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Review

The structure and function of platelet-activating factor acetylhydrolases

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Abstract. Platelet-activating factor acetylhydrolases (PAF-AHs, EC 3.1.1.47) constitute a unique and biologically important family of phospholipase A_2s. They are related to neither the well-characterized secretory nor cytosolic PLA_2s, and unlike them do not require Ca^{2+} for catalytic activity. The distinguishing property of PAF-AHs is their unique substrate specificity: they act on the phospholipid platelet-activating factor (PAF), and in some cases on proinflammatory polar phospholipids, from which they remove a short acyl moiety – acetyl in the case of PAF – located at the *sn*-2 position. Because PAF is found both in the plasma and in the cytosol of many tissues, PAF-acetylhydrolases are equally widely distributed in an animal organism. Recent crystallographic studies shed new light on the complex structure-function relationships in PAF-AHs.

Key words. Platelet-activating factor; acetylhydrolase; α/β hydrolase; phospholipase A_2; molecular modelling.

Introduction

Phospholipids constitute one of the most abundant groups of biological compounds. Studied extensively as key components of biological membranes, they have more recently emerged as a source of diverse signalling molecules involved in many regulatory pathways. Among these biologically active phospholipids, the platelet-activating factor (PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; see fig. 1) is the most potent and best-characterized messenger molecule. It was first discovered as a mediator of anaphylaxis in rabbits [1–3], and its chemical structure was verified by complete synthesis [4]. Like cytokines, PAF binds to specific G protein-coupled receptors and activates a plethora of cells [5–7]. The ensuing cellular responses, primarily in platelets, neutrophils and monocytes [8], are observed with PAF concentrations as low as 10^{-10} M, a manifestation of a unique affinity of the receptor towards this phospholipid.

Although PAF is often referred to specifically as a proinflammatory messenger, it has very diverse physiological and pathophysiological functions, It is involved in allergic reactions and asthma [9–12], and has been implicated in fertilization [13], carcinogenesis [14, 15] and apoptosis [16]. These wide-ranging biological effects of PAF have stimulated a significant body of biomedical research. The number of publications that appeared on the subject – nearly 7500 according to a Medline search conducted in September 1997 – best illustrates the intensity of this effort.

Interestingly, PAF is not the only molecule capable of activating its receptor. Oxidative damage to low density lipoproteins (LDL) generates polar phospholipids with short, often oxidized acyl chains in the *sn*-2 position

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[17]. These molecules are mitogenic in smooth muscle cells, induce neutrophil adhesion and consequently enhance the atherogenic potential of high levels of LDL. Their biological effects are apparently due to the activation of the PAF receptor, as PAF-receptor antagonists abolish them [17]. Because the oxidation of LDL is not controlled, the resulting polar phospholipids can induce inflammatory responses even in the absence of natural stimuli. In contrast, during physiologically normal inflammation, biosynthesis and sectetion of PAF are tightly regulated.

All these effects are mediated by the extracellular pool of PAF. However, many cells contain a substantial cytosolic pool of the messenger, which can be potentially active inside the cells as one of the numerous second messengers. In fact, PAF-synthesizing cells retain the majority of the messenger [18]. Intracellular PAF receptors were found in rat cortex [19], in transformed neurons [20] and in epithelial cells [21]. In some cases, as in the induction of human immunodeficiency virus (HIV) [22], PAF-mediated alterations to gene expression take place via mechanisms independent of the PAF receptor. Further dissection of these intracellular signalling pathways is currently impeded by lack of specific data regarding relevant receptors.

Both intracellular and plasma PAF undergo rapid turnover, due to specific hydrolases which inactivate it through a hydrolytic cleavage of the *sn*-2 ester bond and the release of free acetate and biologically inactive lyso-PAF. Given this specificity and the chemical nature of the substrate molecule, PAF acetylhydrolases (PAF-AHs) are classified as phospholipase A_2s. They constitute a very heterogeneous group, with apparently diverse physiological functions. The first such enzyme was identified in blood plasma and originally referred to as the acid-labile factor [23, 24]. Subsequently, other isoforms were found in brain [25], liver [26] and in erythrocytes [27]. A specific PAF-AH was also described in invertebrates, specifically in nematodes [28]. To date, three acetylhydrolases were cloned and characterized in considerable detail. These are: the brain heterotrimeric PAF-AH(Ib) [29–31], the plasma PAF-AH (pPAF-AH) [32] and intracellular isoform II, or PAF-AH(II) [33]. Interestingly the complementary DNA (cDNA)-derived amino acid sequences of these enzymes show no similarities to any of the previously characterized phospholipase A_2s. This made it initially difficult to dissect specific structure-function relationships. However, recent crystallographic studies in our laboratory [34, 35] allow us to rationalize some of the existing biochemical data in structural terms.

