Characterization of a platelet-activating factor acetylhydrolase secreted by the nematode parasite *Nippostrongylus brasiliensis*

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Nippostrongylus brasiliensis, a small nematode parasite of the gastrointestinal tract of rodents, secretes an enzyme that cleaves the proinflammatory molecule platelet-activating factor to its inactive *lyso*- form. The enzyme activity is Ca^{2+} -dependent and does not exhibit interfacial activation. It does not require the addition of reducing agents for maximal activity, and is not inhibited by thiol-active reagents. Sensitivity to inhibitors suggests the involvement of serine and histidine residues in the enzyme activity. As described for other platelet-activating factor

acetylhydrolases, it cannot cleave, nor is it inhibited by, long-chain diacyl phospholipids that are typical substrates for phospholipases A_2 . The purified enzyme was resolved by SDS/PAGE as a heterodimer composed of two protein subunits with apparent molecular masses of 38 and 25 kDa. The properties of the nematode enzyme thus differ from those described for the mammalian enzymes, but are more closely related to those of an acetylhydrolase than a phospholipase.

INTRODUCTION

Platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is a potent phospholipid mediator of inflammation [1], synthesized by many cell types on appropriate stimulation [2,3]. It mediates a broad spectrum of biological activities such as hypotension, smooth-muscle contraction and an increase in vascular permeability [4–6]. PAF is an important mediator within the mammalian immune system, in which it induces chemotaxis, aggregation and degranulation of neutrophils and eosinophils, and induction of the oxidative burst and production of interleukin-1 and tumour necrosis factor α in macrophages and monocytes [7–11]. PAF also enhances natural killer cell-mediated lysis and augments the IgE-mediated cytotoxic function of eosinophils against larval *Schistosoma mansoni* [12,13].

The activity of PAF is regulated both at the level of synthesis [14] and by rapid metabolism to the inactive product *lyso*-PAF [15,16]. The latter process occurs via hydrolysis of the *sn*-2 acetyl moiety [17], and is effected by a family of acetylhydrolases found in both the plasma, where they are associated with low-density lipoproteins [16,18–20], and the cytosolic fraction of many mammalian tissues [21–24]. These enzymes have been purified from human plasma [19], erythrocytes [22] and bovine brain [23], and recently genes encoding the human plasma enzyme and the catalytic subunit of the bovine brain enzyme have been cloned [24,25]. The enzymes can be discriminated by a variety of criteria, although they all show specificity for short acyl chains at the *sn*-2 position, distinguishing them from phospholipases A_2 [26]. In addition, PAF acetylhydrolases have been demonstrated to remove the *sn*-2 acyl group from oxidatively fragmented phospholipids [22,27], suggesting that their biological role may be greater than previously envisaged, and that they might also serve to limit lipid peroxidation in biological systems.

Nippostrongylus brasiliensis is a small nematode parasite of the gastrointestinal tract of rats. Helminths in general do not possess the ability to undergo antigenic variation, and have evolved sophisticated means to neutralize or down-regulate immune effector mechanisms resulting in the establishment of chronic infection [28]. In experimental situations, *N. brasiliensis* is expelled from its host by an acute immune-mediated inflammatory response that ultimately results in intestinal pathology and dysfunction. The expulsion event is characterized by intestinal mastocytosis and eosinophilia [29], hypersecretion of mucus, damage to the intestinal epithelium and an increase in gut motility [30,31]. A number of inflammatory mediators are associated with these events, including tumour necrosis factor α [32], histamine [33], prostaglandin E_2 [34], 5-hydroxytryptamine [35] and leukotrienes [31]. PAF is released into the intestinal lumen of rats immunized with *N. brasiliensis* and challenged intravenously with parasite antigen [36], a procedure that induces symptoms immunologically synonymous with those seen in the acute inflammatory response associated with the spontaneous cure of infection. These observations have led to the hypothesis that products secreted by parasitic nematodes might target and inactivate this proinflammatory molecule, and we recently demonstrated that a component in the secretions of adult *N. brasiliensis* inactivated PAF by cleavage of its *sn*-2 acetyl group [37]. In this paper, we describe the properties of the PAF acetylhydrolase, and discuss the potential role this enzyme might play in moderating intestinal inflammation and expulsion of parasites.

