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Structure and Activity of Human Mitochondrial Peptide Deformylase, a Novel Cancer Target

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Abstract

Peptide deformylase proteins (PDFs) participate in the N-terminal methionine excision pathway of newly synthesized peptides. We show that the human PDF (HsPDF) can deformylate its putative substrates derived from mitochondrial DNA-encoded proteins. The first structural model of a mammalian PDF (1.7 Å), HsPDF, shows a dimer with conserved topology of the catalytic residues and fold as non-mammalian PDFs. The HsPDF C-terminus topology and the presence of a helical loop (H2 and H3), however, shape a characteristic active site entrance. The structure of HsPDF bound to the peptidomimetic inhibitor actinonin (1.7 Å) identified the substrate-binding site. A defined S1' pocket, but no S2' or S3' substrate-binding pockets, exists. A conservation of PDF–actinonin interaction across PDFs was observed. Despite the lack of true S2' and S3' binding pockets, confirmed through peptide binding modeling, enzyme kinetics suggest a combined contribution from P2' and P3' positions of a formylated peptide substrate to turnover.

Keywords

human deformylase; peptide deformylase; crystal structure

Introduction

Removal of the formyl moiety on methionine of nascent proteins by the metalloprotease peptide deformylase (PDF) is a necessary activity for prokaryotic cell viability.¹ This activity was not believed to be important in eukaryotic cells until recently,² because nuclear encoded proteins are not N-formylated. However, in eukaryotes, mitochondrial protein synthesis may involve the formylation and deformylation of proteins, as evidenced by the presence of the enzymatic machinery to perform these activities in mammals and plants, among other eukaryotes.^{3–5} A human peptide deformylase (HsPDF) was recently shown to be present in mitochondria.^{2,6,7}

The central role of PDF in bacterial protein synthesis, coupled with its previously unrecognized function in mammals, and the need to develop novel antimicrobials have led to significant efforts to discover antibiotics that selectively target bacterial PDFs.^{8,9} PDF inhibitors are a promising drug class, as demonstrated by the broad spectrum activity *in vitro*, of the clinical drug candidates LBM415¹⁰ and BB-83698¹¹ against drug-resistant bacterial strains. The PDF inhibitor BB-83698 has been proposed as a tuberculosis treatment.¹² The discovery of HsPDF has now complicated this drug discovery process. Structural differences between bacterial and human PDFs will likely be key to the design of new selective antimicrobials that are not toxic to mammalian cells.

The physiologic role of PDF in mammalian cells is unclear. The mitochondrial localization of HsPDF, and the N-terminal Met-formylation of mammalian mitochondrial translation products for translation initiation^{13,14} point at the 13 proteins encoded by the mitochondrial genome as putative substrates of HsPDF. The slow enzyme kinetics of HsPDF compared to PDF from other organisms,^{2,6,15} and the N-terminal capping of mitochondrial translation products purified from bovine heart^{16–18} have nevertheless supported the notion that deformylation of methionine does not occur. However, the decrease in human cell growth resulting from siRNA interference of HsPDF and from its pharmacologic inhibition⁷ with the PDF inhibitor actinonin,^{6,19,20} and its analogs, suggests that HsPDF is functional in the mitochondria.

In order to determine how HsPDF-specific residues might contribute to its kinetic properties, we embarked on the structural analyses of HsPDF alone and in complex with the peptidomimetic hydroxamic acid-based inhibitor actinonin. Here, we show that HsPDF can process, *in vitro*, N-Met-formylated peptides of sequences derived from nine proteins encoded by the mitochondrial DNA. We solved the 1.7 Å resolution crystal structure of an N-terminal truncated, catalytically active cobalt-substituted HsPDF, alone and in complex with actinonin and compared them to their non-mammalian counterparts.

The high level of amino acid identity of HsPDF with the PDF predicted for other mammals suggests that the HsPDF crystal structure model presented here is a true representative, and a first, of mammalian PDFs.

Results

HsPDF processes formylated peptides derived from mitochondrial DNA-encoded proteins

HsPDF localizes to the mitochondria and mitochondrial DNA-encoded proteins are N-Met formylated for efficient translation initiation;¹³ therefore, we hypothesized that the 13 mitochondrial-encoded proteins might be the substrates of HsPDF. We measured deformylation of the 11 soluble peptides (Table 1 and Fig. 1) with sequences corresponding to the first four N-terminal residues of proteins encoded by human mitochondrial DNA (NCBI accession number NC_001807). All soluble peptides were deformylated by HsPDF with varying degrees of efficiency. In order to determine whether structural differences between HsPDF and those described for bacterial PDF can account for their kinetic differences,²¹ we resolved the crystal structures of the N-terminally truncated HsPDF in the absence of inhibitor as well as in complex with actinonin (Table 2).

The crystal structure of HsPDF is a dimer

HsPDF was expressed and purified as a Co²⁺ enzyme because this was the only metal that allowed reconstitution of its enzymatic activity.⁶ However, there is no direct evidence that Co²⁺, or any other metal, is the native metal in HsPDF. The metal was confirmed as Co by measuring the EXAFS spectrum of the crystal (data not shown). The asymmetric unit of the HsPDF crystal contained four polypeptide chains that form two pairs, with the metal-binding

site being proximal to the dimer interface. HsPDF molecules in each pair are related by a non-crystallographic 2-fold axis (Fig. 2a), and are indistinguishable from each other, with an average 0.3 Å r.m.s.d. for the inter-monomer comparison including all main chain atoms.

The interaction between the HsPDF monomers in each pair occurs through a hydrophobic core, which is zippered at the edges by intra- and inter-monomer hydrogen bonding, as well as van der Waals interactions (Fig. 2b and c). The carbonyl oxygen of R18 in each monomer forms a hydrogen bond with the amino group of the R173 side chain of the opposite monomer, judging by the proximity between such carbonyl and R173 N^ε. Hydrogen bonding occurs also between the pairs G163-R173 and V12-N177, each of these residues in a different monomer. Intra-monomer hydrogen bonding occurs between the S116 O^γ and the main chain carbonyl of C10, in close proximity to the dimer interface. In addition, intra-monomer salt bridges such as D168-R18 are also present at the dimer interface. The van der Waals contacts occur between the W180 side chain of one monomer and the D14 C^α of the opposite monomer; similarly, T176 O^γ interacts with G13 C^α of the opposite monomer.

The hydrophobic patch at the dimer interface in each monomer is formed by V12, V117, F120, L165, F166, I167, M170, and F175. F120, F175, and M170 of each subunit are positioned at the dimer interface, each one facing its counterpart in the opposite monomer, building the core of the hydrophobic dimer interaction. The hydrophobic core underlies the metal-binding site, with intra-monomer van der Waals contacts between the metal-coordinating residues C114 and H160, and the hydrophobic residues F166 and L165, respectively. At the same time, the side chain of H160 and the carbonyl oxygen of L17, which lies in the hydrophobic cluster, are bound through hydrogen bonding to a water molecule.

