Crystal Structure of Human Plasma Platelet-activating Factor Acetylhydrolase

STRUCTURAL IMPLICATION TO LIPOPROTEIN BINDING AND CATALYSIS

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Human plasma platelet-activating factor (PAF) acetylhydrolase functions by reducing PAF levels as a general anti-inflammatory scavenger and is linked to anaphylactic shock, asthma, and allergic reactions. The enzyme has also been implicated in hydrolytic activities of other pro-inflammatory agents, such as sn-2 oxidatively fragmented phospholipids. This enzyme is tightly bound to low and high density lipoprotein particles and is also referred to as lipoprotein-associated phospholipase A2 (Lp-PLA2). The crystal structure of this enzyme has been solved from x-ray diffraction data collected to a resolution of 1.5 Å. It has a classic lipase α/β-hydrolase fold, and it contains a catalytic triad of Ser273, His511, and Asp296. Two clusters of hydrophobic residues define the probable interface-binding region, and a prediction is given of how the enzyme is bound to lipoproteins. Additionally, an acidic patch of 10 carboxylate residues and a neighboring basic patch of three residues are suggested to play a role in high density lipoprotein/low density lipoprotein partitioning. A crystal structure is also presented of PAF acetylhydrolase reacted with the organophosphate compound paraoxon via its active site Ser273. The resulting diethyl phosphoryl complex was used to model the tetrahedral intermediate of the substrate PAF to the active site. The model of interface binding begins to explain the known specificity of lipoprotein-bound substrates and how the active site can be both close to the hydrophobic-hydrophilic interface and at the same time be accessible to the aqueous phase.

Platelet-activating factor (PAF, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is a phospholipid messenger synthesized by a variety of cells involved in host defense, such as endothelial cells, platelets, neutrophils, monocytes, and macrophages. High levels of PAF are responsible for a variety of human diseases such as inflammation, asthma, necrotizing enterocolitis, and sepsis (2). The enzyme PAF-AH (EC 3.1.1.47) was first identified from the plasma by its ability to hydrolyze and therefore inactivate PAF (3).

The enzyme plasma PAF-AH has been classified as group VIA phospholipase A2 (PLA2) (4), and it hydrolyzes the ester bond at the sn-2 position of phospholipid substrates with a short sn-2 chain. In addition to its role in reducing PAF levels, PAF-AH functions by hydrolyzing other pro-inflammatory agents, such as oxidized lipids of LDL particles (5, 6). Many of these oxidized phospholipids have an oxidatively fragmented sn-2 chain that would orient away from the hydrophobic portion of an LDL particle. These oxidized phospholipid species are present at elevated levels at atherogenic lesions, and the PAF-AH hydrolysis of these species has attracted considerable attention recently as a potential therapeutic target (6–10).

Physiologically, plasma PAF-AH is associated to both LDL and HDL particles and therefore functions on the lipid-aqueous interface and can be considered a peripheral membrane protein. Another generally used name for plasma PAF-AH is lipoprotein-associated PLA2 (7). Kinetic studies have shown that although PAF-AH binds to interfaces, such as LDL particles and vesicles, this binding is not necessary for catalysis (11, 12). This is in contrast to the 14-kDa secreted PLA2 enzymes that are allosterically activated upon interface binding (13). The plasma PAF-AH and the homologous (42% identity) intracellular type II PAF-AH (also known as group VIIIB PLA2) enzymes are calcium-independent and contain a GXSXG motif that is characteristic of neutral lipases and serine esterases (14). Structurally distinct from the 44-kDa plasma PAF-AH and type II PAF-AH enzymes is a 26-kDa intracellular brain catalytic domain that has been designated type I PAF-AH or more commonly PAF-AH-Ib (15). Although the structure of PAF-AH-Ib has been reported (16, 17), there is no sequence homology to the other PAF-AH enzymes. Additionally, the PAF-AH-Ib enzyme has been suggested to have a physiological function other than as a true PAF-AH enzyme (18, 19). Although a structure has been determined for a distantly related lipase from Streptomyces exfoliatus (20), the extent of sequence overlap between this enzyme and the mammalian plasma PAF-AH enzyme is limited to 19% identity over a subset of 228 aligned residues. Hence, a crystal structure of a mammalian PAF-AH was required to understand the relationship between structure and function.

