J. Biochem. **131**, 635–640 (2002)

Platelet-Activating Factor Acetylhydrolase (PAF-AH)

Hiroyuki Arai,*,[1] Hiroyuki Koizumi,* Junken Aoki,* and Keizo Inoue†

Graduate School of Pharmaceutical Sciences, University of Tokyo, 3-1, Hongo-7, Bunkyo-ku, Tokyo 113-0033; and †Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Tsukui, Kanagawa 199-0195

Received February 22, 2002; accepted April 10, 2002

Platelet-activating factor (PAF) is one of the most potent lipid messengers involved in a variety of physiological events. The acetyl group at the sn-2 position of its glycerol backbone is essential for its biological activity, and its deacetylation induces loss of activity. The deacetylation reaction is catalyzed by PAF-acetylhydrolase (PAF-AH). A series of biochemical and enzymological evaluations revealed that at least three types of PAF-AH exist in mammals, namely the intracellular types I and II and a plasma type. Type I PAF-AH is a G-protein-like complex consisting of two catalytic subunits (α1 and α2) and a regulatory β subunit. The β subunit is a product of the *LIS1* gene, mutations of which cause type I lissencephaly. Recent studies indicate that LIS1/β is important in cellular functions such as induction of nuclear movement and control of microtubule organization. Although substantial evidence is accumulating supporting the idea that the catalytic subunits are also involved in microtubule function, it is still unknown what role PAF plays in the process and whether PAF is an endogenous substrate of this enzyme. Type II PAF-AH is a single polypeptide and shows significant sequence homology with plasma PAF-AH. Type II PAF-AH is myristoylated at the N-terminus and like other *N*-myristoylated proteins is distributed in both the cytosol and membranes. Plasma PAF-AH is also a single polypeptide and exists in association with plasma lipoproteins. Type II PAF-AH as well as plasma PAF-AH may play a role as a scavenger of oxidized phospholipids which are thought to be involved in diverse pathological processes, including disorganization of membrane structure and PAF-like proinflammatory action. In this review, we will focus on the structures and possible biological functions of intracellular PAF-AHs.

Key words: brain development, Miller-Dieker syndrome, oxidized phospholipids, PAF.

1. Introduction

Platelet-activating factor (PAF) is one of the most potent lipid messengers involved in a variety of physiological events. The acetyl group at the *sn*-2 position of its glycerol backbone is eminent for its biological activity, and its deacetylation induces loss of activity. The deacetylation reaction is catalyzed by PAF-acetylhydrolase (PAF-AH). The PAF-AH activity was first described in 1980 (*1*). Subsequently, Blank and colleagues reported that this enzyme is widely distributed in mammalian tissues and blood and that it is specific for deacetylation of phospholipids (*2*). They also suggested that PAF-AH activities in the plasma and kidney soluble fraction may be due to separate proteins, since only the latter is inactivated by protease treatment. Following these findings, a series of biochemical and enzymological evaluations revealed that at least two types of PAF-AH exist, namely the intracellular (cytosolic) and extracellular (plasma) type. The extracellular type of PAF-AH was purified from human plasma in 1987 by Stafforini and colleagues (*3*), who also extensively characterized the protein and cloned its cDNA in 1995 (*4, 5*). They also reported on the occurrence of PAF-AH activity in human erythrocytes (*6*) and partially purified the enzyme. However, the cDNA encoding erythrocyte PAF-AH has not been cloned so far. We found that a bovine brain soluble fraction contains at least two types of PAF-AH, namely PAF-AH (I) and PAF-AH (II) (*7*). Similar results were obtained when bovine kidney and liver were analyzed. We purified the PAF-AH (I) from bovine brain in 1993 (*7*) and PAF-AH (II) from bovine liver in 1995 (*8*). We also cloned their cDNAs (*9–12*) and demonstrated that I and II have totally different structures. PAF-AH (I) is a heterotrimeric enzyme composed of α1, α2, and β subunits (*7*), while PAF-AH (II) is a 40-kDa monomer and its amino acid sequence shows 43% identity with that of plasma PAF-AH (*3, 9, 12*). Since excellent reviews on plasma PAF-AH have already been published elswhere (*13, 14*), we focus on the structures and possible biological functions of mammalian intracellular PAF-AHs in this review.

2. Intracellular type I PAF-AH

2-1. General characteristics. Intracellular PAF-AH (I) purified from bovine brain cytosol gives three distinct bands of 45, 30, and 29 kDa, respectively, on SDS-PAGE. Originally we named these polypeptides α, β, and γ (*7*); however, we renamed them β, α1, and α2, since the overall structure of the enzyme was later found to be very similar to that of a trimeric G-protein (*15*). The amino acid sequences of the three subunits show extremely high homologies among the mammalian species (*9–11, 16–19*). The