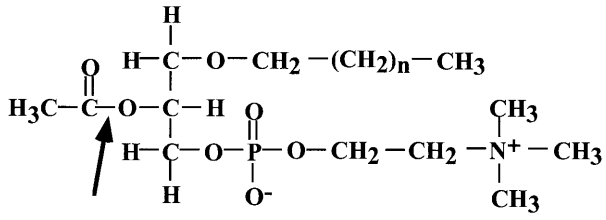

Figure 1. Chemical structure of PAF. The arrow indicates the bond cleaved by PAF-AH.

Here, we review the current knowledge in the field with particular emphasis on the structure-function relationships. First, we describe the properties of plasma and isoform II PAF-AHs. We show that these enzymes are homologous, old on an evolutionary scale and related to the ubiquitous α/β hydrolase superfamily. Second, we discuss the brain heterotrimeric acetylhydrolase and its putative unique role in brain development. Readers interested in more biomedical aspects of the biochemistry of acetylhydrolases should also refer to other recent reviews [36, 37].

Structure-function relationships in pPAF-AH and PAF-AH(II)

Biochemical properties of pPAF-AH

pPAF-AH was successfully cloned from human as well as other sources [32, 38–40]. It is a single polypeptide protein, 45 kDa in size, made up of 441 amino acids. The sequence contains a 17-residue secretion signal and a putative propeptide motif; the N-terminus in mature species varies due to limited proteolysis. The native enzyme is heterogeneous due to N-glycosylation, but the carbohydrate can be removed enzymatically [40]. Up to 70% of pPAF-AH is associated with LDLs, the remainder with HDLs (high-density lipoproteins) [41].

The rate of PAF hydrolysis by purified pPAF-AH shows a sigmoid dependence on the concentration of PAF and becomes hyperbolic above the 5 μM level [42]. Thus, pPAF-AH appears to show preference for micellar rather than monomeric PAF. Similar preference is widespread among phospholipase A_2s and neutral lipases, and is known in the literature as interfacial activation [43, 44]. The consequences of this phenomenon in pPAF-AH are not clear, but it is physiologically advantageous for the enzyme to be regulated in a simple way so that its activity picks up with excess of the insoluble substrate in blood plasma.

With respect to substrate specificity, pPAF-AH shows interesting flexibility. With a five-carbon *sn*-2 acyl group it retains 50% of its hydrolytic activity and up to nine-carbon-long acyl chain is still noticeably cleaved off. However, in the latter case the activity is restored to nearly 50% of that against PAF when an aldehyde

group is added in the ω position [45]. The enzyme is inhibited by diisopropylfluorophosphonate (DFP), a classic serine esterase reagent, and the presence of a lipase/esterase GXSXG sequence motif [46, 47] initially suggested the identity of a putative nucleophile. As expected, site-directed mutagenesis showed that the replacement of Ser273 within the motif with other amino abolishes enzymatic activity [38]. Furthermore, systematic mutagenesis of His and Asp amino acids established that His351 and Asp296, conserved in sequences from other species, are also essential for catalytic activity [38]. This strongly suggested the presence of a catalytic triad, ubiquitous among neutral lipases [48]. The order in which the triad residues occur in the polypeptide chain (Ser-Asp-His) is consistent with the well-known α/β hydrolase fold [49], which is found in virtually all esterases and neutral lipases that have been structurally characterized to date [48]. This fold has been originally identified in four crystal structures of otherwise unrelated enzymes: dienelactone hydrolase [50], haloalkane dehalogenase [51], *Geotrichum candidum* lipase [52] and acetylcholinesterase [53]. It consists of a central, predominantly parallel β sheet with helical connections. The active site in this family is invariably located at the carboxyl edge of the β sheet and contains a constellation of three hydrogen-bonded amino acids – the catalytic triad. The key member in this triad is a residue with the nucleophilic function group. It is typically a serine, although a cysteine or an aspartate can function in this capacity [50, 51]. The nucleophile is situated at the C-terminus of one of the central strands, within a sharp turn preceding a buried α helix [54]. This strand-turn-helix motif is known in the literature as the nucleophilic elbow [49, 54]. The turn itself is made up by the pentapeptide GXS(C,D)XG, which is often considered to be the 'fingerprint' of the fold.