The total buried surface at the inter-subunit interface is approximately 1600 Å². Analytical size-exclusion chromatography in the presence of sodium chloride resolved HsPDF close to the molecular mass of a monomer; however, sedimentation equilibrium of the protein used for crystallization suggested that HsPDF exists predominantly as a dimer in solution, and it is prone to aggregation (data not shown). Whether HsPDF is monomeric or dimeric in its native form in the mitochondria, however, is not determined by these data.

The structure of HsPDF has the conserved PDF fold

Despite the approximately 30% sequence identity between HsPDF and other non-mammalian PDFs (Fig. 3a), such as those of Gram positive and Gram negative bacteria, and plants, the high degree of similarity (average of 50%) of HsPDF to these other PDFs is reflected in the conserved αβ fold displayed by the human PDF. The classification of PDFs has been based primarily on sequence homology, with the PDF family members sharing three signature sequence motifs GXGXAAXQ, EGCLS, and HEXXH.²² These motifs are well conserved in non-mammalian PDFs, but protein sequence alignment of HsPDF with other mammalian PDFs suggests a different consensus in mammals for the motifs CXGX⁵⁷SAPQ, and EG¹¹⁶CES (Fig. 3b).

In HsPDF, an antiparallel β sheet is formed by β strands S1 (G52–S54), S2 (V64–L67), and S3 (R93–V96) (Fig. 3c), while a second antiparallel β sheet is formed by β strands S4 (S99–L103), S7 (A128–L135), and S8 (G139–S147). The two β sheets form an angle, creating a cavity that houses the central α helix H4 (W149–Q162). This α helix is conserved in all PDF members, together with α helix H1 (P32–R48) at the N-terminus. H4 (156–160) contains the key metal-binding and active site residues in the H¹⁵⁶EXDH¹⁶⁰ PDF conserved sequence. The second mammalian conserved primary sequence stretch C⁵⁰XGX⁵⁷SAPQ localizes to the C-terminal portion of the loop that connects α helix H1 and β strand S1, and extends to the 3¹⁰ helix at the C-terminus of this β strand. The third sequence motif conserved among PDFs, E¹¹²G¹¹⁶CES¹¹⁶, can be found in the loop region of the β hairpin formed by strands S5 (107–112) and S6 (122–127), at the C-terminus of β strand S5. Two short α helices, H2 (E71–E76)

and H3 (P79–R85), are also present between β strands S2 and S3, instead of the loop that has been referred to as the CD loop in bacterial PDFs.²³ The H2 and H3 helical loop creates a lid over the entrance to the HsPDF active site. A shorter topologically equivalent loop in the Gram negative bacteria *Leptospira interrogans* PDF, a type 1B PDF, has been shown to adopt “open” and “closed” conformations.²⁴ All monomers in the asymmetric unit of our structural model appear in the open conformation.

Metal coordination and active site in HsPDF

The geometry of the metal in HsPDF is close to tetrahedral. Co^{2+} is kept at the active site by coordination to the side chain N atoms of H156 and H160, the side chain sulfur atom of C114, and a fourth unexpected ligand (Fig. 4a). Other non-mammalian PDF structures have shown a water molecule as the fourth metal ligand.²⁵ Interestingly, however, electron density revealed the presence of two tetrahedral molecules at the active site of HsPDF (Fig. 4b), one of which replaces the water molecule as the fourth metal coordinating molecule at the active site (Fig. 4c). These molecules were modeled as inorganic phosphate, as the crystallization buffer contains this ion. These phosphate molecules were not observed in the actinonin-bound molecule as the inhibitor molecule displaced them. Average *B*-factors for the phosphate closest to the cobalt metal (PO_4^{3-} 1) and that directly above this phosphate (PO_4^{3-} 2), are 15 and 27, respectively, suggesting that both presumed phosphates coexist in an HsPDF protein molecule.

The topology of the metal and coordinating atoms and the metal in HsPDF is comparable between HsPDF and non-mammalian PDFs. However, the position of the oxygen in the phosphate 1 molecule that coordinates the metal (O2) is different from that occupied by the water molecule in other PDFs. O2 in the phosphate is 113.62° relative to the C114 side chain in HsPDF; however, the angle between the equivalent cysteine residue in other PDFs and the water molecule is significantly larger (Table 3). As a result, the metal coordination in HsPDF is closer to tetrahedral than in non-mammalian PDFs.

Differences between HsPDF and non-mammalian PDFs

The hairpin between S5 and S6 in HsPDF is held in place by three anchoring points, through van der Waals and hydrogen bonding interactions (Fig. 5a): at its N-terminal base by contacting residues on H3 and H4 (in cyan), at its tip by contacting residues on H1, the N-terminus, and at the dimer interface (in green) and at its C-terminal side by contacting residues at the C-terminus of HsPDF (in magenta). H3/H4 hairpin interactions involve Q81 N^ε-R85 side chain N, and E112 O^ε-R152 side chain N hydrogen bonds, and L84 C^γ-F110 C^ε and R152-W149 side chain van der Waals contacts. H1/N-terminus–hairpin loop interactions occur through Q11 N^ε-E115 CO, R48 side chain N-E115 CO, and V12 amine N-S116 CO hydrogen bonds. The van der Waals contacts between C114 C^β and V117 C^γ, at the hairpin loop, with F166C^ε and L165 C^δ, at the dimer interface, also anchor the top of the hairpin. The C-terminal side of the hairpin is anchored to the C-terminus of HsPDF through V178 amine N-G119 CO, and T176 CO-L121 amine N hydrogen bonds and van der Waals interaction between N177 C^α and F120 C^δ.

The overall fold in HsPDF, a type 1A PDF, resembles that of other non-mammalian PDFs (Fig. 5b–d). *Escherichia coli* PDF (EcPDF) is representative of type 1B PDFs,^{25–27} *Staphylococcus aureus* PDF (SaPDF) is a representative type 2 PDFs,^{23,28–30} while *Arabidopsis thaliana* PDF (AtPDF) is a representative of the non-mammalian mitochondrial type 1A PDFs.³¹ Nevertheless, the absence of particular anchoring points, or the presence of additional tertiary structures in other PDFs, confers a particular shape to the HsPDF active site entrance.