Following identification of the enzyme in the plasma, it had been subjected to extensive biochemical characterization,
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including the determination of the limits of N- and C-terminal truncations that preserve native functions (14). Here, we report a 1.5-Å ligand-free crystal structure and a 2.1-Å diethyl phosphoryl (DEP) complex crystal structure of a 43.4-kDa construct (residues 47–429) of PAF-AH. This construct represents a form of the enzyme with native-like enzyme activity (14) toward the substrate PAF, as well as native-like LDL binding properties (12).

EXPERIMENTAL PROCEDURES

Protein Preparation and Crystallization—We obtained 160 mg of pure human plasma PAF-AH (PAFase, residues 47–429, NCBI accession Q13093) overexpressed from Escherichia coli from ICOS Corporation. Details of the protein preparation and initial crystallization of the ligand-free form of human PAF-AH have been reported (21) and will be summarized briefly. Prior to crystallization, PAF-AH samples were suspended in appropriate buffer/detergent solutions (see below). PAF-AH was assayed for kinetic activity using the ester substrate p-nitrophenyl acetate, and the reaction was followed by UV absorbance at 402 nm (ε_{402 nm} = 17,700 M⁻¹ cm⁻¹).

Ligand-free PAF-AH Crystals—Our best crystals of ligand-free PAF-AH were obtained at 20 °C starting from a protein solution at 4 mg/ml that contained 10 mM Tris-HCl, 6 mM sodium citrate, 3% (w/v) sucrose, 1.0 mM dithiothreitol, 27 mM n-octyl-β-D-glucopyranoside, 0.04% (w/v) Pluronic F68, 0.002% (w/v) Tween 80, pH 6.7. The crystallization reservoir solution contained 98.5 mM MOPS buffer, pH 6.6, 44.3% (w/w) (NH₄)₂SO₄, 0.394 M Li₂SO₄, 0.985 M sodium acetate, and 1.48% (v/v) 1,4-butanediol. Aliquots of 1.5 μl of protein and crystallization solutions were mixed to form each hanging drop, and protein crystals formed in 3–4 weeks.

To obtain phase information, the ligand-free crystals of PAF-AH were directly soaked with MeHgCl by adding a small grain of solid powder to the hanging drop and incubating it in the drop for 24 h. Derivative crystals were then flash cooled in liquid nitrogen, stored, and transported to the synchrotron for data collection.

Paraoxon Inhibited PAF-AH—PAF-AH protein received from ICOS Corp. was exchanged with the detergent Triton DF16 (0.01% w/v) using a Q-Sepharose column as described previously (21). The protein was then incubated overnight with the organophosphate compound paraoxon (ChemService) to covalently react with the enzyme active site Ser273. Protein was purified using a Centricon 30 membrane (Millipore) at slow speed (2000 rpm) to avoid aggregation and precipitation. The protein component contained 3 mg/ml protein, 1.0 mM dithiothreitol, 27 mM n-octyl-β-D-glucopyranoside. The crystallization solution contained 41% (w/v) (NH₄)₂SO₄, 400 mM Li₂SO₄, 800 mM sodium formate, pH 7.0, 1.24% (w/v) 1,4-butanediol. Aliquots of 1.5 μl of the protein and crystallization solutions were mixed and set as hanging drops. The crystals grew (150 × 120 × 90 μm) within 2 weeks.

X-ray Data Collection—Native and mercury-derivative data sets were collected at synchrotron beamlines using crystals frozen in liquid nitrogen; additional cryo-protectant was not required. The single- and multiple-wavelength anomalous dispersion data sets were collected at Beamline 19ID of the Advanced Photon Source (Argonne National Laboratory, Chicago, IL). The data sets for native ligand-free and paraoxon inhibited crystals were collected at Beamline X29 of the National Synchrotron Light Source (Brookhaven National Laboratory, Upton, NY). The diffraction data were indexed and processed using the program HKL2000 (22).