[1] To whom correspondence should be addressed. Tel: +81-3-5841-4720, Fax: +81-3-3818-3173, E-mail: harai@mol.f.u-tokyo.ac.jp

amino acid sequences of the β subunit from mouse, rat, and cow are identical and only one amino acid substitution is observed in the human β subunit. Similarly, only one amino acid substitution is observed in the human α2 subunit in comparison with the other three species. The sequence identities of the α1 subunits are lower than in the α2 and β subunits, but are still over 95% among these four species. The α1 and α2 subunits show approximately 60% amino acid homology. Both α1 and α2 have a catalytic center (*9, 10*). PAF-AH (I) exhibits pH optima in the neutral to mild alkaline region and is unaffected by EDTA. Diisopropyl fluorophosphate, an inhibitor of serine esterase family, completely inhibited the activity at 0.1 mM. In fact, we identified Ser^{47} of the bovine α1 subunit as an active serine residue. The sequence surrounding it is different from the consensus sequence (Gly-Xaa-Ser-Xaa-Gly) of the serine esterase family. When these subunits are expressed in *Escherichia coli*, they form catalytically competent homodimers (*15, 9*), while the α1 and α2 subunits of the PAF-AH (I) purified from bovine brain formed a α1/α2 heterodimer. The β subunit, which does not possess enzymatic activity, has a unique domain structure called a WD-repeat, that may interact with other proteins (*20, 21*).

2-2. Properties of catalytic subunit homo- and heterodimers. PAF-AH (I) has a strong specificity for the acetyl group attached to the glycerol backbone of PAF, while phospholipids having a head group other than choline are also good substrates for the enzyme (*46*). The α2/α2 homodimer shows altered substrate specificity compared to the α1/α1 homodimer and α1/α2 heterodimer. The substrate specificity of the latter two is similar. Of the 1-*O*-alkyl-2-acetyl-phospholipids, the α2/α2 homodimer hydrolyzes PAF (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphophatidylcholine) and 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphophatidylethanolamine most efficiently. In contrast, both α1/α1 and α1/α2 hydrolyzes 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphophatidic acid more efficiently than PAF. 1-*O*-Alkyl-2-acetyl-*sn*-glycero-3-phosphophatidylethanolamine is the poorest substrate for these enzymes. The β subunit binds to all three catalytic dimers but modulates the enzyme activity depending on catalytic dimer composition. The β subunit strongly accelerates the enzyme activity of the α2/α2 homodimer but rather suppresses the activity of the α1/α1 homodimer and has little effect on that of the α1/α2 heterodimer. Thus, the enzyme activity of PAF-AH (I) may be regulated in multiple ways by altering the composition of the catalytic subunit and by manipulating the β subunit.

2-3. PAF-AH (I) and lissencephaly. It was found unexpectedly that the β subunit gene is identical to the human *LIS1* gene, the causative gene of type I lissencephaly (*11, 22*). Brain development is severely impaired in children with lissencephaly. The specific distribution of neurons within the cerebral cortex is disrupted, a condition that arises from inadequate migration of neuronal progenitors to their cortical destinations. This causes the cortex to be underpopulated with neurons. Consequently, the surface of the brain is smooth rather than folded as it is in normal individuals. The defective cortical development causes severe mental retardation, epilepsy, and usually early death.

The first clue to understand the role of LIS1/β in neuronal migration came from the identification of its homologue outside the vertebrate world, namely *nudF* in *Aspergillus nidulans*. In hyphae of the filamentous fungus, *A. nidulans*, numerous nuclei are evenly distributed within one and the same cytoplasm, and the nuclei migrate outwards as the hyphal tips extend (*23*). Genetic analysis of nuclear movement in fungi has led to the identification of genes (called *nud* genes) required for nuclear migration and positioning (*23, 24*). These include genes encoding α-tubulin, a component of microtubules (*25*), cytoplasmic dynein heavy and light chains which are components of a microtubule associated mechanical motor (*26, 27*), ARP1, a component of the dynactin complex which functions as a dynein activator (*14*), and some other proteins of unknown function (*28, 29*). These data indicate that the nuclei migrate in the cytoplasm of fungi along microtubules by the use of a cytoplasmic dynein motor. Interestingly, one of these genes, the *NudF* gene, was found to encode a mammalian homologue of LIS1 (*30*). This finding directly suggested that LIS1/β may also be involved in nuclear movement during neuronal migration (*31*). The hypothesis that the defective neuronal migration seen in lissencephaly may be a failure of nuclear translocation has also been tested by examining the migratory behaviour of cerebellar granule cell explants from LIS1/β knockout mice (*32*). Wild type explants send out cytoplasmic processes through which the nuclei then migrate. Explants from LIS1/β knockout mice extend processes similar to those of wild-type mice, but migration of the nuclei is impaired. Thus, the LIS1/β knockout mouse model supports the hypothesis that a nuclear migration defect is the cause of the neuronal migration defect in lissencephaly.

A series of studies indicate that LIS1/β not only interacts with the catalytic subunit of PAF-AH (I) but also with a number of proteins including the dynein heavy and intermediate chains (*33, 34*), as well as α-tubulin (*35*), and NUDE (*36–39*), a mammalian homologue of the fungal nudE gene product. These observations show that the β subunit functions as a subunit of intracellular PAF-AH and also exhibits other important cellular functions such as induction of nuclear movement and control of microtubule organization. This leads to the fact that one has to consider if the catalytic subunit of PAF-AH (I) and its substrate, PAF itself, is also involved in these processes. There is no direct evidence so far proving that the PAF-AH catalytic subunit is directly involved in neuronal migration. In the following, some data are presented that support the hypothesis that the PAF-AH (I) catalytic subunits also play a role in brain development and diseases.