The N-terminal base and tip of the hairpin in EcPDF are anchored by interactions with H4, and H1 and the N-terminus, respectively, similar to HsPDF. However, unlike HsPDF, EcPDF lacks

the loop containing α helices H2 and H3, and has a C-terminal α helix, which contains residues that substitute the HsPDF dimer interface-hairpin anchoring points. The absence of H2 and H3 results in a wider atrium of the active site in EcPDF than in HsPDF. Nevertheless, the inner entrance into the active site of HsPDF is more accessible to substrate than that of EcPDF (Fig. 5e and f; Supplementary Data Fig. 1) due to the side chains of residues E87, E95, and R97 (EcPDF numbering), which occlude the EcPDF active site entrance. These residues face the active site cavity opening, and are replaced by P111, G119, and L121, respectively, in HsPDF. P111 is located on β strand S4, on the metal-binding hairpin loop. The other two residues, also found on the loop, localize to the C-terminal side hairpin region that contacts the main chain atoms of the C-terminus.

Type II PDFs such as SaPDF share residues topologically equivalent to those in HsPDF that anchor the β hairpin at its crest to the enzyme's N-terminus and at its base to the central PDF α helix, H4 in HsPDF (Fig. 5c). However, the conformation of the C-terminus in SaPDF creates additional interactions between the C-terminus and the base of the loop. This last feature, in addition to the lack of the H2/H3 helical loop, results in a much wider, an unshielded open cavity, active site entrance in SaPDF compared to that of HsPDF (Fig. 5g).

HsPDF resembles the plant mitochondrial PDF AtPDF, in that both share the hairpin loop anchoring points described above. However, the HsPDF C-terminus is shorter and differs in topology from that of AtPDF (Fig. 5d). The C-terminus of HsPDF is perpendicular to β strand 5. In AtPDF, the C-terminus loops back towards the β strand equivalent to S5 in HsPDF, creating additional hydrogen bonding and van der Waals interactions. As a result, the entrance to the AtPDF active site appears wider than that in HsPDF (Fig. 5h).

Identification of the HsPDF substrate-binding site in the actinonin-bound HsPDF structure model and its comparison to other actinonin-bound PDF structures

The formyl-methionine tripeptide mimetic structure of actinonin (Fig. 6a), a potent natural product antibiotic, enables avid binding to PDFs and leads to inhibition of their activity through chelation and disruption of the metal-dependent catalysis. In the absence of a non-hydrolyzable substrate of HsPDF, we solved the structure of HsPDF in complex with actinonin. The topology of the main chain backbone of HsPDF does not change upon actinonin binding, except for minor shifts in H3 and H4.

In the presence of actinonin, the metal is coordinated by the same residues as in the native HsPDF structure; however, there are two additional ligands, the oxygen atoms in the hydroxamic acid moiety of actinonin (Fig. 6b). The spatial arrangement of the metal-coordinating residues does not change upon actinonin binding; however, small shifts occur in the main chain orientation of residues in R⁷⁵ECPPRQRALRQ⁸⁶, in the H2/H3 α helical region. The van der Waals interaction of the proline-like moiety of actinonin with W149 results in an induced fit where α helix 4 is pushed and rotated by approximately 5°, W149 causes a similar effect on H3. A noteworthy change is that of the I153 side chain, which is pushed away from its position in the native structure to allow for the hydrophobic side chain in actinonin to position itself.

Actinonin fits in the HsPDF active site in a linear conformation, with its backbone kept in place through hydrogen bonds with five hydrogen donors/acceptors in the main chain of HsPDF (HsPDF regions denoted A–E in Fig. 6c) and hydrophobic interactions (denoted by regions F–H on Fig. 6c). Comparison of actinonin-bound HsPDF to a number of representative type 1B, and type 2 PDFs for which an actinonin-bound structure has been elucidated,^{23,24,32,33} shows a similar conformation for actinonin, except for that in *S. aureus*. Similarly, most of the hydrogen bond interactions are conserved across PDFs (Supplementary Data Table 1).

The N atom in the hydroxamic acid moiety of actinonin, N9, hydrogen bonds to the side chain of G52. The main chain N atom of V51 also hydrogen bonds to the carbonyl oxygen, O12, in actinonin. G113 carbonyl forms a hydrogen bond to the actinonin amide nitrogen, N11, and the main chain nitrogen of the same residue hydrogen bonds to the carbonyl oxygen, O20, of the valine residue in actinonin.

The hydrophobic interaction regions between HsPDF and actinonin denoted F, G and H in Fig. 6c, constitute the formyl-Met-peptide substrate side chain binding sites in HsPDF, namely the S1', S2', and S3' pockets, respectively, according to the standard protease nomenclature.^{34,35} S1', S2', and S3' accommodate the P1', P2' and P3' side chains of the first, second, and third amino acids from the N-terminus, respectively, in a formyl-Met-peptide substrate. Although referred to as pockets, the only true pocket in HsPDF is S1'.

The aliphatic pentyl chain of actinonin, analogous to the P1' Met side chain in a substrate, fits in the hydrophobic S1' pocket formed by W149, R152, I153, and H156 (region F in Fig. 6c). The valine side chain in actinonin (C16, C17 and C27) fills a position equivalent to P2' in a formyl-peptide. This valine side chain interacts through van der Waals contacts with the carbonyl and α carbons of G113 (region E in Fig. 6c). Although the only contact with actinonin occurs at G113, G113 is part of a depression delineated by residues G¹¹³CE¹¹⁵, which floor and ceiling are made by V117, G119, and the backbone of F120. The proline-like ring in actinonin (N19, C21–25, O25), analogous to a substrate's P3' side chain, contacts W149 and E112 through van der Waals (region H in Fig. 6c). Despite such interactions of HsPDF with P3', there is hardly a defined S3' pocket in HsPDF. Similar to S2', however, there is a hydrophobic depression in the actinonin P3' vicinity made by residues V51, L69, C77, Q81, M87, and F90.

Comparison of actinonin-bound HsPDF to other non-mammalian actinonin-bound PDFs (Supplementary Data Table 1) shows similar interactions at S1' and S2' with actinonin across type 1B and type 2 PDFs. S3'–actinonin interactions display the most variability across the different PDFs, with interactions being present only in HsPDF and the type 2 PDF of *Bacillus stearothermophilus*, and a single-atom hydrophobic interaction in the type 1 structure of *L. interrogans*. Despite the conserved presence of the S1' pocket, this cleft is narrower in HsPDF than in the bacterial PDFs due to the presence of W149 and R152. A comparison of HsPDF and EcPDF S1' is shown (Fig. 7a). It is not surprising that S1' is the only well defined substrate-binding pocket in HsPDF, given that HsPDF must recognize a formylated methionine-peptide substrate. The hydrophobic depression surrounding S2', but not that in the vicinity of S3', in HsPDF is also conserved across bacterial PDFs. P3' in bacterial PDFs is mostly solvent exposed, without an equivalent of the S3'-like depression in HsPDF.