MATERIALS AND METHODS

Materials

1-*O*-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (PAF; C_{16}), 1-*O*-alkyl-*sn*-glycero-3-phosphocholine (*lyso*-PAF), 1-*O*-alkyl-2-

Abbreviations used: ES, excretory/secretory products; PAF, platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine); cmc, critical micellar concentration; DFP, di-isopropyl fluorophosphate; IEF, isoelectric focusing; 2-propionyl-GPC, 1-*O*-alkyl-2-propionyl-*sn*-glycero-3-phosphocholine; PC, L- α -phosphatidylcholine.

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thioacetyl-*sn*-glycero-3-phosphocholine (2-thio-PAF), and 1-*O*-alkyl-2-propionyl-*sn*-glycero-3-phosphocholine (2-propionyl-GPC) were purchased from Novabiochem. [*acetyl*-³H]PAF was obtained from Du Pont–New England Nuclear. L- α -Phosphatidylcholine (egg yolk PC), 1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine (1,2-dipalmitoyl-GPC), quinacrine and other chemicals were purchased from Sigma. Di-isopropyl fluorophosphate (DFP) was obtained from Aldrich Chemical Co. Reverse-phase chromatography columns (Sep-Pak C₁₈) were obtained from Waters Millipore. Microconcentrators (Centricon 10) were purchased from Amicon.

Parasite recovery and culture

Approx. 5000 infective third-stage larvae were injected subcutaneously into male Sprague–Dawley rats at two sites in the flank. Rats were killed by cervical dislocation 8 days after infection, and the small intestines were removed. The anterior half of each intestine was cut open and placed on to muslin stretched over a Baermann apparatus filled with warm (37 °C) saline. Parasites were collected from the bottom of the tube after 60 min. Before culture, recovered parasites were washed 10 times in 10 vol. of sterile culture medium (serum-free RPMI 1640, 1 % glucose, 2 mM L-glutamine, 100 units/ml penicillin, 100 μ g/ml streptomycin and 20 μ g/ml gentamicin). Parasites were then incubated in sterile medium at 37 °C under 5 % CO₂ for up to 2 weeks. Metabolic labelling was carried out in culture medium supplemented with 200 μ Ci/ml Trans³⁵S-label (New England Nuclear). Medium was routinely changed every 2 days. Pooled excretory/secretory (ES) products were cleared through 0.2 μ m filters and concentrated by passage through Centricon 10 microconcentrators.

Enzyme and inhibition assays

PAF acetylhydrolase activity was assayed as described previously [37], following a protocol outlined by Stafforini et al. [19] using [*acetyl*-³H]PAF as substrate, except that the assays were carried out at a substrate concentration of 8–10 μ M in the presence of 1 mM CaCl₂ in a final volume of 100 μ l unless otherwise indicated in the text. Reactions were terminated by addition of an equal volume of 10 M acetic acid, and cleavage products were recovered and quantified by reverse-phase chromatography on octadecyl silica-gel cartridges (Waters Sep-Pak) as previously described [19,37]. One unit of enzyme activity is defined as the liberation of 1 nmol of acetate/min at 37 °C. For determination of inhibition, the standard PAF acetylhydrolase assay was modified as follows: aliquots of partially purified enzyme (0.1 μ g) after the isoelectric focusing (IEF) step were individually preincubated for 30 min at room temperature with various inhibitors in a volume of 20 μ l. [³H]PAF was then added to the sample to a final concentration of 8 μ M in a volume of 100 μ l. The mixture was incubated at 37 °C for 30 min and assayed for hydrolysis of PAF by reverse-phase chromatography. The effect of a number of analogues of PAF on hydrolysis of [*acetyl*-³H]PAF was assayed by their inclusion in the standard assay mixture at equimolar concentrations (8 μ M).