The original suggestion that pPAF-AH may be similar in its three-dimensional architecture to the α/β hydrolases was based on somewhat circumstantial evidence from mutagenesis experiments, admittedly in the absence of noticeable sequency homology with members of this superfamily. This, and the lack of direct structural data on pPAF-AH precluded further rigorous analysis of the molecular basis of substrate specificity and/or the roots of the observed interfacial properties of the enzyme.

PAF-AH(II): An intracellular homologue of the plasma isoform

The intriguing and elusive functions of intracellular PAF prompted equally intense research into the cytosolic forms of PAF-AHs. The so-called isoform II, or PAF-AH(II), was found to be highly expressed in liver and kidney tissues. It was purified, and the human and bovine cDNAs, 88% identical, were cloned [26, 33]. The enzyme's substrate specificity is similar to that of the plasma enzyme, although the oxidation of the ω-carbon does not appear to raise the specific activity when polar phospholipids are used as substrates [26]. Somewhat surprisingly, the cDNA-derived sequence of PAF-AH(II) showed significant homology with the plasma enzyme – 41% amino acid sequence identity. Consistent with its cytosolic localization, PAF-AH(II) does not contain a secretion signal peptide. All the putative catalytic residues that make up the triad are conserved in the isoform II species, although to date no mutagenesis has been carried out to verify their function.

Extensive computer sequence searches revealed limited stretches of amino acid sequence in the protein with similarities to certain bacterial lipases from the *Streptomyces* and *Moraxella* genera [33]. This was unexpected, because the sequence of the pPAF-AH, a close homologue of PAF-AH(II), was perceived to be unique [32]. It was this fortuitously discovered homology with microbial enzymes that consequently shed new light on the structure-function relationships in both PAF-AHs.

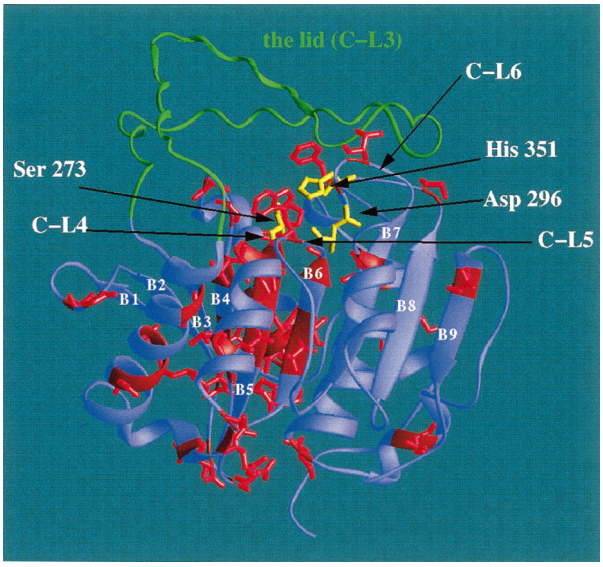

Figure 2. A molecular model of pPAF-AH based on the crystal structure of SeL (PDB accession code 1JFR). The lid subdomain (in green) was modelled from the structure of a similar insert in bromoperoxidase (PDB code 1BRO). Those residues in SeL that are conserved in either pPAF-AH of PAF-AH(II) are shown in red. The residues of the catalytic triad are shown in yellow. Selected C loops (loops on the carboxyl edge of the β sheet) are shown.

Molecular modelling of pPAF-AH and PAF-AH(II) from the crystal structure of the *Streptomyces exfoliatus* lipase

At the time when the cloning of PAF-AH(II) was in progress, we were pursuing in our laboratory in X-ray diffraction study of a lipase purified and then cloned from *Streptomyces exfoliatus* (SeL) [55]. The study was part of an ongoing project aimed at elucidating the catalytic mechanism in lipases. When the crystal structure was in the final stages of crystallographic refinement, we learned of several sequence similarities of this lipase to PAF-AH(II). We realized that these sequence similarities with PAF-AH(II), and consequently pPAF-AH, could be rationalized in terms of the molecular architecture, and that they constituted a genuine reflection of homology. We were thus able to propose a structural model for the PAF-AHs based on the crystal structure of SeL (fig. 2).