Comparison of HsPDF with AtPDF in complex with the catalysis reaction product MAS³¹ (Protein Data Bank ID 1ZY1), highlights differences in the S3' pocket, between these two eukaryotic PDFs. S3' has been defined in AtPDF by residues P46, V48, D66, and Y70 (AtPDF numbering). The lack of sequence conservation between these two PDFs at the positions above, specifically the presence of R49 in HsPDF instead of P46, and L73 rather than Y70 in AtPDF, make this area in HsPDF less concave (Fig. 7b).

Toward understanding HsPDF substrate specificity

The structure of actinonin-bound HsPDF shows that there is no S2' pocket in HsPDF to indicate that substrate specificity may stem from selectivity at the second position from the N-terminus, or P2' of a formylated peptide substrate (Fig. 8a and b). However, the fMLKL peptide, with a P2' position residue equivalent to the valine residue in actinonin, is a poor substrate of HsPDF *in vitro* (Table 1). Because modeling of MTHQ and MFAD peptides binding to HsPDF shows that substrate binding selectivity could stem from the residue at the third or P3' position (Fig.

8c and d) we asked whether the amino acid residue at the third position was relevant for substrate specificity.

We tested a series of peptides with the sequence formyl-Met-Leu-X, where X was F, P, R, D, or E. At concentrations where the first two peptides were soluble in aqueous media, ~12 mM and lower, little activity was observed, whereas no deformylase activity was detected for the other peptides at up to 24 mM except for fMLR ($V_{\max} = 0.0233 \pm 0.00919 \mu\text{mol min}^{-1} \text{mg}^{-1}$; $K_m = 34.4 \pm 20.4 \text{ mM}$; $N = 3$). Contrary to what we expected on the basis of the actinonin-bound-HsPDF structure model, these data suggested that the presence of Leu at P2' does not favor catalysis, and that perhaps the residue following formyl-Met in a substrate affects substrate turnover.

Therefore, to determine if the identity of the second amino acid residue in a formyl-Met substrate dictates substrate processing by HsPDF, we chose to modify the putative substrate fMTHQ (Table 1) because it is one of the better substrates of HsPDF, and it is readily soluble in aqueous solutions. We tested the series fMXHQ, where X = G, V, L, E, R, or F (Table 4). All peptides, except for fMEHQ, were processed by HsPDF with comparable efficiencies, denoted k_{cat}/K_m . Although peptides with residues of longer aliphatic chains on the second position of the substrate, such as V and L, appear to favor binding, K_m values for the peptides tested were not significantly different, as determined by a one-way analysis of variance and the Bonferroni test for comparison of multiple means. The efficient catalysis of the fMLHQ substrate suggests that the identity of the second residue in a substrate alone does not dictate catalysis by HsPDF but rather the combination of residues at the second and third positions might be more important.

Discussion

We present the first X-ray crystal structure model of a mammalian PDF, HsPDF, and direct evidence that HsPDF can deformylate the putative substrates encoded by the human mitochondrial DNA. We compared the structure of HsPDF to those of other non-mammalian PDFs, and identified differences in their tertiary structure arrangements that in turn result in differences at the active site entrance. We describe the structure of HsPDF alone, and in combination with the antibiotic actinonin. The substrate binding site was identified in the actinonin-bound HsPDF structure, and compared to bacterial and plant PDFs. HsPDF has the conserved S1' pocket observed in other PDFs, and though lacking true S2' and S3' pockets, HsPDF has an S3'-like hydrophobic cavity not present in bacterial PDFs. We show also that, despite the lack of an S2' pocket, the combined identity of the second and third amino acids from the N-terminus in a formylated peptide substrate may influence its turnover by HsPDF.

Our kinetic studies show that the binding affinity of HsPDF substrates is similar to that of EcPDF substrates, in the low millimolar range. However the catalytic efficiency, k_{cat}/K_m , of HsPDF substrates is orders of magnitude lower than that measured for most EcPDF substrates, perhaps because so few substrates are present in mammalian cells. It has been shown that residues localized to the PDF family conserved motifs, which differ in HsPDF, contribute to the slow substrate turnover of HsPDF. The combined C50G and E115L mutations in HsPDF ⁵⁰CVGLSAPQ and EGC¹¹⁵ES motifs, increase HsPDF catalytic efficiency 100-fold,² which is closer in magnitude to the deformylation efficiency of EcPDF. Our HsPDF structural model suggests that S54 van der Waals interaction with Q157, and E115 hydrogen bonding to R48 and van der Waals interaction with C50, might impose energetic restrictions on the movement of S54 and E115, which participate in catalysis,²⁵ thus resulting in a higher energetic barrier to catalysis. It should be considered also that other factors, such as the mitochondrial environment, and HsPDF interacting proteins might influence the contribution of these residues to catalysis under physiologic conditions, and that rates *in situ* may be far higher.

HsPDF crystallized as a dimer and sedimentation equilibrium data from the protein in solution showed a dimer as well. While gel-filtration chromatography during the purification of HsPDF showed an apparent molecular mass of approximately 22,000 Da, this is not an accurate method of size determination because it is dependent on size calibrations, the ionic strength and pH of the buffers, and protein hydrophobicity, which may promote protein interactions with the gel support.^{36,37} Two other PDF structures have been described as dimers from crystallography studies, *L. interrogans*³⁸ and *A. thaliana*.³¹ Residues at the positions that mediate the dimerization in HsPDF are highly conserved, with 44% and 55% of them being identical in *L. interrogans* and *A. thaliana*, respectively. The high degree of conservation of the residues at the interface of the HsPDF homodimer together with the extent of the interaction area between the monomers suggests that the observed dimerization of HsPDF is not a result of crystal packing.

Some residues at the hydrophobic dimer interface of HsPDF appear to serve a structural support function. Examples are those residues involved in the anchoring of the β hairpin loop that contains the metal-coordinating residue C114. Other examples are two residues at the dimer interface (L165 and F166) that might help maintain the geometry of the metal coordination site, judging by the van der Waals contacts between them and two of the metal-coordinating residues (H160 and C114, respectively). Examination of the EcPDF structure showed that, despite this enzyme being a monomer, F142 and L141 in EcPDF occupy the same structural positions as F166 and L165 in HsPDF, respectively, and interact through van der Waals contacts with the HsPDF equivalent metal-binding residues. The biological significance of the HsPDF dimerization as a whole, however, is not known.

HsPDF shares the common fold observed in other PDFs, including the β hairpin loop anchoring points that shape the entrance to the active site in the PDF family. The conformation of the C-terminus in HsPDF, in combination with the presence of the H2/H3 α helical loop creates a characteristic entrance to its active site compared to non-mammalian PDFs. The only bacterial PDF found to be a dimer, that of *L. interrogans*, shares C-terminal topology with HsPDF; however, the presence of a short CD loop, creates an active site entrance different from that of HsPDF. Conversely, despite the structural similarity between HsPDF and AtPDF, differences in the topology of their C-terminus results in different active site entrances.