Purification procedure

Centricon 10-concentrated ES proteins from adult *N. brasiliensis* were applied to an FPLC Superose-300 gel-filtration column (2.6 cm $\times$ 90 cm) previously equilibrated with 50 mM phosphate buffer (pH 6.9) and maintained at 4 °C. Elution was effected at a flow rate of 90 ml/h and 4 ml fractions were collected and

assayed for activity and protein content. Protein concentrations were determined by the Micro BCA kit (Pierce) based on the bicinchoninic acid test [38]. Active fractions were pooled and concentrated to 2.5 ml by Centricon 10 filtration before injection into a prefocused Bio-Rad preparative IEF rotofor cell run at constant power (12 W) and equilibrated with 1.2 % (w/v) Bio-lyte 3–10 ampholytes (Bio-Rad). Narrower pH gradients were set up by the addition of 0.3 % Bio-lyte 4–6 and 0.7 % Bio-lyte 3–10 ampholytes in the rotofor cell. Separation was effected in the prefocused pH gradient over 4 h. Fractions were collected from the focusing chamber and analysed for enzyme activity. Peak fractions were collected and equilibrated with 1.0 M NaCl for 1 h before extensive dialysis into 20 mM phosphate buffer/1.0 mM CaCl₂ to remove ampholytes. Pooled fractions were concentrated, equilibrated with 10 % sucrose and separated by electrophoresis for 16 h at 8 mA in 6 % polyacrylamide gels made up in 0.375 M Tris/HCl buffer (pH 8.3)/0.01 % SDS. Gels were then sliced into 25 fragments which were placed individually into 1.5 ml Eppendorf tubes containing 200 μ l of distilled water, and shaken overnight at 4 °C to elute protein. The eluate was then assayed for acetylhydrolase activity and protein content. Active fractions were pooled, concentrated and analysed by reducing SDS/PAGE.

TLC

Substrate specificity was assessed by TLC after incubation of 50 μ g of phospholipids with 1 μ g of partially purified acetylhydrolase for 3 h at 37 °C. Lipids were extracted by the Bligh–Dyer method [39], dried under a stream of N₂, resuspended in methanol and applied to silica-gel plates. A solvent system of chloroform/methanol/acetic acid/water (57:29:9:5, by vol.) was typically used to resolve the samples; the plates were sprayed with either Molybdenum Blue or Rhodamine 6G and the *R_F* values of substrates and cleavage products determined.

RESULTS

PAF acetylhydrolase activity in secreted products

We have previously demonstrated that adult *N. brasiliensis* secrete a product which cleaves PAF by hydrolysis of the *sn*-2 acetyl group [37]. Extending this observation, the hydrolysis of PAF by a crude preparation of adult secreted products was linear with time over the first 30 min of incubation with 5 μ g of protein (Figure 1A). Hydrolysis of a fixed concentration of PAF (10 μ M) was also linear with protein concentration up to 5 μ g of ES per assay, after which the rate of hydrolysis slowed as the substrate became limiting (Figure 1B). If the substrate concentration was increased 10-fold to 100 μ M, however, the rate of hydrolysis was once again linear (results not shown). The enzyme was active over a broad pH range between 6.0 and 9.0, and assays were carried out at an optimal pH of 7.8. The enzyme was stable when stored at –70 °C in phosphate buffer but was very sensitive to freeze–thaw procedures, and a gradual loss of activity was observed on prolonged storage at 4 °C. Preincubation at 65 °C ablated enzyme activity (results not shown).

Enzyme purification

Parasites were incubated in culture medium containing Trans³⁵S-label in order to monitor purification, in view of the extremely limited amount of starting material (1.55 mg of protein). Secreted proteins were collected every 2 days for 2 weeks, concentrated by Centricon filtration and 2.98 units of PAF acetylhydrolase activity was applied to a Superose-300 gel-filtration column.

Fig 1 Hydrolysis of PAF by secretory products of *N. brasiliensis*

(A) Time course of hydrolysis of [acetyl-³H]PAF; (B) dependence on protein concentration. Release of [³H]acetate was measured as described in the Materials and methods section. Incubation was for 30 min at 37 °C, and 5 μg of secreted proteins was used in (A).

Peak activity was eluted in three fractions with an estimated molecular mass between 50 and 70 kDa (Figure 2A). Activity was also identified in higher-molecular-mass fractions which probably resulted from aggregation, as the inclusion of detergent (0.01 % Triton X-100) during chromatography depleted activity in these fractions (results not shown).