2-4. Alteration of the catalytic dimer during brain development. All three subunits of PAF-AH (I) are expressed in embryonic brain, whereas only the α2 and β subunits are detected in the adult brain (*40*). Biochemical analyses revealed that the α1/α2 heterodimer in embryonic and α2/α2 homodimer in adult brain are major catalytic units of PAF-AH. Furthermore, it was found that using primary cultured cells isolated from neonatal rat brain that α1 protein is expressed only in neurons and not in glial cells. In contrast, α2 and β proteins are detected both in neuronal and non-neuronal tissues, showing almost constant expression levels from fetal stages to adulthood. These results demonstrates that α1 expression is restricted to actively migrating neurons and that alteration of catalytic subunits from the α1/α2 heterodimer to the α2/α2 homodimer occurs in these cells during brain development.

It is therefore suggested that these catalytic subunits play a role in neuronal migration.

2-5. PAF-AH (I) catalytic subunit and brain impairment. Nothwang *et al.* reported on the molecular characterization of a translocation t(1;19)(q21.3;q13.2) in a female with mental retardation, ataxia and atrophy of the brain (*41*). Sequence analysis of the breakpoints revealed an Alu-repeat-mediated mechanism of recombination that led to truncation of two genes: the kinase CLK2 and α1 subunit of PAF-AH (I). Two reciprocal fusion genes are present. One expressed fusion gene encodes the first 136 amino acids of the α1 subunit followed by the complete CLK2 protein. The truncated α1 protein lost its hydrolytic activity and potential to interact with the β subunit. These data emphasize the importance of the catalytic subunit PAF-AH (I) in brain development and functioning, demonstrating the first fusion gene apparently not associated with cancer.

2-6. PAF-AH (I) catalytic subunit and lymphoma. Human chromosome 11q23 is a pathologically important region, particularly in haematopoietic malignancies where it can be found rearranged with many different chromosomal regions in a variety of different leukemia subtypes (*42*). Within this region, Lecointe *et al.* identified two different genes, one of which was the human homologue of the α2 subunit of PAF-AH (I) (*43*).

A particularly significant finding is that the translocation breakpoint occurred in the first intron of the α2 subunit gene in several human lymphomas. This recombination event does not affect the coding sequences of the gene but removes the first non-coding exon and places the remaining exons under the control of immunogloblin heavy chain regulatory elements (*44*). This translocation is similar to the t(8;14) translocation which deregulates the *c-myc* gene in human Burkitt's lymphoma. Using transgenic animal models, the deregulation of *c-myc* by loss of the 1st exon and juxtaposition to the immunoglobulin heavy chain enhancer sequence has proven to be oncogenic (*45*). In analogy with this case, it is expected that this t(11;14)(q23;q32) translocation could lead to deregulation of the expression of the α2 subunit in lymphoid cells.

2-7. Tertiary structure of the catalytic subunit. Although findings that the catalytic subunit as well as the β subunit is involved in brain development seem plausible, it is still unclear whether PAF is an endogenous substrate of this enzyme. This is especially the case due to the fact that this enzyme can hydrolyse acetyl groups attached to other lipid substrate besides PAF and sometimes this is done more efficiently than in the case of PAF. The tertiary structure of the catalytic subunit has been resolved by X-ray crystallographic studies (*15, 47*). This infomation may give us clues that will assist in identifying the naturally occuring endogenous substrate for PAF-AH (I).

The crystal structure of the bovine α1 subunit of PAF AH (I) has been resolved at 1.7 Å resolution in complex with a reaction product, namely acetate. The PAF-AH (I) α1 subunit contains a single α/β domain with a central, parallel, 6-stranded β-sheet. This fold is very similar to that found in $p21^{ras}$ and other GTPases. The insertions and deletions in the α1 structure, compared to $p21^{ras}$, can be summarized in the following terms: the turn/β1 fragment, which is unique to $p21^{ras}$ and involved in GTP binding, is absent in the α1 subunit. The first β-strand found in the a subunit of the G-protein transducin and in $p21^{ras}$, which is involved in other protein interactions, is also unique to these proteins. On the other hand, the a1 subunit contains a unique N-terminal α-helix which, together with the loop that follows, is part of the dimer-forming interface. Finally, the catalytic center of α1 is located on two omega loops that are significantly different from their analogues in $p21^{ras}$.

The α1/α1 homodimer has a doughnut-shaped structure with an accessible gorge, about 15 Å deep (Fig. 1). The two active sites are at the bottom of the gorge and only 12 Å apart. The proximity suggests that these sites do not function independently, consistent with the noted functional asymmetry of the heterodimer in which the activity of the α2 subunit can be suppressed by steric effects. PAF-AH (I) is very selective, being 20 times less active towards propionyl-PAF than PAF itself. Three residues, all conserved in the α1 and α2 subunits, come into contact with the acetate's methyl group: these are Leu^{48}, Leu^{194}, and Thr^{103}.