HsPDF lacks the C-terminal α helix observed in EcPDF, which has been shown to mediate binding of EcPDF to the ribosome with contacts with ribosomal proteins L17, L22, and L32.³⁹ Therefore, the mode of EcPDF-ribosome interaction cannot be generalized to HsPDF and the mitoribosome. Whereas *Homo sapiens* mitochondrial ribosomal proteins L17 and L22 are $\geq 47\%$ similar to those of *E. coli*, the sequence homology of the human counterpart of *E. coli* L32 is low ($\sim 21\%$).⁴⁰ Furthermore, studies of the mitoribosome structure suggest two possible peptide exits, thus also two potential modes of interaction with HsPDF, if such interaction occurs.⁴¹ Our model of HsPDF, however, does not provide evidence for a mode of interaction with the mitoribosome.

Despite the presence of two tetrahedral molecules at the active site, one of which coordinates to Co^{2+} instead of the expected water molecule, the presence and topology of the amino acid residues involved in the reaction mechanism and metal coordination are conserved; this suggests that the mechanism of catalysis of HsPDF is also conserved and that HsPDF acts on formylated substrates, as has been demonstrated by the *in vitro* deformylase activity of HsPDF. Since HsPDF was confirmed to be catalytically active up to its crystallization, the presence of the catalytic water molecules described previously, when the protein is in solution, cannot be excluded.

While the HsPDF backbone is similar to that of PDFs of other organisms, our preliminary kinetic studies with HsPDF suggest that its substrate specificity might differ from the broad specificity of EcPDF.²¹ The actinonin-bound HsPDF structural model suggested that P3', but not P2', of a formylated peptide might have a role in determining substrate specificity. However, the fact that fMLHQ and fMVHQ, but not fMLKL or fMLX peptides, are processed by HsPDF shows that both residues at P2' and P3' may have a role in substrate specificity. Despite HsPDF lacking true S2' and S3' substrate-binding pockets, HsPDF residues surrounding the P2' and P3' positions in a putative substrate, such as R48, M87, R85, P111, E115, L121, and W149, which create a hydrophobic depression, could contribute to binding specificity. A comprehensive study of HsPDF structure-substrate specificity will elucidate the role of such residues in substrate binding.

Earlier, we have proposed HsPDF as a potential anti-cancer target.^{7,42} The effects of HsPDF inhibition, mitochondrial membrane depolarization, ATP depletion,⁷ and apoptosis⁴³ suggest that inhibition of HsPDF function affects the energetic balance of the cell. An effective anti-cancer agent targeting HsPDF should optimally not inhibit bacterial PDFs to avoid cytotoxicity to the bacterial flora of humans. Therefore, the contribution of this study towards understanding the structural basis for inhibition of HsPDF in a species-specific way will enable further drug development for cancer treatment as well as aid in the design of more specific antibacterials. The search for guidelines to minimize potential antimicrobial side effects has prompted analyses of putative models of HsPDF structure.^{2,31} Here, we provide the structure of a true representative of mammalian PDFs, as the high level of amino acid sequence identity between HsPDF and the predicted PDF for other mammals (70% to *Mus musculus*, 77% to *Bos taurus*, 79% to *Canis familiaris*, and 73% to *Rattus norvegicus*), suggests that other mammalian PDFs may closely resemble HsPDF.

Structural features that are characteristic of HsPDF can be further exploited for inhibitor design. The narrower S1' pocket in HsPDF and in the plant counterpart, compared to EcPDF, for example, has been exploited for the design of bacterial PDF inhibitors.⁴⁴ Other features in HsPDF, such as the hydrophobic depression in the vicinity of the P3' of a substrate (residues V51, L69, C77, Q81, M87, and F90), could be taken advantage of for the structure-based design of inhibitors. Similarly, the characteristic shape of the active site opening of HsPDF could enable HsPDF-inhibitor interactions that otherwise would not occur with PDFs from other organisms that lack a similar C-terminal conformation and α helices 2 and 3.

Materials and Methods

Cloning, expression, and purification of HsPDF

A truncated HsPDF lacking the first 63 amino acids, which correspond to the mitochondrial targeting sequence, was cloned, and expressed.⁶ As reported earlier, this truncated PDF circumvents the low yields of production in a bacterial host while retaining the key residues for PDF activity.⁵ HsPDF was cloned by PCR from a previous cDNA HsPDF isolate⁶ using adaptor forward and reverse primers to include the NdeI and BamHI restriction sites, respectively. The primer sequences are;

5'GGAATTCCATATGTCATTCTCGCACGTGTGCCAAGTCGGG3'

5'CGCGGATCCTTAGTCATTACCTTCATCCAATAGACGTT3'

(Genelink, Hawthorne NY). The NdeI/BamHI (New England Biolabs, Ipswich, MA) digested PCR fragment was cloned into the expression vector pET-15b (Novagen, San Diego CA), resulting in an N-terminal His₆-tagged truncated HsPDF, with a thrombin cleavage signal sequence plus the insertion of an additional five amino acid, GSHMS, between the tag and the HsPDF sequence. His₆-truncated HsPDF was expressed in BL-21 (DE3) pLys (Invitrogen,

Carlsbad CA). Briefly, transformed bacteria were grown overnight at 37 °C in Lennox LB broth (Fisher Scientific, Fair Lawn NJ) in the presence of 200 µg/mL ampicillin (Sigma, St Louis MO) and 34 µg/mL chloramphenicol (Fisher Scientific, Fair Lawn NJ) and diluted 1:50 (v/v) in fresh medium with the same antibiotic concentrations at 37 °C until A₆₀₀ reached 0.4–0.8. Protein expression was induced with 0.4 mM IPTG (Fisher Scientific, Fair Lawn NJ) and in the presence of 100 µM CoCl₂ for 3 h at 37 °C. Cells were collected by centrifugation at 4690g in a Sorvall SLC-4000 rotor (Sorvall, Asheville, NC) for 30 min at 4 °C. The cell pellet was resuspended in buffer A (20 mM Hepes, 300 mM NaCl, 5% (v/v) glycerol, 20 mM imidazole, pH 7.4) and lysed by sonication. The cell lysate was cleared by centrifugation at 14,000g for 20 min at 4 °C, and the supernatant loaded onto a Ni²⁺ Sepharose high-performance column (Amersham Biosciences, Piscataway NJ). Protein elution was performed with an imidazole gradient using buffer A and buffer B (20 mM Hepes, 300 mM NaCl, 5% glycerol, 500 mM imidazole pH 7.4). HsPDF elution was assessed by measuring the absorbance at 280 nm combined with measurements of HsPDF activity. HsPDF activity was assessed using a fluorescamine-based assay developed in our laboratory.⁴⁵ Fractions containing HsPDF activity were pooled and digested with eight units of thrombin/mg of protein (Amersham Biosciences, Piscataway NJ) overnight at 16 °C. Thrombin was removed by incubation of the digested protein solution with 100 µL of 50% *p*-aminobenzamidase agarose (Sigma, St. Louis MO) for 3 h at 4 °C. Digested truncated HsPDF was precipitated over a range of increasing ammonium sulfate percentages. The protein pellets obtained at each percentage of ammonium sulfate saturation were resuspended in buffer A, and the fraction containing HsPDF determined from the presence of HsPDF activity measured by the formate-dehydrogenase (FDH) assay as described.⁴⁶ The ammonium sulfate in the fraction containing HsPDF was reduced by exchanging the buffer to 20 mM Mes, 20 mM NaCl pH 6.2 using a Superose 12 gel-filtration column (Amersham Biosciences, Piscataway NJ). PDF activity was measured in the single A_{280 nm} peak eluted from the gel-filtration column. The purified HsPDF was stored in 5 mM Tris(2-carboxyethyl)-phosphine (TCEP). The identity of the protein purified was further confirmed by N-terminal Edman degradation of the first nine amino acid residues at the Microchemistry and Proteomics core facility at Sloan-Kettering Institute. The metal at the active site of the pure HsPDF was confirmed as Co²⁺ from the strong anomalous signal in an X-ray fluorescence scan spectrum.

