Communication

Structure of Peptide Deformylase and Identification of the Substrate Binding Site*

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Peptide deformylase is an essential metalloenzyme required for the removal of the formyl group at the N terminus of nascent polypeptide chains in eubacteria. The *Escherichia coli* enzyme uses Fe$^{2+}$ and nearly retains its activity on substitution of the metal ion by Ni$^{2+}$. We have solved the structure of the Ni$^{2+}$ enzyme at 1.9-Å resolution by X-ray crystallography. Each of the three monomers in the asymmetric unit contains one Ni$^{2+}$ ion, and, in close proximity, one molecule of polyethylene glycol. Polyethylene glycol is shown to be a competitive inhibitor with a $K_i$ value of 6 mM with respect to formylmethionine under conditions similar to those used for crystallization. We have also solved the structure of the inhibitor-free enzyme at 2.5-Å resolution. The two structures are identical within the estimated errors of the models. The hydrogen bond network stabilizing the active site involves nearly all conserved amino acid residues and well defined water molecules, one of which ligates to the tetrahedrally coordinated Ni$^{2+}$ ion.

In eubacteria as well as in mitochondria and chloroplasts the amino group of methionyl-tRNA$^{Met}$ is N-formylated by a formyltransferase during initiation of protein synthesis (1). Consequently, all nascent polypeptides are synthesized with the formyl group at the N terminus. During elongation of the polypeptide chain the formyl group is removed hydrolytically by the enzyme peptide deformylase (PDF) (2, 3). For *Escherichia coli*, deletion of the formyltransferase gene leads to a strongly reduced cell growth rate (4), whereas deletion of the PDF gene proves lethal (5). This formylation/deformylation cycle, which appears to be a characteristic feature of eubacteria, does not occur in the cytoplasm of eucaryotic cells. Therefore, PDF is an attractive target for the design of new antibiotics.

PDF from *E. coli*, a monomeric protein of 168 residues, shares the fingerprint motifs HXXH (6), EGCLS, and GXXGAAQ (7) with PDF sequences of other eubacteria, which suggests a common architecture of the catalytic region in these proteins. PDF was reported to be a zinc enzyme (8) that contains the motif HEXXX, known to be involved in zinc binding in metalloproteases (9, 10). Meanwhile, it has been shown that PDF utilizes Fe$^{2+}$ as catalytic metal whereas the Zn$^{2+}$ form is nearly inactive (11, 12). Interestingly, Fe$^{2+}$ can be replaced by Ni$^{2+}$ with a slight reduction in PDF activity (11).

Recently, the structure of the core domain (residues 1–147) of PDF was solved by NMR (8) and the structure of the full-length protein by X-ray crystallography at 2.9-Å resolution (13). Both structures describe the protein as isolated with a tightly bound zinc ion. We report here the structure of the catalytically active enzyme in the nickel-bound form (PDF-Ni) at 2.5-Å resolution and at 1.9-Å resolution in complex with a polyethylene glycol molecule (PDF-Ni/PEG), shown here to be a competitive inhibitor of the enzyme.

**EXPERIMENTAL PROCEDURES**

PDF-Ni (specific activity, 900 units/mg; 0.6 Ni$^{2+}$ ions per protein molecule) was isolated from overproducing *E. coli* cells and crystallized with 2 mM (NH$_4$)$_2$SO$_4$ as precipitant in the presence of 1% (w/v) PEG-1000 as described (2). Crystals were washed in 100 mM MOPS/NH$_4$Cl, 1% (w/v) PEG-1000, 2 mM (NH$_4$)$_2$SO$_4$ at pH 7.4. Three isomorphous derivatives were obtained by soaking crystals in the above buffer with added heavy atom compounds. The mercury derivative crystals were harvested after soaking 12 h in 0.1 mM CH$_3$HgCl and 2 mM K$_2$PtCl$_4$, and the double derivative after soaking 24 h in 2 mM K$_2$PtCl$_4$ and the double derivative after soaking 24 h in 0.1 mM CH$_3$HgCl and 2 mM K$_2$PtCl$_4$. PDF-Ni crystals without PEG have been described (2).