SeL, a single-polypeptide-chain protein made up of 263 amino acids, is a typical α/β hydrolase with a core β sheet consisting of nine strands. The central strand-turn-helix motif conforms to the stereochemistry expected for the nucleophilic elbow, with Ser131 in a strained secondary conformation ($\phi = 67\,°$, $\psi = -125\,°$). His209 and Asp177, along with Ser131, form a hydrogen-bonded constellation virtually identical to the triad found in other neutral lipases and esterases (fig. 3a). The well-established mechanism of ester hydrolysis suggests that the backbone amides of Phe237 and Leu122 [PAF-AH(II) notation] participate in the oxyanion hole, a structural motif that stabilizes the tetrahedral intermediates generated during the course of the reaction [56]. When the amino acid sequences of SeL, pPAF-AH and PAF-AH(II) are aligned, 45 residues in SeL, nearly 20% of the total, have fully conserved counterparts in either pPAF-AH or PAF-AH(II). These residues are specifically clustered in the core of the molecule, close to the nucleophilic elbow, and around the area of the active site. This distribution of conserved amino acids is consistent with the notion of close homology within the family. The amino acid sequence alignment also revealed a major insertion in the PAF-AHs, which spans residues 189–239 in pPAF-AH and 157–202 in PAF-AH(II). This insertion occurs in one of the loops on the carboxyl edge of the central β sheet.

When compared with SeL, in the PAF-AHs two of the three putative catalytic residues – in both cases the serine and the histidine – are conserved. In pPAF-AH these are Ser273 and His351, and in PAF-AH(II), Ser236 and His314. Their catalytic roles, as inferred from the SeL crystal structure, are consistent with mutagenesis studies of pPAF-AH [38]. Surprisingly, the third member of the triad is not conserved. The expected position of the catalytic aspartate is occupied in both PAF-AHs by asparagines. According to current consensus, Asn cannot replace Asp in a chymotrypsin-like catalytic triad without concomitant loss of activity in the enzyme. The clue to the fate of the third member of the triad in PAF-AHs was provided, yet again, by the mutagenesis work in pPAF-AH [38], which showed that another carboxylic acid, Asp296, is essential for enzymatic activity. This second aspartate corresponds in the SeL structure to Thr154, an amino acid located on a loop topologically adjacent to the site of Asp177 at the carboxyl end of strand 5 and immediately downstream of the nucleophilic elbow motif. Our molecular modelling suggests that the substitution of Asp for Thr at this position can yield a favourable hydrogen bond between the Oδ1 atom of the aspartate and the Nε2 of the catalytic histidine (fig. 3b). This bond has an expected donor-acceptor geometry and distance (<3.0 Å), but atypically involves the *anti* electron pair on the oxygen atom, rather than the *syn* pair.

The migration of the catalytic aspartate in the α/β hydrolase fold from one loop at the carboxyl edge of the β sheet to another has been observed previously. It occurs in pancreatic lipase where, interestingly, the 'typical' aspartate (Asp205) is still present [57]. Thus, the stereochemistry of the triad in pancreatic lipase appears to be analogous to the one we proposed on the basis of modelling for PAF-AHs (fig. 3c). Except for the active serine pentapeptide GXSXG motif, pancreatic lipase

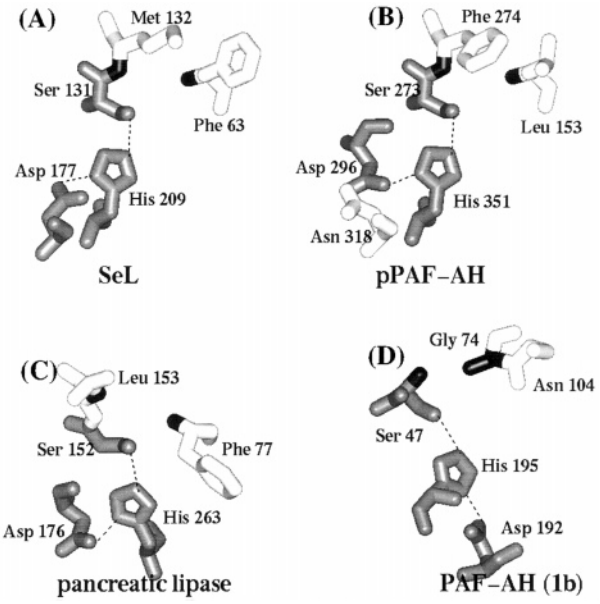

Figure 3. The catalytic tirads in PAF-AHs and related lipases. For further details, see text. The three residues participating in the triad are shaded. The main-chain amide groups which make up the oxy-anion holes are indicated shown in black.

shows no relationship to PAF-AHs, and we presume that the migration of the catalytic aspartate has occurred in the two enzymes independently in the course of evolution.