Crystallization of HsPDF

Crystallization conditions were screened by the hanging-drop method using Hampton Research Crystal Screen, and Crystal Screen 2 (Hampton Research, Aliso Viejo, CA). The hanging drop was a 1:2 (v/v) mixture of HsPDF/mother liquor in a total volume of 3 µL. Reproducible crystals grew in 0.1 M sodium citrate tribasic dihydrate pH 5.6, 1.0 M ammonium phosphate monobasic. To obtain the HsPDF structures in complex with actinonin, an inhibitor stock in dimethylsulfoxide (Sigma, St Louis MO) was diluted to 0.5 mM in 23% (v/v) glycerol in mother liquor, where the crystals were soaked overnight. Crystals were cryoprotected with 25% glycerol in mother liquor upon data collection. Actinonin was synthesized by the Organic Synthesis core facility at Sloan Kettering Institute.

Data collection and structure determination

Crystals belong to space group C2 and diffract to 1.7 Å. Diffraction data were collected at 100 K at the Advanced Photon Source beamline ID24 (Argonne National Laboratories, Argonne, IL). Data were processed with HKL2000.⁴⁷ The structure was solved by molecular replacement using program AMORE.⁴⁸ The search model was built using SWISS-MODEL⁴⁹ with the structure of *A. thaliana* PDF (PDB ID 1ZXZ) as a template. The molecular replacement solution was refined with CNS⁵⁰ and manual fitting was performed with O.⁵¹ The final round of refinement was performed with Refmac⁵² utilizing the TLS and restrained refinement

protocol. Data collection and model refinement statistics are summarized in Table 2. All figures were generated with the program PyMOL†.

HsPDF substrate kinetics

HsPDF activity was measured with a deformylaseformate dehydrogenase coupled assay.⁴⁶ Briefly, a deformylation reaction was started by the addition of His₆-truncated HsPDF, at a final concentration of 10 μ M, to a mixture of substrate, at various concentrations, NAD⁺, and formate dehydrogenase at 4.8 mM and 2U/mL final concentration, respectively, in 50 mM Hepes, 10 mM NaCl, 100 μ M CoCl₂, pH 7.5. All peptide substrates were purchased from Genemed Synthesis (Genemed Synthesis, San Antonio TX). Absorbance readings were taken over 30 min at 340 nm using a SpectraMax M2 spectrophotometer (Molecular Devices, Sunnyvale CA). Time-course absorbance readings were converted into μ mol NADH using the reaction volume and the constant ϵ' (mM⁻¹), which represents the slope of a NADH calibration curve where $A_{340}=\epsilon'$ [NADH] and that was measured in a volume equal to that of the deformylation reaction. Reaction rates at various concentrations of substrate were calculated from linear curve fits of the μ mol NADH *versus* time data. K_m and V_{max} were determined from the slopes at various concentrations of substrate by applying a non-linear curve fit. All analyses were performed using GraphPad Prism version 4.0 for Macintosh (GraphPad Software, San Diego CA).

Protein Data Bank accession numbers

HsPDF structure PDB ID 3G5P. Actinonin-bound HsPDF structure PDB ID 3G5K.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

PDF, peptide deformylase protein; HsPDF, human PDF; EcPDF, *Escherichia coli* PDF; SaPDF, *Staphylococcus aureus* PDF; AtPDF, *Arabidopsis thaliana* PDF.

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Fig. 1. *N*-Met-formylated peptides derived from the N-terminus of mitochondrial DNA-encoded proteins are substrates of HsPDF *in vitro*. Apparent K_m and k_{cat} were measured by a formate dehydrogenase, FDH, NAD^+ -coupled assay (see Materials and Methods). A representative curve for the processing of three substrates is shown. Values represent the mean rate of reaction (in $\mu\text{mol NADH min}^{-1} \text{mg}^{-1}$ of HsPDF) $\pm$ S.E.M. for triplicate samples for each concentration of substrate. (a) HsPDF kinetics with formyl-Met-Ala-His-Ala, fMAHA. (b) HsPDF kinetics with formyl-Met-Asn-Phe-Ala, fMNFA. (c) HsPDF kinetics with formyl-Met-Met-Tyr-Ala, fMYA.

Fig. 2.

The HsPDF asymmetric unit is constituted by two HsPDF dimers. (a) Stereo ribbon view of the HsPDF dimer. Each monomer is shown in a different color, with the N-terminus and C-terminus indicated as N-term, and C-term, respectively. The Co^{2+} atom is a purple sphere. (b) The dimer interface is formed through electrostatic and hydrophobic interactions between the HsPDF monomers. Stereo view, the carbon backbone of each of the monomers is in a different color, green or cyan, with nitrogen atoms in blue, oxygen atoms in red, and sulfur atoms in yellow. Salt bridges are indicated by gray dotted lines. (c) A 2D depiction of the dimer interface in (b). Residues in blue and black correspond to each of the interacting HsPDF monomers.

Residues in gray correspond to the monomer in black, and are located in a different plane, at the core of the dimer interface.

Fig. 3.

