rat liver is resistant to sodium fluoride.

Active Site Labeling with [3H]DFP—

In order to examine the different subunits of trimeric PAF acetylhydrolase Ib (19), we developed a strategy, we tested whether the purified 40-kDa polypeptide has an active serine residue, by incubating the purified enzyme fraction with [3H]DFP, followed by fluorographic analysis. As shown in Fig. 3, the purified 40-kDa polypeptide did incorporate [3H]DFP, confirming it possesses an active serine residue. The specific labeling of the 40-kDa polypeptide with [3H]DFP was seen even in the fraction obtained after the gel filtration step.2 The same bands were also labeled with [3H]DFP when partially purified isoforms II obtained from bovine brain and kidney were used (Fig. 3, lanes C and D). Therefore, the peak II fractions from the kidney and brain probably contained isoform II with the same polypeptide structure as that of the liver.

Partial Amino Acid Sequence—The N-terminal amino acid sequence of the purified 40-kDa polypeptide could not be determined, possibly because some modification of the N-terminal amino group occurred. Therefore, we treated this polypeptide with lysyl endopeptidase, as described under "Experimental Procedures." The sequence of the N-terminal residues of one fragment was (K)EEWPHROQIE (Fig. 4). A search using a protein sequence data bank indicated that there were no perfect matches of this stretch of amino acids with any sequences present in the data bank.

Substrate Specificity—In order to examine the different characteristics of PAF acetylhydrolases II and Ib, various PAF derivatives were tested for their substrate activity (Fig. 5). First, both isoforms II and Ib were found to have little or no hydrolytic activity when phosphatidylcholine with two long acyl chains was the substrate, irrespective of the presence of calcium ions, a common activator of phospholipase A2. Next, derivatives of [14C]lysophosphatidylcholine containing sn-2 fatty acyl residues of increasing chain length were synthesized and tested as substrates for the purified PAF acetylhydrolases II and Ib. The purified PAF acetylhydrolase II hydrolyzed acyl groups that were 3 (propionyl) and 4 (butyroyl) carbon atoms long about equally. 

Table I

| Step                  | Total protein | Total activity | Specific activity | Purification | Yield |
|-----------------------|---------------|----------------|-------------------|--------------|-------|
|                       | mg            | μmol/min       | nmol/min/mg       | -fold        | %     |
| Cytosol               | 46,000        | 73.5           | 1.6               | 1            | 100   |
| Butyl-Toyopearl       | 680           | 16.3           | 24                | 15           | 22    |
| Q-Sepharose FF        | 72.4          | 8.96           | 124               | 78           | 12    |
| Bio-Gel A-1.5m        | 6.93          | 7.38           | 1060              | 670          | 10    |
| Hydroxylapatite       | 3.45          | 5.29           | 1530              | 960          | 7.2   |
| Mono Q FPLC           | 0.3           | 2.16           | 7200              | 4500         | 2.9   |

3 K. Hattori, M. Hattori, H. Adachi, M. Tsujimoto, H. Arai, and K. Inoue, unpublished observation.

3 K. Hattori, M. Hattori, H. Adachi, M. Tsujimoto, H. Arai, and K. Inoue, submitted for publication.

FIG. 3. [3H]DFP labeling of PAF acetylhydrolase II. PAF acetylhydrolase II was partially purified from various tissues by sequential column chromatography using butyl-Toyopearl, DEAE-Sepharose, and Bio-Gel A-0.5m columns. Then, 5 nmol/min enzyme were incubated with [3H]DFP (5 μCi, 0.58 nmol) at 25°C for 30 min and subjected to SDS-PAGE. A, Coomassie Brilliant Blue-stained gel after SDS-PAGE of the purified PAF acetylhydrolase II from liver. B, fluorograph of labeled PAF acetylhydrolase II from liver. C, fluorograph of labeled PAF acetylhydrolase II from brain. D, fluorograph of the labeled PAF acetylhydrolase II from kidney. For details, see "Experimental Procedures."

The addition of a carboxylic acid function to the ω-terminal carbon of the sn-2 fatty acid residue did not seem to increase the substrate hydrolytic activity of the enzyme, although these substrates can be hydrolyzed by the enzyme at a significant rate. When the sn-2 residue was longer than 2 carbon atoms, the catalytic efficiency of PAF acetylhydrolase Ib was dramatically reduced. The PAF homologue with a propionyl residue at the sn-2 position was hydrolyzed only 10% as efficiently as PAF and that with a butyroyl residue had virtually no substrate activity. This substrate specificity shows that PAF acetylhydrolase Ib behaves as a specific acetylhydrolase. Therefore, PAF acetylhydrolases II and Ib can be differentiated from each other by their different substrate specificities.