In SeL, and by implication in the two PAF-AHs, the chirality of the triad is such that the hydroxyl of Ser131 attacks the *re*-face of the ester bond [58], like other lipases and esterases. The Cahn-Ingold-Prelog nomenclature defines the ester's *re*-face as topologically opposite to the *re*-face of a peptide bond; it is the latter which is susceptible to the attack from serine proteinases of the subtilisin and chymotrypsin families. This interesting selectivity is brought about by the relative disposition of the seryl and histidyl residues in the various triads. We will return to this point later, when we discuss the brain intracellular acetylhydrolase.

Given that SeL is a neutral lipase, what is the molecular basis of the unique substrate specificity in pPAF-AH and PAF-AH(II)? Based on the structure of SeL we suggested [35] that the answer lies in the insert within the third loop on the carboxyl edge of the β sheet. With the notable exception of SeL and cutinase [59], all α/β hydrolases contain inserts in one or more of the loops in that area; these inserts can fold into independent subdomains, or alternatively make up readily identifiable secondary structure elements that typically fold over the active site. They are known to confer on individual α/β hydrolases such properties as substrate specificity and, in lipases, interfacial activation [60–62]. The preference of pPAF-AH, and probably also of PAF-AH(II), for micellar PAF is also readily rationalized by the presence of the insert. Unfortunately, the unique amino acid sequence precludes any detailed modelling studies. We can only speculate based on secondary structure prediction, that the insert is mostly helical and contains either two or four antiparallel helices.

The biological functions of pPAH-AH and PAF-AH(II)

The biological role of pPAF-AH appears to be the regulation of inflammation through the inactivation of PAF and polar phospholipids. In this capacity pPAF-AH acts as a signal terminator [63]. This physiological function was tested and verified in rats in a pleurisy model, where vascular leakage occurred in response to PAF [32]. The rats that were pretreated with recombinant pPAF-AH had a $>80\%$ reduction in leakage compared with control animals. A similar biological function has also been demonstrated for pPAF-AH with respect to oxidized phospholipids. Human LDL, pretreated with a pPAF-AH inhibitor DFP and oxidized by cupric sulphate, caused significant neutrophil adhesion, whereas untreated samples showed much lower potency [17]. Thus, pPAF-AH functions as a scavenger of all proinflammatory phospholipids in an animal system.

The biological function of PAF-AH(II) is less clear at the moment. Recently published data suggest that, like the plasma isoform, it can serve as protection against oxidative damage to the cell [64]. This may be particularly important in liver and kidney, organs rich in PAF-AH(II), where there are high levels of superoxide dismutase [26].

The brain intracellular PAF-AH(Ib) complex

The catalytic subunits of PAF-AH(Ib)

pPAF-AH is not found in the brain in any significant amounts. However, the brain does contain high levels of PAF-AH activity, originally attributed to three different intracellular isoforms, Ia, Ib and II [25], although there are only low levels of PAF-AH(II). The most abundant brain acetylhydrolase is a heterotrimeric protein complex Ib [25]. It contains two homologous catalytic subunits, henceforth referred to as the α subunits, and a third subunit, a 45 kDa β chain, whose function will be described elsewhere in this review. The Ia isoform is in fact the catalytic heterodimer. Thus, strictly speaking, PAF-AH activity resides in the brain in three different polypeptide chains that are found on their own or as a part of a complex.

The PAF-AH(Ib) complex can be dissociated into the catalytic α_1/α_2 heterodimer and the β subunit. All the catalytic activity resides in the former, but interestingly, labelling with radioactive inhibitor [^{3}H]DFP indicates that α_1 is significantly more active than α_2 [25, 30, 31]. Both catalytic subunits were cloned from bovine, mouse and human tissues and characterized at the DNA and protein levels [30, 31, 65–67]. They are 63% identical, but what is even more remarkable is the nearly complete conservation between mammalian species: the bovine and human α_2 chains, for example, are identical [67]. Site-directed mutagenesis in the α_1 subunit suggested the identity of the nucleophile: Ser47 (α_1 notation), which resides within the GDSLV pentapeptide, dissimilar from the esterase/lipase GXSXG consensus sequence. A histidyl residue, His195, was also implicated in the catalytic process, but the identity of the third member of a putative triad was not clear, because neither Asp75 nor Asp192 could be mutated without loss of activity (K. Inoue, personal communication). All these residues are conserved in the α_2 subunit.