In general, catalysis by neutral lipases and secretory phospholipase A2 is markedly enhanced at substrate con-

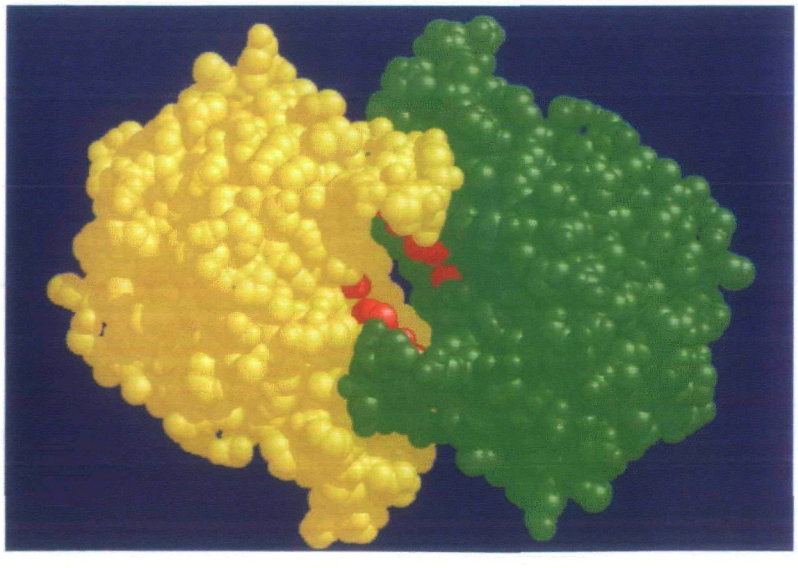

Fig. 1. **Tertiary structure of α1/α1 homodimer.** Yellow and green molecules represent each α1 subunit. Red represents a catalytic center of each α1 subunit.

centration above their critical micellar concentrations (CMCs). Plasma PAF-AH is significantly more active at a PAF concentration above its CMC. The structure of the α1 homodimer shows that the entrance to the active gorge is not particularly hydrophobic, and there is no flexible secondary structural element that might function as a 'lid', a structural motif in serine-dependent lipases that are active at the interface. The crystal structure is therefore consistent with the enzyme being active when in contact with a monomeric substrate.

2-8. Structural similarity with other proteins. Among eukaryotes, only *Drosophila* has so far been shown to harbor a gene coding a homologue of the α subunit of PAF-AH (I) (*48*). However, the protein is catalytically inert, at least with PAF-related substrates and probably also with other esters, given that two of the three residues in the active site triad are altered. In contrast to the mammalian protein, the *Drosophila* homologue is largely monomeric, as judged by gel filtration experiments and does not associate with the *Drosophila* LIS1 homologue.

Recent literature suggests that the catalytic subunit of PAF-AH (I) is distantly related to a diverse family of microbial hydrolases, that have complex saccharides as their substrate (*49–51*). Rhamnogalacturnan is one of the complex polysaccharides in the pectin substances that constitute the primary cell-wall of higher plants. The backbone of its main component, rhamnose and galacturonic acid residues form the dimer repeat unit. Galacturonic acid residues can be acetylated in the C2 and/or C3 position. *Aspergillus aculeatus* produces enzymes that work on the degradation of this complex polysaccharide, each having their own specialized function. One of the first steps in the degradation is the removal of the acetyl group from the galacturonic acid residues, which is an essential for the subsequent action of the enzymes that cleave the glycosidic bond. Rhamnogalacturonal acetylhydrolase from *A. aculeatus* possesses this function. The crystal structure of the rhamnogalacturonan acetylhydrolase from *A. aculeatus* (*49*) has recently been determined at 1.55 Å resolution. Structural and sequence comparisons have revealed that despite very low sequence similarity, this enzyme is related to the α1 subunit of PAF-AH (I) and several other proteins of lower organisms. This new serine esterase family is characterized by four conserved blocks of residues, each containing one completely conserved residue, namely Ser, Gly, Asp, and His, respectively. Each of the four residues plays a role in catalysis. The sequence order of the catalytic residues in these enzymes is Ser-Asp-His, as in the conventional α/β serine esterase. The last two residues in the α/β esterase are quite far away from each other, while in the new serine esterase family described here they are separated by only two residues. This family includes a bacterial pectin acetylhydrolase from *Erwinia chrysanthemi*, a cephalosporine C acetylhydrolase from *Bacillus* sp., and the α1 subunit of PAF-AH (I). It should be noted that all the enzymes function as acetylhydrolases. Among these families, the structure of rhamnogalacturonal acetylhydrolase and the PAF-AH (I) α subunit show great overlapping in tertiary structure. The active site of rhamnogalacturonal acetylhydrolase is at the bottom of an open cleft, making it easily accesible to a large complex polysaccharide as a substrate. In sharp contrast to this finding, the catalytic dimer of PAF-AH (I) contained the active site deep at the bottom of a small gorge, suggesting different, possibly a small molecule as a substrate of this enzyme.

