

## Platelet-activating Factor Acetylhydrolases\*

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Platelet-activating factor acetylhydrolases are structurally diverse isoenzymes that catalyze the hydrolysis of the acyl group at the second position of glycerol in unusual, bioactive phospholipids (Fig. 1). Thus, as categorized by enzymatic activity, they are phospholipases A<sub>2</sub> (Groups VII and VIII, Ref. 1), which often initiate signal transduction and are regulated by the state of the cell activation. However, the platelet-activating factor (PAF,<sup>1</sup> 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) acetylhydrolases have the opposite role; they were discovered by investigators who focused on the inactivation of PAF (2). The phospholipid PAF has diverse physiological and pathological functions, and its inactivation was identified as an important step in regulating the overall biological function; indeed PAF acetylhydrolase has been termed a signal terminator (3). However, an immediate problem is apparent; phospholipases A<sub>2</sub> are a common component of venoms, and their role in this context is to facilitate the spread of toxins by degrading membrane phospholipids; thus, how could there be such an active enzyme circulating in blood and present in the cytoplasm of cells? The answer came when it was shown that the PAF acetylhydrolases have marked selectivity for phospholipids with short acyl chains at the *sn*-2 position; with chains longer than nine carbons there was essentially no measurable activity (Fig. 2) (4, 5). Thus, normal membrane phospholipids are protected from hydrolysis by an intrinsic property of the enzyme. Subsequently, it was discovered that certain forms of PAF acetylhydrolases have a broader spectrum of action; they hydrolyze phospholipids containing relatively long *sn*-2 acyl chains (up to 9 methylene groups, Ref. 5). However, in this case, the suitability of a phospholipid as a substrate depends on another form of unusual *sn*-2 acyl group, one that contains a carbonyl group at the  $\omega$ -end of the acyl chain (5). The markedly restricted substrate specificity of PAF acetylhydrolases is unusual among phospholipases A<sub>2</sub>, and the unifying feature of the substrates utilized is that they have potent biological actions, which can lead to pathological events when they accumulate inappropriately. The nature of the substrates hydrolyzed by PAF acetylhydrolases points at key roles for these activities in physiology and pathology, and it has provided important clues into what is currently thought to be the main function of these enzymes, which is to act as scavengers of bioactive phospholipids.

The initial studies on PAF acetylhydrolases were performed on the secreted form found in mammalian plasma; this isoform circulates in blood as a complex with lipoproteins (6, 7). In addition, PAF acetylhydrolase activities were identified in the cytosolic fraction of various mammalian tissues (8, 9) and human blood cells (9, 10). These activities have substrate specificities quite similar to that of

the plasma PAF acetylhydrolase and thus belong to the same group of calcium-independent phospholipases A<sub>2</sub>. However, the plasma (11) and intracellular (12–14) forms of PAF acetylhydrolase are encoded by individual genes, and the identity among these varies widely, depending on the intracellular isoform. It is not yet precisely known how each domain affects the function of each PAF acetylhydrolase, but the diversity in gene structure most likely serves to determine specific roles played by each member of the group.

PAF, the phospholipid that led to the discovery of PAF acetylhydrolase, is a mediator of a wide range of immune and allergic reactions (15, 16). It activates inflammatory cells at very low concentrations (10<sup>-10</sup>–10<sup>-12</sup> M) through a G-protein-linked, serpentine receptor, and it is synthesized in a regulated pathway(s) in response to a variety of agonists. If the mechanism for PAF inactivation is impaired in some way, the return to basal conditions may be compromised, resulting in prolonged inflammation and inappropriately long recruitment of effector cells to sites of injury. The second group of compounds hydrolyzed by PAF acetylhydrolases, the oxidatively fragmented phospholipids (17, 18), also has short acyl groups at the *sn*-2 position of glycerol, but they are derived from oxidation of polyunsaturated fatty acids that occupy this position in the phospholipids of cellular membranes. These compounds apparently mimic the structure of PAF closely enough to bind to its receptor and thereby elicit the same responses. The similarities between PAF and the oxidatively fragmented phospholipids are contrasted by one essential difference: the synthesis of PAF is highly controlled (16, 19, 20) whereas oxidized phospholipids are produced in an unregulated manner. Therefore, the extent to which products of phospholipid oxidation will accumulate depends very heavily on the rate at which they are catabolized. The fact that PAF acetylhydrolases are maximally active in the basal state and do not require calcium for activity ensures that these activities provide an immediate defense mechanism against toxic effects mediated by fragmented phospholipids.