The peak fractions from gel filtration were further separated using a preparative IEF rotofor apparatus. One major and one minor peak of enzyme activity were detected, at pI values of 5.3 and 6.5 respectively (Figure 2B). We pooled fractions containing the major activity and dialysed them against phosphate buffer to remove ampholytes. Attempts to purify the enzyme activity further by preparative native PAGE were unsuccessful. This was overcome by the inclusion of a low concentration of SDS (0.01 %) in the gel and running buffer, and this step resulted in resolution of the enzyme into a single peak of 64 kDa (Figure 2C). Analysis of a boiled preparation of the purified 64 kDa protein by reducing SDS/PAGE revealed the presence of two polypeptides with masses of 38 and 25 kDa. Given the resolution of enzyme activity by gel filtration and PAGE, the data suggest that the nematode PAF acetylhydrolase is a heterodimer of 38 and 25 kDa subunits.

The overall purification was 33.3-fold with a 10.7 % recovery and a specific activity in the final preparation against PAF of 64 nmol/min per mg. Although this would appear to represent a relatively modest level of purification, the starting material (parasite-secreted products) has a very restricted protein profile. The final yield of purified protein was sufficient only for resolution

of subunit composition. However, the profile of the pooled fractions from the IEF step (Figure 2B) was almost identical with that of the final purified enzyme (Figure 2C), and we therefore utilized the former preparation, designated as partially purified enzyme, for further experiments.

Kinetic properties of the enzyme

Two different classes of enzyme have been described that hydrolyse PAF at the *sn*-2 position: phospholipases A₂ and a specific esterase known as PAF acetylhydrolase. Each class can be distinguished by several biochemical criteria. Most phospholipases exhibit a profound increase in the rate of hydrolysis of substrate when the latter exceeds the critical micellar concentration (cmc), a phenomenon termed interfacial activation. To determine whether the nematode enzyme exhibited interfacial activation, the rate of hydrolysis of PAF was assayed over a range of substrate concentrations above and below the cmc (2.5 μM) [16]. Figure 3 illustrates that the nematode enzyme did not exhibit interfacial activation, but showed kinetic properties akin to those described for mammalian PAF acetylhydrolases [19,22].

Despite the lack of interfacial activation, mammalian PAF acetylhydrolases have been observed to display surface dilution kinetics in the presence of detergents, due to dilution of substrate concentration at the micellar surface [19,22]. We therefore assayed enzyme activity in the presence of increasing concentrations of Triton X-100, with PAF at a constant concentration of 8 μM. Figure 4(A) demonstrates an initial increase in enzyme activity on addition of detergent, but progressive depletion of activity above 80 μM Triton X-100. To assay for a surface dilution effect, the substrate concentration was increased proportionately with that of Triton X-100 so that the abundance at the micellar surface remained constant, as described for mammalian PAF acetylhydrolases [19,22]. In contrast with the properties of these enzymes, however, the acetylhydrolase activity of the nematode enzyme progressively declined, indicating direct inhibition by high concentrations of Triton X-100 (Figure 4B).

Cation-dependence

Ca²⁺ is an obligatory cofactor for most phospholipases A₂, whereas all PAF acetylhydrolases described thus far act in a Ca²⁺-independent manner. The effects of a number of bivalent cations and chelators on parasite enzyme activity were therefore tested. Table 1 illustrates that the partially purified enzyme required Ca²⁺ for activity (this property was also determined before purification). A titration curve (not shown) indicated that 1 mM CaCl₂ was sufficient for maximal hydrolysis. Moreover, both EDTA and EGTA inhibited activity. The addition of MgCl₂ had minimal effect on enzyme activity.