HsPDF has the conserved PDF fold observed in other non-mammalian PDFs. (a) Protein sequence alignment of HsPDF to non-mammalian PDFs: *Escherichia coli* PDF, (EcPDF, Genbank protein ID gi15803814), *Staphylococcus aureus* PDF (SaPDF, Genbank protein ID gi22219286), and *Arabidopsis thaliana* PDF (AtPDF, Genbank protein ID gi11320952). HsPDF and AtPDF sequences both belong to N-terminal truncated PDFs. Alignment is colored according to the percentage identity of residues among the sequences. Dark blue, >80%; blue, >60%; light blue, >40%; and no color, <40%. Alignment was generated with ClustalW⁵³ and Jalview.⁵⁴ (b) Protein sequence alignment of HsPDF to predicted mammalian PDFs. PDF of *Bos taurus* (BtPDF Genbank ID XP_001255524.1), *Canis familiaris* (CfPDF Genbank ID

XP_853641.1), *Rattus norvegicus* (RnPDF Genbank ID XP_001073696.1), and *Mus musculus* (MmPDF Genbank ID AK075957.1). Sequence alignment was done as described for a. (c) Stereo view of HsPDF monomer. Secondary structure elements are numbered. H, α Helix; S, β strand. The Co^{2+} atom is a purple sphere. Regions of HsPDF with structural similarity to non-mammalian PDFs are shown in gray.

Fig. 4.

The active site metal, Co^{2+} , is coordinated to four ligands, three of them residues of HsPDF and a fourth unexpected ligand. (a) The geometry of the metal in HsPDF is close to tetrahedral. Bond angles between the active site Co^{2+} and the residues in HsPDF that coordinate it are shown. A representation similar to that reported for EcPDF⁵⁵ is shown for ease of comparison. (b) The fourth metal coordinating ligand in the HsPDF structure model is a phosphate molecule. Electron density of the HsPDF active site is shown in stereo view. $F_o - F_c$ is contoured at 5σ in yellow. The four ligands of the Co^{2+} atom, in purple, are H_{156} , H_{160} , C_{114} , and a fourth molecule modeled as phosphate, in orange. The distances of the presumed oxygen atoms in the phosphate molecule to neighboring atoms are shown by dotted lines. (c) Diagram depiction

of the metal coordination site in HsPDF, including surrounding residues at the active site. Carbon, oxygen, nitrogen, sulfur and phosphorus atoms are shown as black, red, blue, yellow and green spheres, respectively. Distances (in Å) between atoms are depicted as broken green lines and labels. Atom labels are in black. The diagram was generated with LigPlot v.4.4.2.⁵⁶

Fig. 5.

Differences between HsPDF and non-mammalian PDFs. HsPDF shares the β hairpin loop anchoring points that shape the entrance to the active site in the PDF family. However the conformation of the C-terminus in HsPDF, in combination with the presence of the H2/H3 α -helical loop creates a characteristic entrance to its active site compared to non-mammalian PDFs. The HsPDF Co^{2+} metal is represented as a purple/pink sphere in all panels. Structural alignments in b–d were done with PyMol. Residues included in the C^α RMSD calculations in these panels are shown as gray regions in HsPDF in Fig. 2. RMSD values were obtained with the program O.⁵¹ Structures in b–h are shown in the same orientation. (a) Stereo representation of the HsPDF dimer, highlighting the S5/S6 hairpin anchoring points. Hairpin anchoring at its N-terminal base by residues on H3 and H4 are shown in cyan, anchoring at the crest of the hairpin by residues on H1, the N-terminus, and at the dimer interface are shown in green, and anchoring at the C-terminal side of the hairpin by residues at the C-terminus of HsPDF are shown in magenta. (b) HsPDF, red in b–d, aligned to type 1B EcPDF (PDB entry 1BS7) green. ⁵⁵RMSD is 1.252 Å for the C^α atom alignment of the HsPDF/EcPDF pair. (c) HsPDF alignment to type 2 SaPDF (PDB entry 1LQW),²³ blue. RMSD is 1.314 Å for the C^α atom alignment of the HsPDF/SaPDF pair. (d) HsPDF alignment to type 1A AtPDF monomer (PDB entry 1ZXZ),³¹ cyan. The RMSD is 0.917 Å for the C^α atom alignment of the HsPDF/AtPDF pair. (e) HsPDF active site entrance. (e–h) Surface views of PDF from various organisms in gray, with oxygen atoms in red, nitrogen atoms in blue and sulfur atoms in yellow. The PDF structures correspond to the same PDB entries as those in b and c. (f) EcPDF, a PDF type 1B, active site entrance.

(g) SaPDF, a type II PDF, active site entrance. (h) AtPDF, a representative plant type 1A PDF, active site entrance.

Fig. 6. Identification of the HsPDF substrate-binding site in the actinonin-bound HsPDF structure model. (a) Structure of the peptidomimetic inhibitor actinonin with assigned atom numbers. (b) Stereo image of the active site of HsPDF with actinonin. HsPDF residues, in gray, contacting actinonin, yellow, are shown as sticks. Hydrogen bonding is represented by black dotted lines with interatomic distances also shown. (c) Ball and stick 2D diagram of actinonin interactions at the HsPDF active site. Hydrogen bonds at six points of actinonin with corresponding donors/acceptors in HsPDF are denoted as areas A–E, in light blue. Distances (in Å) between the hydrogen bonding atoms are shown in green and denoted by broken green lines. Regions of hydrophobic interaction between actinonin and HsPDF are shown as light blue regions F–H. Hydrophobic interactions of actinonin with HsPDF are shown as red radiating lines surrounding the atoms, with their respective partner in HsPDF also indicated

by name and red radiating lines. Interactions at the A–H regions in non-mammalian PDFs are described in detail in Supplementary Data Table 1.

Fig. 7.

The substrate-binding pockets of HsPDF differ from those of EcPDF and AtPDF. (a) The S1' binding pocket in HsPDF is narrower than that of EcPDF. The upper stereo pair shows the HsPDF S1' pocket with the protein residues in gray, and the actinonin molecule in yellow. The bottom stereo pair shows the same pocket for EcPDF (1G2A),³² also in gray. (b) HsPDF has no well-defined S3' pocket. AtPDF has an S3' pocket formed by the residues shown in the bottom stereo pair, where the side chain of the product MAS, yellow, fits, (PDB 1ZY1). However, the equivalent depression in HsPDF is less concave, upper stereo pair. The MAS product was modeled from that of AtPDF (PDB 1ZY1).

Fig. 8.

There is no S2' binding pocket in HsPDF. Actinonin-bound HsPDF and virtual peptide-binding modeling, all shown in the same orientation, indicate lack of an S2' binding pocket. Despite the lack of an S3' substrate-binding pocket, actinonin-bound HsPDF and the virtual models suggest that residues in HsPDF surrounding P3' of a substrate might influence substrate specificity. (a) Actinonin-bound HsPDF stereo image. Actinonin, yellow sticks, interacts with HsPDF, in gray. Residues in HsPDF surrounding P2' of actinonin are shown as sticks. Distances (in Å) between the valine side chain in actinonin and the closest residues in HsPDF, G113 and R48, are shown as dotted pink lines. (b) Actinonin-bound HsPDF surface view in stereo mode. The image represents the same structure and orientation as in a, but in surface view. (c) Met-

Thr-His-Gln, MTHQ, bound HsPDF virtual model. MTHQ, in cyan, was modeled into HsPDF based on the actinonin-bound HsPDF structural model using the program CNS.⁵⁰ (d) Met-Phe-Ala-Asp, MFAD, bound HsPDF virtual model. MFAD, in green, was modeled into HsPDF based on the actinonin-bound HsPDF structural model. Modeling was done as in c.