3. Intracellular Type II PAF-AH

3-1. General characteristics. In some tissues the predominant PAF-acetylhydrolase activity is due to PAF-AH (II) rather than PAF-AH (I). Intracellular PAF-AH (II) was initially purified from the bovine liver cytosol (*8*) and consists of a single 40 kDa polypeptide chain. The amino acid sequence, deduced from the cDNA of PAF-AH (II), has no homology with any subunit of PAF-AH (I). However, the amino acid sequence of PAF-AH (II) shows 42% identity with that of the extracellular, plasma PAF acetylhydrolase (*4, 5, 8, 12*). PAF-AH (II) has an *N*-myristoylation signal at the N-terminus (NH_2-Met-Gly-X-X-X-Ser-). In fact, PAF-AH (II) is myristoylated and distributed in both the cytosol and membrane fractions similar to other myristoylated protein (*52*). On the other hand, the PAF-AH (I) catalytic subunits are exclusively located in the cytosol. PAF-AH (II) contains a characteristic lipase/esterase catalytic center (Gly-X-Ser-X-Gly motif) with Ser as the attacking nucleophile, and His and Asp likely forming the other two elements of the catalytic triad. In contrast to PAF-AH (I), PAF-AH (II) is easily inactivated by sulfydryl agents such as 5,5′-dithiobis(2-nitrobenzoic acid), suggesting the presence of a free cystein residue essential for catalysis. A hydrophobic portion comprising the first 20 residues of plasma PAF-AH appears to form the signal sequence for secreted proteins. When the amino acid sequences of both the plasma PAF-AH and PAF-AH (II) were aligned to maximize matching, PAF-AH (II) shows lacking of the first 50 residues of the plasma enzyme. PAF-AH (II) has a *N*-myristoylation signal instead of a signal sequence for secretion, confirming its intracellular location.

3-2. Substrate specificity. PAF-AH (II) shows substrate specificity which is essentially very similar to plasma PAF-AH. Substrate specificity of plasma PAF-AH as well as PAF-AH (II) was extensively studied by Min *et al.* (*53*). Both enzymes hydrolyze short-chain diacylglycerols, triacylglycerols, and acetylated alkanols, and display phospholipase A1 activity. On the basis of these substrate specificity results, it seems to be the case that the minimal structural requirement for a good substrate is the portion of a glyceride derivative that includes an *sn*-2 ester and a reasonably hydrophobic chain in the position occupied by the *sn*-1 chain. Both enzymes do not distinguish between an ester or an ether at the *sn*-1 position of PAF or PAF analogues (*28*), and both can hydrolyze phospholipids with short to medium length *sn*-2 acyl chains including truncated chains derived from oxidative cleavage of long-chain polyunsaturated fatty acyl groups (*28*). Activity of these enzymes toward phospholipids with two long (14–18 carbons) fatty acyl chains is negligible (*28–30*). The broad specificity of these enzymes raises the possibility that PAF and phospholipids with oxidatively truncated *sn*-2 chains are not the only physiological substrates for these enzymes.

3-3. Cellular functions. PAF-AH (II) has been shown to be an antioxidant phospholipase in a number of systems. It plays a role in the protection of oxidative stress induced cell death, as demonstrated by Matsuzawa *et al.* (*52*). It has been shown that the enzyme translocates to the membrane during oxidative stress, and that it inhibits oxidative stress-induced apoptosis, presumably by scavenging oxi-

dized phospholipids. Overexpression of the enzyme resulted in protection against oxidative cytotoxicity. Thus, PAF-AH (II) recognizes the redox state of the interior environment of the cell, changes location in response to cellular requirements, and prevents lipid peroxidation damage. In other words, PAF-AH(II) is a scavenger of oxidized phospholipids which are thought to be involved in diverse pathological processes, including disorganization of membrane structure and PAF-like proinflammatory action.

Recently it has been reported that mammalian PAF-AH (II) possesses transacetylase activity as well as hydrolase activity under certain conditions (*54*). In an *in vitro* assay, the enzyme can transfer an acetyl group from PAF to acceptor lipids such as lysophospholipids and sphingosine. Analysis of a series of site-directed mutant PAF-AH (II) proteins showed that transacetylase activity was decreased,whereas PAF-AH activity was not affected in Cys^{120}Ser and Gly^{2}Ala mutant protein, suggesting that Cys^{120} and myristic acid attached to the N-terminal have a function in the transacetylase but not in the acetylhydrolase. When PAF is added exogenously to cultures of CHO-K1 cells that overexpress this enzyme, the acetyl group of PAF is efficiently transferred to certain endogenous acceptor lipid(s). This raises the possibility that one of the physiological roles of PAF-AH (II) is to diversify the biological function of PAF by producing different lipid mediators such as analogs of PAF and C2-ceramide.

4. Conclusion

Significant progress on the molecular structures of intracellular PAF-AH as well as plasma PAF-AH has been achieved in the past decade. As a result, we found that both intracellular PAF-AHs (I) and (II) are a very unique serine esterase. The catalytic subunits of PAF-AH (I) have no significant homology with any other mammalian proteins. In contrast, its tertiary structure unexpectedly resembles that of the G-protein family such as $p21^{ras}$ and Gα. In addition, the tertiary structure surrounding the catalytic center has significant homology with a series of bacterial polysaccharide acetylhydrolases. Although the evidence in favor of physiological relevance of the association of LIS1 and the catalytic subunits of PAF-AH (I) appears very strong, it is not clear what role PAF plays for instance in neuronal migration. Recent studies have shown that the catalytic subunits will hydrolyze substrates similar to PAF, except for their head groups. Furthermore, it is now known that the α subunit is a member of an old family of hydrolytic proteins, identified some years ago as a family of lipolytic enzymes. It is therefore conceivable that the natural substrate of the presumed PAF-AH (I) could be an as yet unidentified molecule with an acetyl group attached via an ester bond. PAF-AH (II) is also a unique serine esterase with a myristic acid attached at the N-terminal. Although its function in the protection of oxidative stress induced cell death has been elegantly demonstrated, recent work suggested that it also functions as a transacetylase. It therefore is evident that following experiments must elucidate whether only PAF itself or possible other endogenous substrates are of major importance for the physiological functioning of PAF-AH (I) and (II).