Effect of inhibitors

The majority of phospholipases A₂ are sensitive to the addition of reducing reagents [40]. Table 2 indicates that the activity of the nematode enzyme was also marginally affected by the addition of 1 mM dithiothreitol. Activity was unaffected by pretreatment with iodoacetic acid and dithiobis-(2-nitrobenzoic acid), reagents that react with free thiol groups. In addition, the enzyme was insensitive to quinacrine at 1 mM, a concentration that generally inhibits phospholipases A₂. *p*-Bromophenacyl bromide, a nucleophilic reagent that derivatizes histidine residues in the catalytic sites of lipases and esterases [41], substantially inhibited the activity of the nematode acetylhydrolase. A mild inhibitory effect was obtained with 1 mM DFP, as observed for the mammalian

Fig 2 Purification of PAF acetylhydrolase

(A) FPLC Superose-300 gel filtration. Adult ES (1.55 mg) was loaded on to a Superose-300 column (2.6 cm × 90 cm) equilibrated with 50 mM phosphate buffer, pH 6.9. Elution took place at a flow rate of 90 ml/h and fractions (4 ml) were collected and examined for activity and protein content. Molecular mass calibration markers (aldolase, 158 kDa; BSA, 67 kDa; ovalbumin, 43 kDa) are arrowed. The profile of ³⁵S-labelled proteins in the pooled active fractions resolved by SDS/PAGE under reducing conditions is shown. Molecular mass (kDa) is displayed on the vertical axis. (B) Preparative IEF rotor separation. Active fractions eluted from the Superose-300 column were injected into a prefocused Bio-Rad preparative IEF rotor cell run at constant power (12 W) and equilibrated with 1.2% (w/v) Bio-Lyte 3–10 ampholytes. Separation was effected over 4 h. Twenty fractions were collected from the focusing chamber and analysed for PAF acetylhydrolase activity.

Fig 3 Dependence of PAF hydrolysis on substrate concentration

The hydrolysis of PAF above and below cmc by a partially purified preparation (1 μ g) of enzyme was assayed as described in the Materials and methods section.

Fig 4 Effect of Triton X-100 on PAF acetylhydrolase activity

(A) Effect of Triton X-100 at concentrations above and below the cmc of PAF. Partially purified nematode PAF acetylhydrolase (1 μ g) was incubated with [3 H]PAF at either 1 or 8 μ M and the effect of inclusion of Triton X-100 (0–320 μ M) was investigated. The cmc of Triton X-100 is 240 μ M. Incubations were effected as described in the Materials and methods section. $\square$, [PAF] = 8 μ M; $\diamond$, [PAF] = 1 μ M. (B) Effect of increasing Triton X-100 and PAF at a constant ratio. Partially purified nematode PAF acetylhydrolase (1 μ g) was incubated with [3 H]PAF between 8 and 48 μ M and Triton X-100 between 50 and 300 μ M. Incubations were effected as described in the Materials and methods section.

Table 1 Cation-dependence of PAF acetylhydrolase

Preparations of partially purified acetylhydrolase (0.1 μ g) were preincubated for 30 min on ice in the presence or absence of cations and chelators listed at the final concentration in the assay mixture. Assays proceeded in the presence of 80 μ l of 10 μ M [3 H]PAF for 30 min at 37 $^{\circ}$ C (final reaction volume of 100 μ l). Control activity in these experiments was 17.2 nmol/min per mg. Results are expressed as means $\pm$ S.D. for triplicate assays.

CaCl ₂ (mM)	Additions (mM)			Relative activity (%)
	MgCl ₂	EDTA	EGTA	
1.0	—	—	—	100
0.01	—	—	—	13.2 $\pm$ 4.1
—	1.0	—	—	8.6 $\pm$ 2.7
1.0	1.0	—	—	98.3 $\pm$ 3.5
1.0	—	10	—	0
1.0	—	—	10	0

Table 2 Effect of various compounds on the activity of the PAF acetylhydrolase

Preparations of partially purified acetylhydrolase (0.1 μ g) were preincubated for 30 min at room temperature in the presence or absence of the compounds listed at the final concentration in the assay mixture. Assays proceeded in the presence of 80 μ l of 10 μ M [3 H]PAF for 30 min at 37 $^{\circ}$ C (final reaction volume of 100 μ l). Control activity in these experiments was 20.6 nmol/min per mg. Results are expressed as means $\pm$ S.D. for triplicate assays. pBPP, *p*-bromophenacyl bromide; DTT, dithiothreitol; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid); IAA, iodoacetic acid.