Crystal Structure Solution—A single-wavelength anomalous dispersion data set to a resolution of 2.71 Å of a MeHgCl derivative of PAF-AH was used to phase the structure. Eight mercury sites in the crystallographic asymmetric unit were identified and used to generate initial phases using the programs SOLVE and RESOLVE (23). Two subunits of PAF-AH are in the asymmetric unit. A noncrystallographic symmetry axis was identified from six of the mercury sites, with three similar sites per protein subunit. The noncrystallographic symmetry axis was used to improve initial electron density maps using the CCP4 suite program Dm (24). These medium resolution electron density maps, which contained regions of well defined α-helices and β-sheets, allowed the initial model building of roughly half the 766 amino acids/asymmetric unit with the program COOT (25). The partial model was then refined in the nonisomorphous native data set using data to a resolution of 2.6 Å. The partial model was next used as initial phases together with the native data set to a resolution of 1.5 Å for the automated program ARP/wARP (26). Within 60 cycles, the program had traced and auto-built side chains for 97% of the model. Refinement was initially carried out with the program REFMAC5 (24) and was finished using the program SHELXL (27).

Structure of PAF-AH-DEP Complex—A single subunit of the ligand-free PAF-AH structure was used as a search model. Molecular replacement was performed using the program MOLREP (24) to solve the structure of the PAF-AH-DEP complex, which had been crystallized using paraoxon inhibited PAF-AH. The structure of the PAF-AH-DEP complex was refined using the program REFMAC5 (24). A CIF parameter file for the DEP group, which is covalently attached to Ser273, was prepared using the monomer library sketcher module of the program CCP4 (24).

RESULTS

Overall Structure of PAF-AH—We have solved the crystal structures of human plasma PAF-AH in a ligand-free form and as a complex with the organophosphate compound paraoxon reacted with the active site Ser273 (Fig. 1). The protein displays a classic α/β-hydrolase fold, typical of other GXSG lipases. The structure of plasma PAF-AH was submitted to the DALI website to obtain structural homologues (28); the S. exfoliatus lipase structure (20) was the top hit with a Z score of 23.8, Cα RMSD of 2.4 Å, and 19% identity over a subset of 228 amino acids aligned. The topology diagram of the PAF-AH structure is shown in supplemental Fig. S1. In both of the crystal structures reported here, there are two protein subunits in the asymmetric unit. The construct of PAF-AH crystallized contained 383 residues (47–429). A summary of the data collection, phasing and refinement of the crystal structures is presented in Table 1.
Quality of the Structures—As expected, the higher resolution 1.5 Å ligand-free structure has better statistics than the 2.1 Å DEP complex structure. The Ramachandran plots of the ligand-free complex and the DEP complex had 91.6 and 89.5% of residues in the most favored region, respectively. Only residue Ser273, which is the active site nucleophile, was observed to be outside of the generously allowed region (phi, 65°; psi, −116°). The electron density of this region, as shown for the DEP complex structure in Fig. 1D, is very distinct, supporting its proper modeling. The same holds true for the electron density difference map of the 1.5 Å ligand-free structure. The strained conformation of this serine is consistent with other α/β hydrolase enzymes that have nucleophilic serines in a strained ε-conformation (29). The overall B-factor and deviations from ideal geometry are likewise slightly smaller in the ligand-free structure. One other noteworthy outlier in each subunit of both structures reported is the presence of a cis-peptide bond between Phe72 and Asp73. The electron density of this region is very distinct, thereby supporting the presence of this unusual cis-peptide bond.

Disordered Residues and Side Chains—The N- and C-terminal limits of ordered residues observed in our crystal structures are consistent with functional observations of the protein. Plasma PAF-AH functions as a 43-, 44-, or 45-kDa protein with a heterogeneous N terminus starting at residue Ser35, Ile42, or Lys55 (14). In our structures, seven residues (positions 47–53) are missing from the N terminus in each subunit of both the ligand-free and DEP-bound structures. In each case, our structures begin with residue 54, which is close to the endogenous N-terminal start site at Lys55. In previous studies, the limits of N- and C-terminal truncations had been characterized that still ensure the presence of the full native functions of the enzyme (14). In our crystal structures, the C terminus has several disordered residues in the ligand-free structure (A, 426–429; B, 428–429) and DEP complex (A, 427–429; B, 424–429). The ordered C-terminal end of our structures is close to the functional limit tolerated by C-terminal truncation. A subtle difference exists between the two subunits of the ligand-free crystal structure in the crystallographic dimer. In subunit B the electron density of residues 88–92 was weak; therefore their side chain atoms have been truncated to correspond to an alanine side chain. Similarly His114 and Trp115 are missing in subunit A and Trp115 and Leu116 are missing in subunit B; therefore each of these side chains have been truncated.