Diffraction data were collected at room temperature by rotation/directory and recorded by an electronic area detector (x-rays: CuK$_\alpha$, focused by Franks double-mirror optics; generator: GX-18, Elliot/Enraf-Nonius, Delft, operated at 35 kV/50 mA; detector: X100, Siemens/Nicolet, Madison, WI; crystal to detector distance: 10 cm; rotation/image: 0.0417° or 0.0893°). Integrated intensities were extracted from the rotation images by the program XDS (15), which includes routines for space group determination from the observed diffraction pattern (16).

Inhibition of PDF by PEG-1000 was determined by the following procedure. PDF activity was measured at 30 °C with formyl-Met (100 mM) at pH 7.2 (100 mM MOPS/NaOH, 2 mM Li$_2$SO$_4$, 100 mM TCEP) in a total volume of 50 μl. The reaction was started by 420 ng of PDF-Ni (5 μl) and stopped after 5 min by 4% HClO$_4$ (50 μl). The amount of hydrolyzed substrate was determined according to the method of Fields (17) using 2,4,6-trinitrobenzolsulfonic acid ($K_{aeq}$ = 22 mM $^{-1}$ cm$^{-1}$). For inhibition studies, up to 10 mM PEG-1000, pretreated with TCEP (350 mM PEG/35 mM TCEP, pH 7.2) for 1 h, was included in the assay mixture. V$_{max}$ and apparent $K_{eq}$ values were estimated from double-reciprocal plots ($1/\text{versus } 1/V_{max}$), and the $K_{eq}$ value was calculated from secondary replots of the slopes versus PEG-1000 concentrations.

**RESULTS**

**Structure Determination—**Crystallographic data used for structure determination are summarized in Table I. The structure of PDF-Ni/PEG crystals was solved by the multiple isomorphous replacement method and exploitation of the 3-fold redundancy of the electron density in the asymmetric unit. The atomic model was obtained by several rounds of model building.

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‡ The abbreviations used are: PDF, peptide deformylase; PEG, polyethylene glycol; MOPS, 3-morpholinopropanesulfonic acid; TCEP, tris(2-carboxyethyl)phosphine; r.m.s., root mean square.

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and correction followed by refinement (19) and map calculation. Atomic coordinates for about 50% of the residues were restrained to obey non-crystallographic symmetry. Unexpected density near the metal ion occurred in all three independent monomers and improved to a clear feature during the refinement process. It was then assumed to be an ordered part of a PEG molecule, consistent with subsequent biochemical studies showing that PEG is a competitive inhibitor of PDF. Only parts of the PEG-1000 molecules (HO(C₂H₄O)ₙH, n ≈ 22) are found in the map at a density above 5% of the map maximum. The visible part is modeled with n = 10 for the molecule length. The final model consists of 4122 (3 × 1346 + 84 alternate locations) non-hydrogen protein atoms, 3 Ni²⁺ ions, 2 SO₄²⁻ ions, 3 PEG, and 205 water molecules. The estimated coordinate error is 0.21 Å, and r.m.s. deviations from ideal geometry are 0.01 Å and 1.2° for bond length and bond angles, respectively.

Structure determination of PDF-Ni crystals was based on the above atomic model. The refined structure consists of 4038 (3 × 1346) non-hydrogen protein atoms, 3 Ni²⁺ ions, 2 SO₄²⁻ ions, and 100 water molecules. The estimated coordinate error is 0.30 Å, and r.m.s. deviations from ideal geometry are 0.015 Å and 1.4° for bond length and bond angles, respectively. In both structures, coordinates for residues Arg-167 and Ala-168 are ill defined, and Pro-9 is found in a cis-conformation.