Additions	Relative activity (%)
DFP (1 mM)	63.8 $\pm$ 6.1
PMSF (1 mM)	46.0 $\pm$ 5.4
pBPP (1 mM)	11.6 $\pm$ 0.8
Quinacrine (1 mM)	86.1 $\pm$ 2.9
DTT (1 mM)	84.3 $\pm$ 2.8
DTNB (2 mM)	98.5 $\pm$ 1.6
IAA (2 mM)	91.9 $\pm$ 2.6

plasma acetylhydrolase [42], and even greater inhibition was effected by 1 mM PMSF, which has been documented to inhibit other PAF acetylhydrolases [43,44]. Thus, on the basis of inhibitor specificity, the nematode enzyme more closely resembles an esterase than a phospholipase despite its requirement for Ca²⁺. The PAF acetylhydrolase activity was distinct from nematode secretory acetylcholinesterases, as neither acetylcholine nor butyrylcholine were cleaved by the purified enzyme (results not shown).

Substrate specificity

The substrate specificity of the acetylhydrolase was assessed with PC, 1,2-dipalmitoyl-GPC, 2-propionyl-GPC and PAF. Table 3 illustrates that the nematode enzyme did not cleave PC or 1,2-dipalmitoyl-GPC, but showed specificity for short acyl chains esterified at the *sn*-2 position, as it was able to cleave 2-propionyl-GPC in addition to PAF. *Lyso*-PAF was resolved by TLC alongside the samples which confirmed that the cleavage products

The profile of labelled proteins in pooled active fractions resolved by SDS/PAGE under reducing conditions is shown. Molecular mass (kDa) is displayed on the vertical axis. (C) Preparative PAGE. Active fractions from IEF were loaded on to a 6% preparative polyacrylamide gel containing 0.01% SDS, resolved as described in the Materials and methods section, fractions recovered and assayed for enzyme activity. The molecular mass of marker proteins (kDa) is arrowed. The profile of labelled proteins in pooled active fractions resolved by SDS/PAGE under reducing conditions is shown. Molecular mass (kDa) is displayed on the vertical axis.

Table 3 Substrate specificity of PAF acetylhydrolase analysed by TLC

Assays were carried out with 1 μ g of partially purified enzyme and 50 μ g of substrate for 3 h at 37 °C in the presence of 1 mM CaCl₂ and separated by TLC as described in the Materials and methods section.

Substrate	<i>R_f</i> values	
	— PAF acetylhydrolase	+ PAF acetylhydrolase
PC	0.73	0.73
Dipalmitoyl-GPC	0.67	0.67
Propionyl-GPC	0.43	0.21
PAF	0.40	0.21
<i>Lyso</i> -PAF	0.21	0.21

were the *lyso*-derivatives of the substrates. A number of PAF analogues were also tested for their ability to inhibit hydrolysis of PAF competitively. Addition of equimolar amounts of 1-*O*-alkyl-2-thioacetyl-GPC and 1-*O*-alkyl-2-propionyl-GPC inhibited the hydrolysis of PAF by 48 and 26 % respectively, whereas PC had no effect (results not shown).

DISCUSSION

These studies demonstrate that *N. brasiliensis* secretes an enzyme that hydrolyses the proinflammatory molecule PAF to its *lyso*-form. The enzyme does not exhibit interfacial activation, and like the PAF acetylhydrolases previously described, cannot cleave, nor is it inhibited by, long-chain diacyl phospholipids that are typical substrates for phospholipases A₂ [19,22,43,45]. The properties of the nematode acetylhydrolase therefore suggest that it is distinct from phospholipases A₂, although the activity differs from mammalian PAF acetylhydrolases in that it is dependent on Ca²⁺. Further studies with inhibitors suggest the involvement of histidine and serine residues in enzyme activity, but no apparent role for thiol groups. DFP is generally a potent inhibitor of PAF acetylhydrolases, although a mild inhibitory effect analogous to that described for the nematode enzyme has been documented for the human plasma enzyme [42]. Greater inhibition was observed with PMSF, which sulphonates active-site serine residues. The enzyme has restricted substrate specificity analogous to the mammalian PAF acetylhydrolases in that it has an absolute requirement for a short fatty acyl chain at the *sn*-2 position of the glycerol backbone [19,22,23].