### Plasma (Secreted) PAF Acetylhydrolase

The cloning of a cDNA encoding the plasma form of PAF acetylhydrolase was reported in 1995 (11) and subsequently was confirmed by other groups (21, 22). The gene is expressed in thymus, tonsil, and placenta but not in heart, kidney, or cerebral cortex. However, there is expression of this form of PAF acetylhydrolase in other areas of the brain (11). This is interesting since PAF has been implicated as a physiological messenger in long term potentiation (23) and, when present in excess, as a pathological mediator, for example in seizures (24). However, whether the plasma form of PAF acetylhydrolase is responsible for regulating the amount of PAF in the brain is not clear since mRNAs for intracellular PAF acetylhydrolases occur in specific brain regions as well (12, 13).

The cDNA for human plasma PAF acetylhydrolase encodes a protein whose first 17 residues (Met-1 to Ala-17) are hydrophobic and presumably target the protein for secretion. The next 24 predicted residues (Val-18 to Lys-41) were not found in the protein purified from plasma, and it is not yet clear whether these represent a prepro-peptide or whether the protein isolated from human plasma had undergone proteolytic degradation. The primary structure of plasma PAF acetylhydrolase is unique and includes only a small region of homology, a GX<sub>2</sub>SXG motif found in serine esterases, including many lipases. The active site of such esterases contains a catalytic triad composed of a serine, an acidic residue (usually aspartate), and a histidine residue. In plasma PAF acetylhydrolase, the components of the active site triad have been identified by site-directed mutagenesis; Ser-273, Asp-296, and His-351 are essential for catalytic activity (25). From these observations it is likely that the structures of the active sites of this enzyme and several neutral lipases are similar.

Several observations indicate that synthesis and secretion of the

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<sup>1</sup> The abbreviations used are: PAF, platelet-activating factor; LDL, low density lipoprotein.

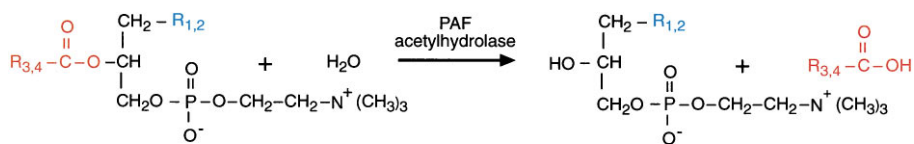


FIG. 1. Reaction catalyzed by PAF acetylhydrolases. PAF acetylhydrolase recognizes PAF and also a family of structurally related phospholipids, some of which contain oxidized functionalities at the second position of the glycerol backbone. The type of linkage at the *sn*-1 position does not affect catalytic activity. The reaction products are lyso derivatives of phospholipid substrates and short fatty acids.

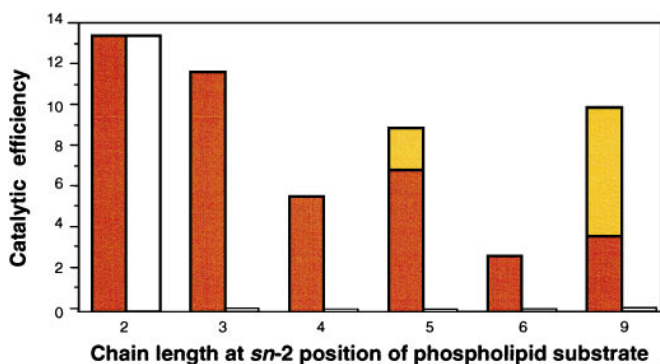
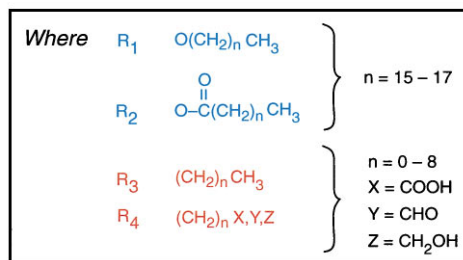