REFERENCES

1. Farr. R.S., Cox, C.P., Wardlow, M.L., and Jorgensen, R. (1980) Preliminary studies of an acid-labile factor (ALF) in human sera that inactivates platelet-activating factor (PAF). *Clin Immunol. Immunopathol.* **15**, 318–330
2. Blank, M.L., Lee, T., Fitzgerald, V., and Snyder, F. (1981) A specific acetylhydrolase for 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine (a hypotensive and platelet-activating lipid). *J. Biol. Chem.* **256**, 175–178
3. Stafforini, D.M., Prescott, S.M., and McIntyre, T.M. (1987) Human plasma platelet-activating factor acetylhydrolase. Purification and properties. *J. Biol. Chem.* **262**, 4223–4230
4. Tjoelker, L.W., Eberhardt, C., Unger, J., Trong, H.L, Zimmerman, G.A., McIntyre, T.M., Stafforini, D.M., Prescott, S.M., and Gray, P.W. (1995) Plasma platelet-activating factor acetylhydrolase is a secreted phospholipase A2 with a catalytic triad. *J. Biol. Chem.* **270**, 25481–25487
5. Tjoelker, L.W., Wilder, C., Eberhardt, C., Stafforini, D.M., Dietsch, G., Schimpf, B., Hooper, S., Trong, H.L., Cousens, L.S., Zimmerman, G.A., Yamada, Y., McIntyre, T.M., Prescott, S.M., and Gray, P.W. (1995) Anti-inflammatory properties of a platelet-activating factor acetylhydrolase. *Nature* **374**, 549–552
6. Stafforini, D.M., Rollins, E.N., Prescott, S.M., and McIntyre, T.M. (1993) The platelet-activating factor acetylhydrolase from human erythrocytes. Purification and properties. *J. Biol. Chem.* **26**, 3857–3865
7. Hattori, M., Arai, H., and Inoue, K. (1993) Purification and characterization of bovine brain platelet-activating factor acetylhydrolase. *J. Biol. Chem.* **268**, 18748–18753
8. Hattori, K., Hattori, M., Adachi, H., Tsujimoto, M., Arai, H., and Inoue, K. (1995) Purification and characterization of platelet-activating factor acetylhydrolase II from bovine liver cytosol. *J. Biol. Chem.* **270**, 22308–22313
9. Hattori, M., Adachi, H., Tsujimoto, M., Arai, H., and Inoue, K. (1994) The catalytic subunit of bovine brain platelet-activating factor acetylhydrolase is a novel type of serine esterase. *J. Biol. Chem.* **269**, 23150–23155
10. Hattori, M., Adachi, H., Aoki, J., Tsujimoto, M., Arai, H., and Inoue, K. (1995) Cloning and expression of a cDNA encoding the beta-subunit (30-kDa subunit) of bovine brain platelet-activating factor acetylhydrolase. *J. Biol. Chem.* **270**, 31345–31352
11. Hattori, M., Adachi, H., Tsujimoto, M., Arai, H., and Inoue, K. (1994) Miller-Dieker lissencephaly gene encodes a subunit of brain platelet-activating factor acetylhydrolase. *Nature* **370**, 216–218
12. Hattori, K., Adachi, H., Matsuzawa, A., Yamamoto, K., Tsujimoto, M., Aoki, J., Hattori, M., Arai, H., and Inoue, K. (1996) cDNA cloning and expression of intracellular platelet-activating factor (PAF) acetylhydrolase II. Its homology with plasma PAF acetylhydrolase. *J. Biol. Chem.* **271**, 33032–33038
13. Stafforini, D.M., Prescott, S.M., Zimmerman, G.A., and McIntyre, T.M. (1996) Mammalian platelet-activating factor acetylhydrolases. *Biochim. Biophys. Acta* **1301**, 161–173
14. Stafforini, D.M., McIntyre, T.M., Zimmerman, G.A., and Prescott, S.M. (1997) Platelet-activating factor acetylhydrolases. *J. Biol. Chem.* **272**, 17895–17898
15. Ho, Y.S., Swenson, L., Derewenda, U., Serre. L., Wei, Y., Dauter, Z., Hattori, M., Adachi, T., Aoki, J., Arai, H., Inoue, K., and Derewenda, Z.S. (1997) Brain acetylhydrolase that inactivates platelet-activating factor is a G-protein-like trimer. *Nature* **385**, 89–93
16. Adachi, H., Tsujimoto, M., Hattori, M., Arai, H., and Inoue, K. (1995) cDNA cloning of human cytosolic platelet-activating factor acetylhydrolase gamma-subunit and its mRNA expression in human tissues. *Biochem. Biophys. Res. Commun.* **214**, 180–187
17. Adachi, H., Tsujimoto, M., Hattori, M., Arai, H., and Inoue, K. (1997) Differential tissue distribution of the beta- and gamma-subunits of human cytosolic platelet-activating factor acetylhy-