The two α subunits can be overexpressed separately in *Escherichia coli* using a variety of expression systems. Under these conditions, the proteins always form stable homodimers, both of which are catalytically competent [31]. Recently, in our laboratory, we determined the three-dimensional structure of the α_1 homodimer at 1.7-Å resolution. This study permitted us to make sense of some of the biochemical results [34].

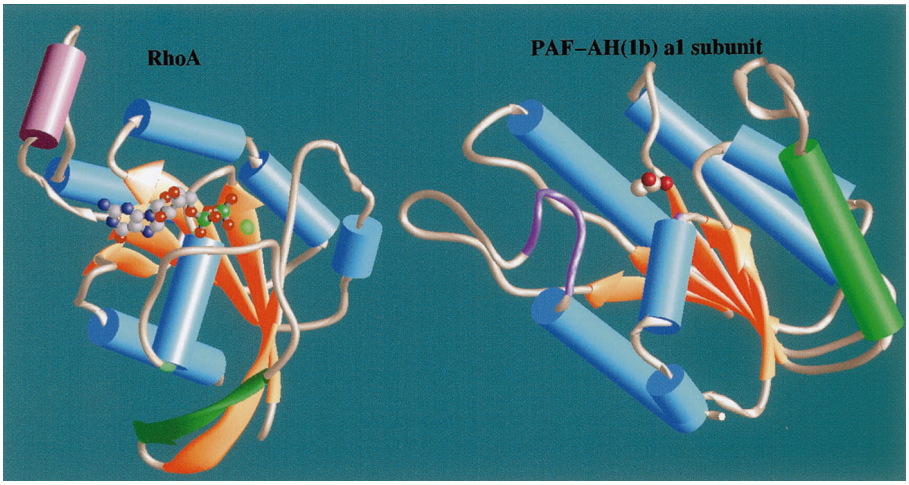

Figure 4. A comparison of the three-dimensional folds of a typical cytosolic GTPase, RhoA (PDB code 1FTN) and the α_1 subunit of PAF-AH(Ib). The GDP and acetate molecules are shown to illustrate the same topological location of the active sites. The Rho-specific insert helix is shown in magenta. The effector-binding strand unique to GTPases is shown in green. The helix which in the α_1 subunit is involved in dimerization and has no counterpart in GTPases is also shown in green.

The α_1 subunit has a tertiary structure that is closely similar to that of $p21^{Ras}$[68] and other small guanosine 5′-triphosphate hydrolases (GTPases) like RhoA [69] (fig. 4). It is the only lipolytic enzyme known to have such a tertiary fold. As expected, the pentapeptide GDSLV containing the active site Ser47 shows no structural similarities to the nucleophilic elbow. However, the enzyme does contain a triad, made up of a hydrogen-bonded constellation of the side chains of Ser47, His195 and Asp192. The high-resolution crystal structure also allowed us to clarify the ambiguous results of the mutagenesis of Asp75. This residue is partly buried in the crystal structure and makes numerous hydrogen bonds utilizing the full potential of its side-chain carboxyl group. A substitution of Asn or Ala for Asp75 will seriously disrupt this network and destabilize the fold, thus indirectly abolishing activity. The two tetrahedral intermediates, formed during the course of the reaction, are stabilized by an oxyanion hole made up of three hydrogen-bond-donating groups: two main-chain groups, Leu48 and Gly74, and the side-chain amide of As104. A three-point oxyanion hole has been identified in other esterases, but it remains unclear if it has any functional advantage over the more common two-point arrangement [70].

In spite of the different tertiary structures of pPAF-AH and PAF-AH(II), on the one hand, and the α_1PAF-AH(Ib), on the other, the triads in all three show the same chirality (fig. 3d). This is achieved despite the fact that the imidazole of the catalytic histidine in α_1 is rotated 180 ° around the axis bisecting the Cε–Nε–Cδ angle with respect to its analogue in the other acetylhydrolases. Thus, PAF is likely to bind to all PAF-AHs in the same general fashion, with the *re*-face of the *sn*-2 ester attacked in an S_N2-type reaction by the hydroxyl group of the catalytic serine. This observation reinforces the hypothesis that all lipolytic enzymes are likely to have evolved so as to attack the *re*-face of the ester bond, regardless of the tertiary fold [58].