FIG. 2. PAF acetylhydrolases have marked specificity for PAF and structurally similar phospholipids. The ability of PAF acetylhydrolases to recognize phospholipid substrates varies with each isoform. The plasma (secreted) PAF acetylhydrolase and intracellular isoform II recognize PAF and phospholipids with up to 9 methylene groups at the *sn*-2 position of phospholipid substrates. In addition, the suitability of a phospholipid increases if the *sn*-2 acyl group has an oxidized functionality, such as an aldehydic or carboxylic group. In contrast, intracellular isoform Ib, which includes catalytically active subunits  $\beta$  and  $\gamma$ , is strictly specific for PAF hydrolysis. These subunits do not recognize phospholipids with medium length *sn*-2 chains, regardless of the presence of oxidized functionalities, and their function is limited to precisely regulate the levels of PAF. Orange bar, extracellular PAF acetylhydrolase and intracellular isoform II; white bar, intracellular PAF acetylhydrolase isoform Ib ( $\beta$  and  $\gamma$  subunits); gold bar, oxidized functionality.

plasma form of PAF acetylhydrolase are hormonally regulated *in vivo*. Miyaura *et al.* (26) showed that estrogens decrease secretion by macrophages and that progestins have the opposite effect. These studies were complemented by the observation that the PAF acetylhydrolase activity in maternal plasma decreased dramatically before parturition (27) and returned to basal levels soon afterward. Based on these findings, Johnston and colleagues (27) proposed that during early pregnancy the high PAF acetylhydrolase levels ensure that the concentrations of PAF will be low; this protects against premature uterine contraction since PAF is a potent agonist for this response. In contrast, the fall in enzymatic activity late in pregnancy may allow PAF to accumulate and initiate parturition. The conclusion that estrogen has a regulatory role is supported by population studies in which it has been found that women have a lower level of plasma PAF acetylhydrolase activity than men (28).<sup>2</sup> In addition to the sex hormone regulation, dexamethasone, an anti-inflammatory glucocorticoid, was found to increase PAF acetylhydrolase levels in the plasma of rats (29). Another example of regulation of this activity is developmental; at birth the levels are very low, but there is a marked increase during the first weeks of life (30).<sup>2</sup> The basis for this is unknown. In adults, there is a strong correlation between the plasma activity and the cholesterol or low density lipoprotein (LDL) level (31–33); again the basis is undetermined. This may be important since the enzyme circulates bound to lipoproteins and the fraction associated with LDL seems to be more active under some conditions (34). This may

result from an allosteric effect of the binding or may represent an effect of the lipoprotein environment on substrate availability. Finally, Satoh *et al.* (35) showed that PAF itself can induce the synthesis and secretion of the plasma PAF acetylhydrolase, a response that may indicate a form of feedback regulation that serves to protect the organism against excessive signaling by PAF and related lipids.