DISCUSSION

In the current study, we purified another type of intracellular PAF acetylhydrolase from bovine liver and showed that it was a single 40-kDa polypeptide. Moreover, we found that the relative ratios of these two enzymes in bovine tissues differed. We demonstrated previously that the PAF acetylhydrolase activity present in the cytosolic fraction of bovine brain cortex separated chromatographically into three peaks (designated peaks Ia, Ib, and II) and that the enzyme in peak Ib was a heterotrimer comprising 29-, 30- and 45-kDa polypeptides (19). The enzyme belong to a novel serine esterase family: both the 29- and 30-kDa subunits have a catalytic serine residue (20). In contrast, PAF acetylhydrolase II (in the peak II fraction) appears to be a monomeric enzyme. It is, however, too early to be absolutely certain that this is the case, as we could obtain only a sharp single peak of activity by gel filtration chromatography in the presence of a certain detergent (CHAPS). Consistent with these data, Stafforini et al. (14) showed that PAF acetylhydrolase from rat liver cytosol was eluted in a relatively broad peak when subjected to Sephadex G-200 gel filtration chromatography. The chromatographic behavior on a butyl-Toyopearl hydrophobic column suggested that PAF acetylhydrolase II is more hydrophobic in nature than PAF acetylhydrolase II.
drolase Ib. The former enzyme may be associated with some other cytosolic component(s). It should be noted that human plasma PAF acetylhydrolase, a 44-kDa monomer, is associated with plasma lipoproteins, such as low and high density lipoproteins, and cannot be dissociated from them without detergent (24, 25).

The suggestion that PAF acetylhydrolases Ib and II are distinct enzymes is also supported by the amino acid sequence data. The catalytic components of PAF acetylhydrolase Ib, the 29- and 30-kDa subunits, are novel proteins with no significant homology with other known proteins. The partial amino acid sequence of the 40-kDa PAF acetylhydrolase II matches neither those of these polypeptides nor any other protein reported so far. Very recently, the cDNA for human plasma PAF acetylhydrolase was cloned and sequenced (26). Both plasma and intracellular (isoform Ib) enzymes belong to the serine esterase family, but otherwise these proteins showed no sequence similarities, and no identical sequences in the plasma enzyme and intracellular enzyme isoform II were observed. Northern blot analysis of human tissues using the cDNA for the plasma enzyme revealed that signals was hardly detected in the human liver or kidney, whereas in both these bovine tissues, PAF acetylhydrolase II is the most abundant isoform. These findings also support the hypothesis that the plasma enzyme and isoform II are distinct proteins. The N-terminal amino acid sequence of PAF acetylhydrolase from human erythrocytes has been reported (18). Despite repeated attempts to determine the N-terminal amino acid sequence of the purified PAF acetylhydrolase II, we were unable to do so, possibly because some modification of the N-terminal amino group occurred. The erythrocyte enzyme appears to be a homodimer consisting of the 25-kDa polypeptide (18). Moreover, the elution positions of the liver and erythrocyte PAF acetylhydrolases indicated that these two activities could be differentiated from each other. All these data support the concept that PAF acetylhydrolase from erythrocytes is not identical to PAF acetylhydrolase II. Aarsman et al. (27) reported that two lysophospholipases (I and II) existed in bovine liver cytosol and that lysophospholipase II, the molecular mass of which SDS-PAGE showed was 63 kDa, had intrinsic PAF acetylhydrolase activity, although deacylation of PAF was five times slower than deacylation of lysospholipid. We tested whether the purified PAF acetylhydrolase II possessed lysophospholipase activity using 1-palmitoylglycerophosphocholine as a substrate and found that this enzyme was unable to split acyl chains attached to the sn-1 position of glycerophospholipid. According to our purification data, over 70% of the original activity was lost during the first chromatographic step, when lysophospholipase II might have been separated from PAF acetylhydrolase II.

Recently, we found that peak Ia obtained after hydroxylapatite chromatography of peak I was a 60-kDa enzyme consisting of 29- and 30-kDa polypeptides. These polypeptides are immunologically identical to the corresponding subunits of isoform Ib, indicating that these two isoforms are mutually related. Thus, at present, it can be concluded that the intracellular type of PAF acetylhydrolase comprises at least three groups of enzymes, PAF acetylhydrolases I (Ia and Ib), PAF acetylhydrolase II, and erythrocyte-type PAF acetylhydrolase.

We showed previously that one subunit (45 kDa) of isoform Ib is identical to the product of the causative gene for Miller-Dieker lissencephaly, a human brain malformation that manifests a smooth cerebral surface and abnormal neuronal migration (28). This study raised the possibility that the migration of neuronal cells during brain development is regulated by PAF
(or a PAF-like phospholipid), and a defect in PAF acetylhydrolase Ib results in defective or unregulated PAF metabolism in the cells. It should be noted that the catalytic subunit of isoform Ib possesses a sequence of about 30 amino acids located 6 residues downstream from the active serine site, which exhibits significant homology to the first transmembrane region of the PAF receptor (20), whereas plasma PAF acetylhydrolase does not possess such a region (26). Although it is not clear whether this domain is involved in substrate binding, such a sequence in the catalytic subunit may confer the property of specific recognition of PAF on isoform Ib. The function of isoform II is unknown at present. Isoform II was found to have a specific recognition of PAF on isoform Ib. The function of isoform II may recognize and degrade oxidized species without acting on toxic products of lipid peroxidation, such as oxidatively damaged phospholipids produced in the cells. It should be noted that the catalytic subunit of isoform II is known to be rich in superoxide dismutase. The next challenge is to clarify the structural and functional differences of the two distinct intracellular PAF acetylhydrolases.

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