drolase (isoform I). *Biochem. Biophys. Res. Commun.* **233**, 10–13
18. Albrecht, U., Abu-Issa, R., Ratz, B., Hattori, M., Aoki, J., Arai, H., Inoue, K., and Eichele, G. (1996) Platelet-activating factor acetylhydrolase expression and activity suggest a link between neuronal migration and platelet-activating factor. *Dev. Biol.* **180**, 579–593
19. Watanabe, M., Aoki, J., Manya, H., Arai, H., and Inoue, K. (1998) Molecular cloning of cDNAs encoding alpha1, alpha2, and beta subunits of rat brain platelet-activating factor acetylhydrolase. *Biochim. Biophys. Acta* **1401**,73–79
20. van der Voorn, L. and Ploegh, H.L. (1992) The WD-40 repeat. *FEBS Lett.* **307**, 131–134
21. Neer, E.J., Schmidt, C.J., Nambudripad, R., and Smith, T.F. (1994) The ancient regulatory-protein family of WD-repeat proteins. *Nature* **371**, 297–300
22. Reiner, O., Carrozzo, R., Shen, Y., Wehnert, M., Faustinella, F., Dobyns, W.B., Caskey, C.T., and Ledbetter, D.H. (1993) Isolation of a Miller-Dieker lissencephaly gene containing G protein beta-subunit-like repeats. *Nature* **364**, 717–721
23. Morris, N.R,, Efimov, V.P., and Xiang, X. (1998) Nuclear migration, nucleokinesis and lissencephaly. *Trends Cell. Biol.* **8**, 467–470
24. Xiang, X., Zuo, W., Efimov, V.P., and Morris, N.R. (1999) Isolation of a new set of *Aspergillus nidulans* mutants defective in nuclear migration. *Curr. Genet.* **35**, 626–630
25. Willins, D.A., Xiang, X., and Morris, N.R. (1995) An alpha tubulin mutation suppresses nuclear migration mutations in *Aspergillus nidulans*. *Genetics* **141**, 1287–1298
26. Xiang, X., Beckwith, S.M, and Morris, N.R. (1994) Cytoplasmic dynein is involved in nuclear migration in *Aspergillus nidulans*. *Proc. Natl. Acad. Sci. USA* **91**, 2100–2104
27. Beckwith, S.M., Roghi, C.H., Liu, B., Ronald, and Morris, N. (1998) The "8-kD" cytoplasmic dynein light chain is required for nuclear migration and for dynein heavy chain localization in *Aspergillus nidulans*. *J. Cell. Biol.* **143**, 1239–1247
28. Osmani, A.H., Osmani, S.A., and Morris, N.R. (1990) The molecular cloning and identification of a gene product specifically required for nuclear movement in *Aspergillus nidulans*. *J. Cell. Biol.* **111**, 543–551
29. Efimov, V.P. and Morris, N.R. (2000) The LIS1-related NUDF protein of *Aspergillus nidulans* interacts with the coiled-coil domain of the NUDE/RO11 protein. *J. Cell. Biol.* **150**, 681–688
30. Xiang, X., Osmani, A.H., Osmani, S.A., Xin, M., and Morris, N.R. (1995) NudF, a nuclear migration gene in *Aspergillus nidulans*, is similar to the human LIS-1 gene required for neuronal migration. *Mol. Biol. Cell.* **6**, 297–310
31. Morris, N.R. (2000) Nuclear migration. From fungi to the mammalian brain. *J. Cell Biol.* **148**, 1097–1101
32. Hirotsune, S., Fleck, M.W., Gambello, M.J., Bix, G.J., Chen, A., Clark, G.D., Ledbetter, D.H., McBain, C.J., and Wynshaw-Boris, A. (1998) Graded reduction of Pafah1b1 (Lis1) activity results in neuronal migration defects and early embryonic lethality. *Nat. Genet.* **19**, 333–339
33. Smith, D.S., Niethammer, M., Ayala, R., Zhou, Y., Gambello, M.J., Wynshaw-Boris, A., and Tsai, L.H. (2000) Regulation of cytoplasmic dynein behaviour and microtubule organization by mammalian Lis1. *Nat. Cell Biol.* **2**, 767–775
34. Faulkner, N.E., Dujardin, D.L., Tai, C.Y., Vaughan, K.T., O'Connell, C.B., Wang, Y.l., and Vallee, R.B. (2000) A role for the lissencephaly gene LIS1 in mitosis and cytoplasmic dynein function. *Nat. Cell Biol.* **2**, 784–791
35. Sapir, T., Elbaum, M., and Reiner, O. (1997) Reduction of microtubule catastrophe events by LIS1, platelet-activating factor acetylhydrolase subunit. *EMBO J.* **16**, 6977–6984
36. Kitagawa, M., Umezu, M., Aoki, J., Koizumi, H., Arai, H., and Inoue, K. (2000) Direct association of LIS1, the lissencephaly gene product, with a mammalian homologue of a fungal nuclear distribution protein, rNUDE. *FEBS Lett.* **479**, 57–62
37. Feng, Y., Olson, E.C., Stukenberg, P.T., Flanagan, L.A., Kirschner, M.W., and Walsh, C.A. (2000) LIS1 regulates CNS lamination by interacting with mNudE, a central component of the centrosome. *Neuron* **28**, 665–679