Although the nematode enzyme thus qualifies as a specific acetylhydrolase, it differs from PAF acetylhydrolases described thus far in subunit composition, Ca²⁺-dependence and inhibition by Triton X-100. Considerable heterogeneity has already been observed for mammalian PAF acetylhydrolases with respect to the former criterion. Thus the enzyme from human plasma has an apparent molecular mass of 43–45 kDa [19,24], whereas the erythrocyte enzyme is a homodimer of 25 kDa subunits [22]. The PAF acetylhydrolase from bovine brain has a molecular mass of approx. 100 kDa, resulting from a heterotrimeric association of proteins with apparent masses of 45, 30 and 29 kDa when resolved by SDS/PAGE [23]. Genes encoding the plasma enzyme and the catalytic subunit of the enzyme from bovine brain have recently been cloned, and the deduced protein sequences show no similarity other than a shared motif at the active-site serine [24,25]. The nematode enzyme described here was characterized as a heterodimer comprised of two protein subunits with apparent molecular masses of 38 and 25 kDa,

revealing further diversity in the composition of PAF acetylhydrolases. The majority of work described to date has been carried out on mammalian enzymes, and our data indicate that invertebrate organisms also synthesize enzymes of this class. An acetylhydrolase activity has been detected in the protozoan *Tetrahymena pyriformis* [43], although details of the purified enzyme have not yet been reported.

The most obvious function for the nematode-secreted acetylhydrolase is inactivation of PAF generated locally in mucosal tissues of the gastrointestinal tract during infection. Moqbel et al. [36] have shown that in rats immune to *N. brasiliensis*, PAF is released into the intestinal lumen and its concentration elevated systemically after intravenous challenge with parasite antigen. In primary infections, however, no significant elevation of PAF concentration was observed until expulsion of the worms was complete [46]; this observation may be due to high levels of acetylhydrolase secreted by the parasites. Secretion of the enzyme appears likely to function in an anti-inflammatory capacity by limiting the recruitment of leucocytes responsive to PAF, and consistent with this is the observation that elevated numbers of eosinophils in the jejunum are detectable only after expulsion [46]. Endothelial cells treated with exogenous PAF or sensitized as a result of endogenous synthesis become adhesive for neutrophils [47], and thus the parasite acetylhydrolase might directly inhibit extravasation of granulocytes. A recombinant form of the human plasma PAF acetylhydrolase was recently demonstrated to block the ability of PAF to activate polarization and spreading of neutrophils *in vitro*, in addition to limiting vascular leakage *in vivo* [24]. PAF acetylhydrolase probably represents one of a number of anti-inflammatory molecules secreted by the parasite, and recent work [48] has highlighted a protein from *Ancylostoma caninum* (canine hookworm) that blocks the adhesion of neutrophils to vascular endothelial cells and inhibits the release of hydrogen peroxide from neutrophils activated with *N*-formyl-methionyl-leucyl-phenylalanine (fMLP).

Mammalian PAF acetylhydrolases also hydrolyse phospholipids that contain short *sn*-2 acyl chains generated by oxidative fragmentation of polyunsaturated fatty acids, but are incapable of hydrolysing *sn*-2 fatty acyl chains longer than six carbons unless they have been oxidatively modified by the addition of an aldehyde moiety to the terminal carbon residue [27]. Thus fragmented species derived from phospholipids containing a 9,10-unsaturated bond, the most common *sn*-2 unsaturation, can be effectively removed after oxidative fragmentation [49]. Selective detoxification of oxidatively fragmented membrane phospholipids might thus function to protect parasite cuticular membranes, limiting the chain reaction of lipid peroxidation and allowing for the subsequent reacylation of *lyso* derivatives to restore membrane integrity.

In summary, we have purified and characterized a PAF acetylhydrolase secreted by a gastrointestinal nematode parasite. Trickle infections of rats with *N. brasiliensis* result in the establishment of a chronic infection which persists for several months [50]. In this context, inactivation of PAF is likely to be an important factor in down-regulating the immune system to the protracted advantage of the parasite.

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