The plasma form of PAF acetylhydrolase has been the target of many studies testing its association with inflammatory diseases (reviewed in Ref. 36). Modest changes in plasma activity have been described in asthma, hypertension, vascular disease, atherosclerosis, sepsis, necrotizing enterocolitis, and others (36). In most cases, the activity increased during the acute phase, suggesting that this may be a physiological response to inflammatory stimuli, perhaps PAF itself as described above. A limiting feature of these studies is that they were restricted to measurements of enzymatic activity since antibodies have only recently become available. This may be important since several groups have shown that the plasma PAF acetylhydrolase is inactivated by oxidants and many of the disorders studied would include oxidant and/or free radical formation (37). Paradoxically, the most important substrates of this enzyme are the products of oxidative reactions, and these compounds inactivate the enzyme themselves. Thus, although informative, studies that determine changes in the overall levels of PAF acetylhydrolase activity are difficult to interpret precisely because the final level of activity is the result of diverse factors with (sometimes) opposite effects. Animal studies using recombinant PAF acetylhydrolase have provided the best clues into the role of this enzyme in disease; pretreatment with recombinant PAF acetylhydrolase blocks PAF-induced edema (11), prevents asthma-related symptoms, and protects animals from septicemia.<sup>2</sup> Thus, this enzyme prevents not only inflammatory conditions induced by administration of exogenous PAF but also diseases in which the production of PAF and/or oxidized phospholipids is suspected to occur.

An important medical area in which plasma PAF acetylhydrolase may play a protective role is in oxidative processes that are thought to be a component of early vascular disease. The phospholipids in LDL particles are known to undergo oxidation under experimental conditions, and evidence for this *in vivo* has been presented as well (38). The oxidized phospholipids are substrates for PAF acetylhydrolase, and addition of the enzyme prevents the formation of minimally modified and modified LDL *in vitro* (39, 40). The products of hydrolysis, lysophosphatidylcholine and fragments of fatty acids, are water-soluble and can be further metabolized to products that are not toxic. In contrast, the phospholipids with an oxidized fatty acid, perhaps with an aldehyde at the  $\omega$ -end, could be detrimental in two ways; these phospholipids mimic PAF to support inflammation but, in addition, could covalently modify the apolipoprotein. This results in unregulated cholesterol ester accumulation by macrophages, which become the foam cells characteristic of atherosclerosis. LDL-associated PAF acetylhydrolase can prevent the modification of LDL to an atherogenic particle (39), likely by the hydrolysis of reactive oxidatively fragmented phospholipids before they can derivatize apoB-100 to a form recognized by scavenger receptors. The lysophosphatidylcholine formed in the PAF acetylhydrolase reaction also can provoke pathological reac-

<sup>2</sup> D. M. Stafforini, T. M. McIntyre, G. A. Zimmerman, and S. M. Prescott, unpublished results.

TABLE I  
Secreted and intracellular PAF acetylhydrolases

Type	Isoform	Source	Active site	Molecular weight
Secreted	--	Plasma	G H S F G	45,000
Intracellular	II	Liver & kidney	G H S F G	40,000
Intracellular	Ib, $\gamma$ subunit	Brain	G D S L V	29,000
Intracellular	Ib, $\beta$ subunit	Brain	G D S M V	30,000

tions in the cells of the vascular wall, but this effect occurs only at concentrations at least 1000-fold higher than that required for the effects of PAF and oxidized phospholipids. Thus, PAF acetylhydrolase provides LDL particles with an intrinsic protective mechanism that minimizes the damaging effects caused by lipid peroxidation reactions.

It has been known since 1988 that approximately 4% of the Japanese population has undetectable levels of plasma PAF acetylhydrolase activity, and the prevalence is even higher in children with severe asthma (41). The molecular basis for the deficiency was discovered recently, and it results from a mutation that converts Val-279, which is conserved in five species examined, to Phe (42). In Japan, this mutation occurs as a heterozygous trait in 27% of the population, which indicates that the population is in Hardy-Weinberg equilibrium for this trait. Surprisingly, given the remarkable prevalence in Japan, this mutation was not found in North American subjects and appears to be restricted to Asia. Several groups are now testing whether this mutation increases the susceptibility to inflammatory or allergic diseases. One possibility is that the plasma PAF acetylhydrolase functions normally as an anti-inflammatory safety net, much as do the plasma anti-proteases. If this is true, then deficiency of the enzyme should be a risk factor for developing relevant diseases (or enhancing their severity), but the disease might be manifest only when the mutation occurs in conjunction with other genetic predisposition or if the individual encounters the appropriate environmental factors.