38. Sasaki, S., Shionoya, A., Ishida, M., Gambello, M.J., Yingling, J., Wynshaw-Boris, A., and Hirotsune, S. (2000) A LIS1/NUDEL/cytoplasmic dyenein heavy chain complex in the developing and adult nervous system. *Neuron* **28**, 681–696
39. Niethammer, M., Smith, D.S., Ayala, R., Peng, J., Ko, J., Lee, M.-S., Morabito, M., and Tsai, L.H. (2000) NUDEL is a novel Cdk5 substrate that associates with LIS1 and cytoplasmic dynein. *Neuron* **28**, 697–711
40. Manya, H., Aoki, J., Watanabe, M., Adachi, T., Asou, H., Inoue, Y., Arai, H., and Inoue, K. (1998) Switching of platelet-activating factor acetylhydrolase catalytic subunits in developing rat brain. *J. Biol. Chem.* **273**, 18567–18572
41. Nothwang, H.G., Kim, H.G., Aoki, J., Geisterfer, M., Kubart, S., Wegner, R.D., van Moers, A., Ashworth, L.K., Haaf, T., Bell, J., Arai, H., Tommerup, N., Ropers, H.H., and Wirth, J. (2001) Functional hemizygosity of PAFAH1B3 due to a PAFAH1B3-CLK2 fusion gene in a female with mental retardation, ataxia and atrophy of the brain. *Hum. Mol. Genet.* **10**, 797–806
42. Young, B.D. (1992) Cytogenetic and molecular analysis of chromosome 11q23 abnormalities in leukaemia. *Baillieres Clin. Haematol.* **5**, 881–895
43. Lecointe, N., Meerabux, J., Ebihara, M., Hill, A., and Young, B.D. (1999) Molecular analysis of an unstable genomic region at chromosome band 11q23 reveals a disruption of the gene encoding the alpha2 subunit of platelet-activating factor acetylhydrolase (Pafah1a2) in human lymphoma. *Oncogene* **18**, 2852–2859
44. Meerabux, J.M., Cotter, F.E., Kearney, L., Nizetic, D., Dhut, S., Gibbons, B., Lister, T.A., and Young, B.D. (1994) Molecular cloning of a novel 11q23 breakpoint associated with non-Hodgkin's lymphoma. *Oncogene* **9**, 893–898
45. Adams, J.M., Harris, A.W., Pinkert, C.A., Corcoran, L.M., Alexander, W.S., Cory, S., Palmiter, R.D., and Brinster, R.L. (1985) The c-myc oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. *Nature* **318**, 533–538
46. Manya, H., Aoki, J., Kato, H., Ishii, J., Hino, S., Arai, H., and Inoue, K. (1999) Biochemical characterization of various catalytic complexes of the brain platelet-activating factor acetylhydrolase. *J. Biol. Chem.* **274**, 31827–31832
47. Ho, Y.S., Sheffield, P.J., Masuyama, J., Arai, H., Li, J., Aoki, J., Inoue, K., Derewenda, U., and Derewenda, Z.S. (1999) Probing the substrate specificity of the intracellular brain platelet-activating factor acetylhydrolase. *Protein Eng.* **12**, 693–700
48. Sheffield, P.J., Garrard, S., Caspi, M., Aoki, J., Arai, H., Derewenda, U., Inoue, K., Suter, B., Reiner, O., and Derewenda, Z.S. (2000) Homologs of the alpha- and beta-subunits of mammalian brain platelet-activating factor acetylhydrolase Ib in the *Drosophila melanogaster* genome *Proteins* **39**, 1–8
49. Molgaard, A., Kauppinen, S., and Larsen, S. (2000) Rhamnogalacturonan acetylesterase elucidates the structure and function of a new family of hydrolases. *Struct. Fold Des.* **8**, 373–383
50. Brick, D.J., Brumlik, M.J., Buckley, J.T., Cao, J.X., Davies, P.C., Misra, S., Tranbarger, T.J., and Upton, C. (1995) A new family of lipolytic plant enzymes with members in rice, arabidopsis and maize. *FEBS Lett.* **377**, 475–480
51. Upton, C. and Buckley, J.T. (1995) A new family of lipolytic enzymes? *Trends Biochem. Sci.* **20**, 178–179
52. Matsuzawa, A., Hattori, K., Aoki, J., Arai, H., and Inoue, K. (1997) Protection against oxidative stress-induced cell death by intracellular platelet-activating factor-acetylhydrolase II. *J. Biol. Chem.* **272**, 32315–32320
53. Min, J.H., Wilder, C., Aoki, J., Arai, H., Inoue, K., Paul, L., and Gelb, M.H. (2001) Platelet-activating factor acetylhydrolases: broad substrate specificity and lipoprotein binding does not modulate the catalytic properties of the plasma enzyme. *Biochemistry* **40**, 4539–4549
54. Bae, K., Longobardi, L., Karasawa, K., Malone, B., Inoue, T., Aoki, J., Arai, H., Inoue, K., and Lee, T. (2000) Platelet-activating factor (PAF)-dependent transacetylase and its relationship with PAF acetylhydrolases. *J. Biol. Chem.* **275**, 26704–26709