The specificity window of the α subunits of PAF-AH(Ib) is narrower than that of the previously discussed acetylhydrolases. The specific activity falls dramatically after the acyl chain in the *sn*-2 position is extended by a single methylene [25]. The α_1 subunit was crystallized from a solution containing sodium acetate buffer. This resulted in the acetate ion being bound in the active site, a fortuitous situation tantamount to the crystallization of an enzyme-product complex. We were therefore able to directly analyse the molecular basis of this specificity. Three methyl groups, from the side chains of Leu48, T103 and Leu194 form a socket, which accommodates acetate's methyl but creates steric hindrance when a longer acyl group is present in the same site (fig. 5).

It is important to consider the potential biological consequences of this strict specificity in PAF-AH(Ib). In general terms, hydrolytic enzymes that evolved as general degrading enzymes typically show specificity only towards the type of *bond* they disrupt, but not necessarily the rest of the substrate, For example, digestive proteinases are promiscuous and will hydrolyse proteins at multiple sites. Also, lipases that provide free fatty

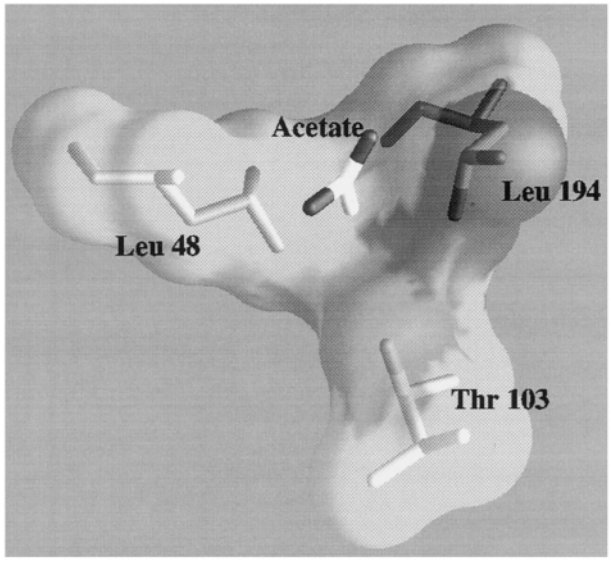

Figure 5. The specificity pocket in the α_1 subunit. For details, see text. The acetate molecule is shown in front with the two oxygen atoms shaded. The surfaces generated by two critical methyl groups (side chains of Leu194 and Thr103) are shown in grey.

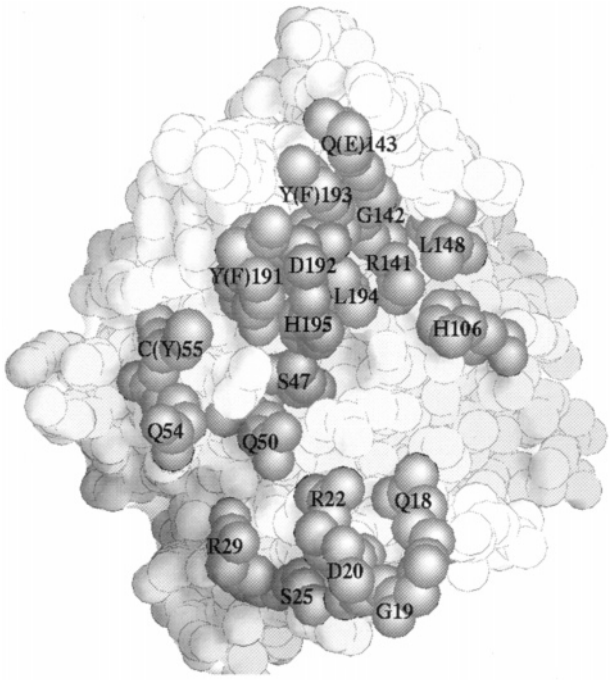

Figure 6. The dimer-forming interface in the α_1 subunit of PAF-AH(Ib). The catalytic residues and those directly involved in the interface are shaded and labelled. In parentheses the identities of the homologous residues in α_2 are given for the non-conserved amino acids.

acids as a carbon source are typically nonspecific and will often remove acyl chains of varying lengths and from varying positions. In contrast, enzymes that are involved in signalling and regulatory pathways are very specific. Thus, for example, caspase and viral proteinases are far more specific than trypsin or pepsin. Can this argument be extended to PAF-AHs? We suggest that this is indeed the case and that the brain PAF-AH(Ib) is a signalling hydrolase, rather than a general housekeeping enzyme like the plasma and isoform II acetylhydrolases. We will return to this point when we discuss the putative function of the Ib complex.