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TABLE 1
Data collection, phasing, and refinement statistics of PAF-AH

The values in parentheses are for the highest resolution shell. The RMSD bond angles are reported as angle distances using the program SHELXL and as degrees in the program REFMACS.

| Crystal parameters | MeHgCl | Native | DEP complex |
|--------------------|--------|--------|-------------|
| Space group        | C2     | C2     | C2          |
| Unit cell dimensions a, b, c (Å), β (°) | 114.7, 79.0, 96.5, 115.3 | 116.2, 83.1, 96.7, 115.1 | 117.2, 78.7, 97.3, 101.6 |
| Beamline           | APS-19ID | NSLS-X29 | NSLS-X29 |
| Data collection    | Peak   | 1.006a | 1.000a | 1.072a |
| Wave length (Å)    | 50-2.7 (2.8-2.7)a | 50-1.5 (1.55-1.50) | 50-2.1 (2.18-2.10) |
| Completeness (%)   | 98.6 (97.4)a | 96.4 (75.7) | 94.9 (73.8) |
| Redundancy         | 2.1 (2.1)a | 3.6 (1.8) | 3.4 (2.1) |
| Rmerge             | 18.3 (2.1)a | 19.2 (1.9) | 19.6 (3.4) |
| Rmerge/ Rfree      | 0.047 (0.387)a | 0.057 (0.283) | 0.053 (0.193) |

| Phasing statistics | SHELXL | REFMACS |
|--------------------|--------|---------|
| Number of mercury sites | 8a | 8a |
| Phasing power anomalous | 0.603a | 0.298a |
| R factor (FC vs. FP)b | 0.131/0.179 | 0.208/0.264 |
| Figure of merit (acentric/centric/all) | 0.70/0.77/0.71a | |

| Refinement statistics | SHELXL | REFMACS |
|-----------------------|--------|---------|
| Resolution program    | 10.0-1.5 | 50.0-2.1 |
| Rfree                 | 0.131/0.179 | 0.208/0.264 |
| Number of atoms (non-hydrogen) | 6692 | 6366 |
| B-factor main chain   | 24.0 | 28.3 |
| B-factor side chain   | 31.3 | 31.1 |
| RMSD bond lengths (Å) | 0.01 | 0.02 |
| RMSD bond angles (Å)  | 0.03 Å | 1.87° |
| Ramachandran plot (most favored) | 91.6% | 89.5% |
| Ramachandran plot (most favored and additionally allowed region) | 99.5% | 99.7% |

)a These are the peak values.

b The R factor compares structure factors calculated from density modification (FC) to those measured (FP).

as well. In addition to these residues mentioned above, several other surface-accessible and polar side chains from the ligand-free crystal structure were disordered and therefore not modeled. In contrast, the DEP-bound crystal structure had well ordered side chain positions for all residues modeled, including residues His114–Leu116. Consequently, the DEP-bound crystal structure had well ordered side chain positions for all residues modeled, including residues His114–Leu116. The disorder of the residues His114–Leu116, described above, can be explained because of the flexibility of these residues on the surface of the protein. Additionally, these residues are on an α-helix (114–126) that is predicted to be a component of the interfacial binding surface of the protein that accesses the lipoprotein particle. Specific residues shown to be important for binding to LDL by site-directed mutagenesis were Tyr205, Trp115, Leu116, and to a lesser extent Met117 (30). A second short helix (residues 362–369) has hydrophobic residues positioned to insert into the hydrophobic portion of the aqueous-lipid interface. Recently, residues near this helix were predicted to be important for HDL binding (31). The predicted interfacial-binding residues of PAF-AH are shown in Fig. 1B relative to the active site.

Active Site of PAF-AH—As originally predicted (14, 32) the active site of PAF-AH contains a catalytic triad of Ser273, His351, and Asp296. Ser273 is located at the N terminus of an α-helix and on the conserved GXXG motif found in other lipases (14). The amide nitrogens of Phe274 and Leu153 are well poised to stabilize the negative charge of a tetrahedral intermediate of the reaction mechanism, thereby acting as the oxanion hole of the enzyme. The other two catalytic triad residues are appropriately positioned to activate the nucleophilic Ser273 for catalysis; Asp296 is located on the C-terminal end of a β-sheet, and His351 is located at the N terminus of an α-helix. Like other lipases, the catalytic triad lies within a hydrophobic pocket that is oriented toward its substrate (Fig. 1, B and C). Notably, the orientation of the catalytic triad and hydrophobic residues Leu153 and Phe274 were previously predicted based on modeling from the distantly related S. exfoliatus lipase structure (32). From previous functional and kinetic characterization of PAF-AH, it was suggested that the active site would allow substrates to enter from lipoproteins and the aqueous phase (11, 12). The placement of the active site observed in the structure is consistent with these previous findings.