#### Intracellular PAF Acetylhydrolases

In addition to the extracellular plasma enzyme, intracellular PAF acetylhydrolase activities have been reported to regulate the accumulation of PAF under some circumstances; for example, in macrophages (43, 44) and platelets (45, 46) the level of PAF accumulation is determined by the PAF acetylhydrolase activity. The intracellular PAF acetylhydrolase activities present in brain, kidney, and liver have been extensively characterized. Inoue's group described enzymatic activities that they designated isoforms I and II, and isoform I proved to have more than one enzyme so it was subdivided. Subsequently, they have isolated three different cDNAs encoding catalytically active, intracellular PAF acetylhydrolases (Table I) (47). The minimal essential elements determining the catalytic reaction *per se* are shared by all PAF acetylhydrolases, including secreted and intracellular forms: the GX SXG or GX SXV motif characteristic of lipases and esterases (Table I) (47). Features such as localization, structure of the protein, and substrate binding site are likely to be defined by regions of the gene that differ widely among isoforms.

Hattori *et al.* (14) have studied isoform II extensively and recently reported the cloning of a cDNA encoding this enzyme. This isoform, which is expressed in liver and kidney, has active site serine and cysteine residues, and its substrate specificity is similar to that of the secreted, plasma activity; isoform II catalyzes the hydrolysis of phospholipids with acyl chains containing up to five methylene groups. This suggests that one function of isoform II may be to scavenge oxidatively fragmented phospholipids (48), much like the plasma PAF acetylhydrolase. Interestingly, the amino acid sequence of isoform II shows 41% identity with the plasma PAF acetylhydrolase (Fig. 3A) (14). These results indicate that the regions shared by these PAF acetylhydrolases include determinants of substrate specificity and that the two activities may share a common physiologic function.

The most thoroughly studied of the intracellular enzymes is isoform Ib, which has three subunits of molecular masses 45, 30, and 29 kDa ( $\alpha$ ,  $\beta$ , and  $\gamma$ , respectively) (49). This isoform is entirely

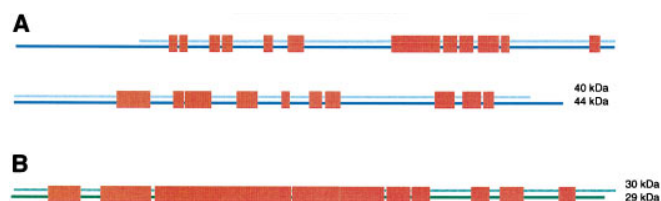


FIG. 3. Comparison of PAF acetylhydrolase isoforms at the amino acid level. A, the plasma (secreted form,  $M_r = 44,000$ ) and one intracellular form of PAF acetylhydrolase (isoform II,  $M_r = 40,000$ ) share a high degree of identity (41%) at the amino acid level. The homologous regions are clustered in discrete domains. Since these two enzymes also share the ability to recognize a wide range of substrates, it is likely that some of these homologous regions represent substrate recognition domains. B, PAF acetylhydrolase intracellular isoform Ib is formed of a regulatory and two catalytic subunits. The catalytic subunits are similar in size ( $\gamma$  subunit,  $M_r = 29,000$ ;  $\beta$  subunit,  $M_r = 30,000$ ) and have the same substrate specificity. The two subunits are highly similar at the amino acid level (63.2% identity) but share no homology with the plasma form or with intracellular isoform II. Orange bar, homologous and conserved domains; light blue line, intracellular isoform II; dark blue line, plasma PAF acetylhydrolase; light green line,  $\beta$  PAF acetylhydrolase; dark green line,  $\gamma$  PAF acetylhydrolase.