Table 1

Kinetics of HsPDF with formylated mitochondrial peptide substrates

Mitochondrial-encoded protein	Peptide substrate	$V_{\max}$ ($\mu\text{mol NADH min}^{-1} \text{mg}^{-1}$)	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($\text{s}^{-1} \text{M}^{-1}$)
Cytochrome <i>c</i> oxidase subunit I	fMFAD	0.1508±0.0134	1.3510±0.7565	0.0570	42.1648
NADH dehydrogenase subunit 3	fMNFA	0.1329±0.0087	2.4430±0.7388	0.0502	20.5497
Cytochrome <i>c</i> oxidase subunit III	fMTHQ	0.1165±0.0112	2.2870±1.054	0.0440	19.2426
NADH dehydrogenase subunit 5	fMTMH	0.0960±0.00480	2.1350±0.527	0.0362	16.9784
NADH dehydrogenase subunit 6	fMIMYA	0.1709±0.0354	4.0850±2.80	0.0646	15.8035
Cytochrome <i>b</i>	fMTPM	0.0462±0.0018	1.1810±0.3103	0.0174	14.7678
Cytochrome <i>c</i> oxidase subunit II	fMAHA	0.2199±0.0088	8.1400±0.8869	0.0831	10.2048
NADH dehydrogenase subunit 2	fMNPL	0.0559±0.0039	12.4400±1.902	0.0211	1.6974
ATP synthase F0 subunit 8	fMPQL	0.0748±0.0245	24.0900±13.3	0.0283	1.1735
NADH dehydrogenase subunit 4	fMLKL	0.0130±0.0007	5.7600±0.8827	0.0049	0.8506
ATP synthase F0 subunit 6	fMNEN	Low activity	—	—	—
NADH dehydrogenase subunit 1	fMPMA	Poor solubility	—	—	—
NADH dehydrogenase subunit 4L	fMPLI	Poor solubility	—	—	—

Apparent K_m and k_{cat} were measured by a formate dehydrogenase, FDH, NAD^{+} -coupled assay where oxidation of the released formyl group to CO_2 produces NADH (see Materials and Methods). Values represent mean rate of reaction (in $\mu\text{mol NADH min}^{-1} \text{mg}^{-1}$ of HsPDF) and K_m (mM)±S.E.M. for triplicate samples per concentration of substrate.

Table 2

Data collection and model refinement statistics

Data set	HsPDF native	Actinonin bound HsPDF
Wavelength (Å)	0.97922	0.97922
Resolution range (Å)	50–1.7 (1.76–1.70)	50–1.7 (1.76–1.70)
Space group	C2	C2
Cell parameters		
<i>a</i> (Å)	116.329	116.158
<i>b</i> (Å)	77.823	77.884
<i>c</i> (Å)	111.039	110.598
α (deg)	90	90
β (deg)	107.926	107.409
γ (deg)	90	90
Redundancy	5.2 (5.1)	4.6 (4.6)
Completeness	100 (100)	99.9 (100)
<i>R</i> _{sym}	0.061 (0.38)	0.060 (0.31)
<i>R</i> _{rim}	0.053 (0.11)	0.051 (0.073)
<i>R</i> _{pim}	0.023 (0.049)	0.023 (0.033)
<i>I</i> /σ _{<i>I</i>}	38.6 (5.1)	39.8 (5.5)
Molecules/ASU	4	4
Reflections with <i>I</i> >0	99,992 (9317)	100,609 (9235)
Working set	94,945 (8818)	95,548 (8760)
Test set	5047 (489)	5061 (475)
<i>R</i> _{work}	0.165 (0.217)	0.162 (0.200)
<i>R</i> _{free}	0.190 (0.240)	0.188 (0.227)
Number of atoms	6539	6445
Protein	5780	5780
Water	715	553
Ion	44	4
Hetero compound	–	108
Mean <i>B</i> value (Å ²)	19.1	18.9
RMS deviation from ideal		
Bond lengths (Å)	0.029	0.028
Bond angles (deg)	2.182	2.422
ESU based on ML (Å)	0.049	0.054

Numbers in parentheses refer to the highest resolution shell. *R*_{rim} and *R*_{pim} were calculated with the program RMERGE⁵⁷ (see Materials and Methods).

Angles between the metal and the metal coordinating residues in HsPDF compared to non-mammalian PDFs

[illegible]

Non-mammalian PDFs are the same as referenced in Fig. 5. Numbering shown for residues in HsPDF, residues in the equivalent positions were used for angle measurements in non-mammalian PDFs, similarly PO_4^{3-} 1(O2) was replaced by a water molecule in these PDFs.

Table 4

The identity of the second amino acid residue on a formyl-Met substrate does not determine processing by HsPDF

Peptide	$V_{\max}$ ($\mu\text{mol NADH min}^{-1} \text{mg}^{-1}$)	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($\text{s}^{-1} \text{M}^{-1}$)
fMLHQ	0.00728 $\pm$ 0.00139	0.521 $\pm$ 0.150	0.00275 $\pm$ 0.000525	4.45 $\pm$ 2.41
fMVHQ	0.0154 $\pm$ 0.00246	0.738 $\pm$ 0.104	0.00581 $\pm$ 0.000929	4.01 $\pm$ 4.00
fMTHQ	0.0733 $\pm$ 0.0432	1.56 $\pm$ 0.722	0.0277 $\pm$ 0.0163	16.4 $\pm$ 2.86
fMRHQ	0.0534 $\pm$ 0.00224	2.97 $\pm$ 0.523	0.0202 $\pm$ 0.00224	6.80 $\pm$ 1.42
fMFHQ	0.0356 $\pm$ 0.00818	3.01 $\pm$ 0.596	0.0134 $\pm$ 0.00309	4.45 $\pm$ 0.146
fMGHQ	0.0411 $\pm$ 0.00084	5.14 $\pm$ 3.97	0.0155 $\pm$ 0.000317	7.57 $\pm$ 5.91
fMEHQ	0.0248 $\pm$ 0.0115	42.9 $\pm$ 28.7	0.00937 $\pm$ 0.0115	0.218 $\pm$ 0.305

HsPDF enzyme kinetics with fMTHQ-OH derived sequences. Values represent the mean $\pm$ standard error, $N=3$ for at least one independent experiment.