DEP Complex of PAF-AH—To gain insights into the active site properties of PAF-AH, we solved the crystal structure of a covalent complex with the organophosphate compound paraoxon. The enzyme was quickly inactivated by paraoxon, as determined by enzymatic assay. Crystal set-ups led to crystals in the C2 space group, just as with the ligand-free structure, but with slightly different cell dimensions (Table 1). An overall alignment of the ligand-free and DEP complex crystal structures shows only subtle differences of side chain positions. A comparison of the ligand-free and DEP complex structures shows an overall Cα RMSD of 0.3 Å. The active site residues are virtually unchanged, aside from the covalent attachment of DEP to Ser273 as depicted in Fig. 2; the complex with DEP-bound (panel A) serves as a mimic of the tetrahedral intermediate of the esterolysis reaction (panel B). The catalytic triad residues and neighboring active site residues are positioned around the PAF-AH-DEP complex in a manner consistent with a tetrahedral intermediate complex (Fig. 1D). Contact distances between active site residues and the bound DEP group are shown in Table 2. Notably, the amide nitrogens of Phe274 and Leu153 make H-bonds with the O-3 oxygen of the DEP moiety,
which would correspond with the enolate oxygen of a tetrahedral intermediate. The positions of the two ethoxy groups of the bound DEP group are well ordered, as shown in the electron density maps shown in Fig. 1D. 

**DISCUSSION**

Recently, there has been a lot of attention focused on plasma PAF-AH because of its apparently contradictory anti-inflammatory, anti-atherogenic, pro-atherogenic, and pathological importance (8, 34, 35). Early work on the enzyme focused on its role to inactivate the G protein-coupled receptor ligand PAF, and therefore PAF-AH was believed to be a beneficial enzyme (3). The enzyme had been developed as a potential protein therapeutic by the company ICOS Corporation to treat patients deficient in the enzyme and who suffered severe inflammation conditions related to elevated PAF levels (3). In contrast, the lipoprotein-associated function of PAF-AH to hydrolyze oxidatively fragmented phospholipids led others to propose that the enzyme is a significant contributor to pathological conditions, such as atherosclerosis (7, 8, 36). Here, the development of specific and tight binding inhibitors of the enzyme would serve as effective treatments of these pathological conditions (10, 37, 38). In support of serving a pro-atherogenic role, PAF-AH has been shown to be an independent predictor of coronary heart disease (6, 39, 40). Additionally, epidemiological studies have demonstrated a correlation between the polymorphism at residue 279 and coronary artery disease (41) as well as the occurrence of heart attacks (42). Our crystal structure of PAF-AH lends a structural framework that enhances an understanding of previously published work and further opens the door for further studies to explore its functions.

**Polymorphism in PAF-AH**—Several polymorphic alleles of PAF-AH have been discovered that have either shown a loss of plasma PAF-AH activity (V279F and Q281R) (43) or have been shown to be linked to human disease (I198T, A379V, and R92H) (41, 44). The position of each of these polymorphic sites is shown in Fig. 3. Our crystal structure of PAF-AH includes the most common variant at each polymorphic position, with the exception of the Val379 variant, which has a frequency of 0.24 in European populations (42). The two mutations (V279F and Q281R) that lead to a loss of function in 4% of the Japanese population (43) are core residues. The Val279 residue is a conserved hydrophobic core residue and is critical for proper folding of the enzyme and its function. A loss of function of plasma PAF-AH had also been observed in the Q281R variant. Residue 281 is in the middle of an α-helix that also contains the active site Ser273. Therefore, the Q281R variant is believed to disrupt the active site.