specific for PAF hydrolysis, *i.e.* it does not recognize oxidized phospholipids as substrates, which indicates that its function is to precisely modulate PAF levels exclusively of other substrates (49). The 29-kDa subunit is catalytically active, exhibits no overall homology with other proteins, and contains a modified version of the consensus sequence of the serine esterase family that has been found in other PAF acetylhydrolases (Table I). It has been crystallized and a high resolution structure determined; a key finding was that the tertiary fold of this protein is markedly similar to that found in  $p21^{ras}$  and other GTPases (50). In addition, the active site is made of a trypsin-like triad of Ser-His-Asp, and its chirality is the same as that found in other esterases and neutral lipases. The 30-kDa subunit is homologous (63.2% identity) with the 29-kDa subunit (Fig. 3B), and it contains the same catalytic triad present in the 29-kDa subunit (13). The 45-kDa subunit of isoform Ib has no catalytic activity, and it has been proposed that it may regulate the activity, location, or turnover of the holoenzyme (50). This subunit of isoform Ib is one of the most conserved proteins known, and it has received much attention because mutations in the human gene (*LIS-1*) are responsible for Miller-Dieker lissencephaly (51), a devastating neurological disease whose hallmark is severe brain malformation manifested by a smooth cerebral surface (52). The cellular basis of the disease appears to be abnormal neuronal migration during development (52). The finding that a subunit of intracellular PAF acetylhydrolase plays an important role in brain development may be related to the work of Kato *et al.* (23), who concluded that the substrate, PAF, is a retrograde messenger in long term potentiation. A current model proposes that PAF acetylhydrolase serves to modulate PAF levels, which can be toxic at high concentrations, during brain development (24) and that hydrolysis of PAF may induce conformational changes in the heterotrimeric PAF acetylhydrolase complex that affect the ability of the 45-kDa subunit to interact with cytoskeletal proteins (50). Three lines of evidence support the existence of PAF acetylhydrolase Ib as a heterotrimer *in vivo*. First, the three subunits co-purify throughout several chromatographic steps. Second the crystal structure of the 29-kDa subunit is reminiscent of a G-protein-like heterotrimer. Third, mRNAs for the three subunits are co-expressed in the developing brain (53). Thus, it is likely that the heterotrimer is representative of the structure that this isoform adopts *in vivo* and that this complex serves to carefully modulate the levels of PAF in the brain. The fact that this isoform has an even higher specificity for PAF than the others suggests that it may have a specific role in a signal transduction pathway, whereas the plasma enzyme and intracellular isoform II may have a more fail-safe role, *i.e.* to inactivate PAF and oxidized phospholipids that have been generated in the wrong quantities or the wrong place and are likely to cause damage.

The human erythrocyte PAF acetylhydrolase activity has been purified and shown to be composed of two identical 25-kDa subunits; it exhibits surface dilution kinetics and has biochemical properties that differentiate it from the plasma activity (54). However, this activity is an isozyme of the PAF acetylhydrolase family

because it is calcium-independent and hydrolyzes phospholipids containing short and/or oxidized acyl groups at the *sn*-2 position. The enzyme is a serine esterase and requires reducing agents for maximal activity, much like isoform II. The most likely role of the erythrocyte PAF acetylhydrolase *in vivo* is the hydrolysis of the phospholipid products of oxidative fragmentation of membrane phospholipids. This step may serve to hinder further oxidative reactions and to reduce the toxic effects of oxidized lipids. Moreover, hydrolysis allows subsequent restoration of the membrane integrity by reacylation of the lyso derivatives with long chain fatty acyl groups, as can also occur in red blood cells (55).

### Conclusions and Perspectives

Much has been learned about the PAF acetylhydrolases; the last 3 years have been particularly fruitful because the tools generated (cDNAs, recombinant proteins, and antibodies) will allow additional dissection at a molecular level. How are the PAF acetylhydrolase genes regulated? What is the role of each of the isoforms? Do they serve a general antioxidant role? What is the structural basis for the marked substrate specificity? What structural features determine the binding of the plasma PAF acetylhydrolase to specific lipoproteins in the blood? What functional benefit is conferred by the trimeric structure of the intracellular type Ib? In addition, the next few years will see the development of genetically engineered animals, as well as clinical studies of whether these enzymes have the postulated protective effects against inflammatory and allergic diseases.

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