As already pointed out, the crystals described in our study [34] are those of the recombinant α_1 homodimer. However, given the homology of the two α chains, this structure is representative of the heterodimer. The interface which is buried when the monomers associate is shown in fig. 6. There are several interesting points with respect to the structure of the dimer. The two active sites are in close proximity at the bottom of a gorge formed between the two monomers. There appears to be little space to accommodate two molecules of PAF for simultaneous catalysis, and it follows that only one site can be active at any time. This is consistent with the observation that only the α_1 subunit is active in the physiological heterodimer. However, the structural basis of this selectivity is not yet understood. Its explanation will have to await the elucidation of the crystal structure of the heterodimer, a project currently under way in our laboratory.

The product of the *LIS-1* gene: the β subunit of brain PAF-AH(Ib)

When the bovine β subunit cDNA was cloned, the inferred amino acid sequence was shown to be nearly identical to that derived from the *LIS-1* human gene, the causative gene of Miller-Dieker lissencephaly [29, 71]. The 45 kDa protein is ubiquitously expressed [71, 72] and contains seven so-called WD repeats, characteristic of a large family, whose best-known members are the β subunits of trimeric G proteins [73]. The structural consequences of the presence of the WD repeats, so named after the tandem of Trp and Asp residues repeated approximately every 40 amino acids, were beautifully clarified by crystallographic investigations of β subunits of G proteins [74, 75]. The seven WD repeats form a seven-blade propeller structure, with each blade made up of four antiparallel β strands. The invariant Trp and Asp residues occupy key structural positions in this fold. In general, the WD-repeat proteins are now believed to constitute a family that mediate protein-protein interactions.

When the *LIS-1* gene is disrupted, the development of the brain is seriously affected and the cortex is not formed [76]. Unlike familial genetic diseases, this genetic disorder occurs spontaneously and results in

Miller-Dieker syndrome, which leads to an early death. The role of the LIS-1 protein in brain development has been unambiguously shown using laboratory mice [72]. The mechanism by which the *LIS-1* gene product, or the PAF-AH(Ib) β subunit, controls the mechanism of cortex formation is not known, although direct interaction of LIS-1 with tubulin suggests that signalling to cytoskeleton during neuronal migration is involved [77]. It is interesting to note that the only known homologue of this protein, the fungal *NudF* gene product, is responsible for the migration of the nuclei to their target location after division [78]. It has been suggested that during cortex development the migration of nucleus precedes the migration of the entire neuron [79, 80]. This hypothesis is consistent with the pattern of expression of the β subunit in the developing brain [72].

The putative function of the PAF-AH(Ib) complex

In situ hybridisation studies show that in the developing mouse brain tissues the three subunits of PAF-AH(Ib) are coexpressed, and the expression pattern is consistent with neuronal defects characteristic of Miller-Dieker lissencephaly [66]. Furthermore, of the two α subunits, α_1 is dominant in the developing brain and does not appear in other tested fetal tissues [66]. It follows that the interaction of the α_1 and β subunits may be unique to fetal brain tissue. These observations are consistent with the entire heterotrimeric PAF-AH(Ib) complex being involved in cortex development. The question to be answered is: What functional correlation is there between the PAF-hydrolase activity of the α subunits and the signalling mediated by the β subunit? Recent data indicate that PAF analogues capable of inhibiting PAF-AH activity also inhibit migration in granule cells from rat cerebellum [81]. What is most suggestive is that analogues with both agonistic and antagonistic PAF-receptor activity show this effect, as long as they inhibit the acetylhydrolase. PAF-receptor agonists without PAF-AH inhibitor activity show no effect with regard to cell migration. It is tempting to speculate that there is intersubunit communication within the trimeric protein, such that the hydrolysis of PAF activates the β subunits, perhaps by releasing them from the complex. To date, no such signalling has been experimentally demonstrated.

Conclusion

It is clear from this short review that recent years have seen a dramatic enhancement of our understanding of structure-function relationships in enzymes associated with the metabolism of PAF and PAF-mediated signalling. In spite of this progress, much remains to be done. To date, only one PAF-AH has been studied directly by crystallography, and only one of its catalytic subunits has so far been characterized. The diversity of enzymes with acetylhydrolase activity will require many more structural studies before the mysteries of substrate specificity and catalytic mechanisms are fully unravelled. It must be noted here that PAF-AHs also offer interesting opportunities for therapeutic applications. For example, pure recombinant PAF-AH might be useful in in situ treatment of severe acute inflammation. It has been suggested that recombinant PAF-AH is, in general terms, a useful alternative to PAF antagonists, which are also looked upon as important chemotherepeutics. These possibiliites justify further efforts in this interesting field of enzymology.

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