Three polymorphic sites are solvent-exposed and distant from the active site (Fig. 3); therefore these polymorphisms most likely exhibit their phenotypic differences through interactions with lipoproteins or other binding partners. Notably, the Val379 variant is common in European populations (frequency of 0.24 overall) and has been implicated to be correlated
with a decreased risk of heart disease (41, 42). In our PAF-AH structure, Val379 is located on a surface α-helix, is 39% solvent-accessible (45) and is 15 Å away from the catalytic His351. The Ile198 polymorphic site is 71% solvent-accessible and is 35 Å from the active site Ser273. Previous kinetic work suggested that the I198T and A379V variants show an increased apparent $K_m$ value (14 and 42 μM, respectively) when compared with the wild type (7 μM), although the $V_{max}$ parameter of these two variants essentially remained constant with a slight increase for the Val379 variant (44). Our PAF-AH structure raises doubts about whether the previously reported kinetics for the A379V and R92H variants reflect significant differences of these polymorphisms (V279F and Q281R) that lead to a loss of function in 4% of Japanese individuals are core residues. This figure was rendered using the program PyMOL (51).

FIGURE 3. Polymorphic sites of PAF-AH shown in gray ball and stick relative to the active site Ser273 and in a view looking directly at the interface binding surface of the enzyme. Three of the polymorphic sites (I198T, A379V, and R92H) are solvent-accessible. Two loss of function polymorphisms (V279F and Q281R) that lead to a loss of function in 4% of Japanese individuals are core residues. This figure was rendered using the program PyMOL (51).

FIGURE 4. A model of the tetrahedral intermediate of C$_{18}$-PAF (cyan) bound to PAF-AH with catalytic triad residues (Ser273, His351, and Asp296) in green and interface-binding residues (Thr113, His114, Trp115, Leu116, Met117, Ile120, Leu123, Leu124, Ile364, Ile365, Met368, and Leu369) in yellow. The coordinates of PAF-AH show a potentially important region of PAF-AH for specific lipoprotein interactions. Fig. 4 reveals a potentially important region of PAF-AH for specific lipoprotein interactions. Fig. 4 (B and D) displays the neighboring acidic and basic patches. A cluster of
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10 carboxylate residues (Asp374, Asp376, Asp382, Asp401, Asp403, Asp406, Glu410, Asp412, Asp413, and Glu414), which is shown in Fig. 4B, is adjacent to a group of basic residues (Lys55, Arg58, and Lys363). Although the functional and structural relevance of these residues is not clear, we believe the following are noteworthy. The cluster of 10 carboxylate residues and three basic residues are mainly conserved when compared across several mammalian sequences of plasma PAF-AH (supplemental Fig. S2). However, most of these residue are not present in the alignment with the intracellular homologue type II PAF-AH (42% identity, 62% similarity). This suggests a potential role of these residues toward LDL/HDL partitioning. Also, the A379V polymorphic site, which has been correlated with heart disease (41, 42), is located at the base of this acidic patch, adjacent to the predicted plane of lipoprotein binding. It is plausible that a switch between alanine and valine at this position may influence LDL/HDL partitioning. In addition to possible interactions with lipoproteins, these residues may maintain electrostatic interaction with charged or polar head groups of phospholipids on the surface of the lipoproteins, a process followed by hydrophobic interaction of the hydrophobic cluster discussed above. Studies are underway to explore the structural and functional relevance of the residues of these acidic and basic patches.

PAF-AH Substrate Specificity—The predicted mode of lipoprotein binding presented in Fig. 4 shows an open channel, and active site residues are positioned in such a way that they can access substrates from the aqueous phase. Gelb and co-workers (11, 13) hypothesized a mechanism in which plasma PAF-AH accesses its substrate from the aqueous phase, and only substrate in the aqueous phase can bind to the catalytic site of the enzyme at the membrane-water interface. Our model is consistent with this hypothesis if one merely adds that the long hydrophobic tail, of for example C18-PAF, remains partly consistent with this hypothesis if one merely adds that the long hydrophobic portion of the interface. Our model is consistent with the hypothesis if one merely adds that the long hydrophobic tail, of for example C18-PAF, remains partly embedded in the hydrophobic portion of the interface, as depicted in Fig. 4. In this model PAF-AH and lipophilic substrates are brought together by their common affinity for lipoproteins. However, the active site faces the aqueous phase and sits just above the interface.

The DEP complex crystal structure allows us to predict how a substrate would access and bind to the active site. The covalent complex of DEP with Ser273 from our crystal structure reminiscient of the fold found in lipases and esterases. Our crystal structures of the enzyme in both a ligand-free state and with a tetrahedral intermediate mimic have provided a working model of how plasma PAF-AH may bind to lipoproteins and catalyze the hydrolysis of both PAF and oxidized phospholipid substrates.

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