| PDF-Ni/PEG | Resolution bins (Å) | Space group C2, a = 140.7 Å, b = 63.4 Å, c = 86.9 Å, β = 120.6° |
|-----------|---------------------|---------------------------------------------------------------|
| Native     | Rsym (%)             | Overall 6–6.0 6.0–4.0 4.0–3.0 3.0–2.0 2.0–1.9 |
| Rsym (%)   | Mean redundancy (%)  | 7.5 5.5 5.9 7.2 13.6 24.7 |
| Completeness (%) | 97.9 98.1 99.5 99.7 97.9 95.3 |
| (R/σ) (%) | R (%) | 21.8 28.6 21.8 20.6 21.1 20.2 |
| Rfree (%) | Mean figure of merit | 0.60 0.81 0.67 0.52 |
| EMP       | Completeness (%)     | 85.6 87.0 91.2 82.4 |
| Phasing power (%) | 2.1 3.3 2.0 1.3 |
| K₃PtCl₄   | Completeness (%)     | 97.4 97.3 99.3 96.4 |
| Phasing power (%) | 4.4 28.7 44.4 57.1 |
| CH₃HgCl/K₃PtCl₄ | Completeness (%) | 98.6 94.9 98.9 99.2 |
| Phasing power (%) | 1.5 2.2 1.4 1.1 |
| Rc (%)    | Rfree (%)            | 45.2 26.2 48.7 62.6 |

| PDF-Ni   | Resolution bins (Å) | Space group C2, a = 143.4 Å, b = 64.0 Å, c = 84.5 Å, β = 123.0° |
|-----------|---------------------|---------------------------------------------------------------|
| Native     | Rsym (%)             | Overall 6–6.0 6.0–4.0 4.0–3.0 3.0–2.6 2.6–2.5 |
| Rsym (%)   | Mean redundancy (%)  | 8.6 5.9 6.1 9.5 22.6 33.3 |
| Completeness (%) | 97.2 98.5 99.4 98.4 95.5 94.3 |
| (R/σ) (%) | R (%) | 17.4 48.3 35.4 16.5 5.0 3.6 |
| Rfree (%) | Mean figure of merit | 22.5 18.4 21.2 28.7 32.7 |
| EMP       | Completeness (%)     | 98.6 94.9 98.9 99.2 |
| Phasing power (%) | 45.6 26.2 48.7 62.6 |
| K₃PtCl₄   | Completeness (%)     | 97.4 97.3 99.3 96.4 |
| Phasing power (%) | 45.2 26.2 48.7 62.6 |
| CH₃HgCl/K₃PtCl₄ | Completeness (%) | 98.6 94.9 98.9 99.2 |
| Phasing power (%) | 1.5 2.2 1.4 1.1 |
| Rc (%)    | Rfree (%)            | 45.2 26.2 48.7 62.6 |

Rsym(%) = \frac{\sum h |I_{h}-\langle I_{h}\rangle|}{\sum h |I_{h}|}, \text{ where } h \text{ are unique reflection indices and } I_{h} \text{ are the intensities of symmetry equivalent reflections giving a mean value of } I_{h}.

Multiplicity of measurements of each unique reflection.

R = \frac{\sum |F_{o}-F_{c}|^2}{\sum F_{o}^2}, \text{ where } F_{o} \text{ and } F_{c} \text{ are observed and atomic model structure factor amplitudes, respectively. Reflections at a lower resolution than 6 Å are excluded from the summation.}

R factor calculated for 10% of randomly chosen reflections, which were excluded from the refinement.

Phasing power is the mean value of the heavy atom structure factor amplitude divided by the residual lack-of-closure error.

Cullis R factor for centric reflections.

FIG. 1. Stereo view of superimposed C-α traces of the three crystallographically independent copies of peptide deformylase. The numbers refer to amino acid residues. The transformations for optimal superposition were determined from equivalent C-α atoms using molecule A as a reference and applied to the NiCl₂ ion (marked as Ni) and the PEG molecule as well.
Overall Structure—Basically confirming the results of Chan et al. (13), PDF-Ni is an $\alpha + \beta$ protein consisting of a five-stranded antiparallel $\beta$-sheet ($\beta_1$, $\beta_2$, $\beta_3$, $\beta_6$, $\beta_7$), a two-stranded $\beta$-ribbon ($\beta_4$, $\beta_5$), three regular $\alpha$-helices, and three short $\beta_\alpha$ helices (Figs. 1 and 2). It has been noticed (8) that this overall arrangement of secondary and tertiary structure is quite different from other metalloproteases such as thermolysin (20) or stromelysin-1 (21), which also contain parallel $\beta$-strands.

The structures of the three molecules in the asymmetric unit are rather similar except for the more flexible regions 62–68 and 164–168 (Fig. 1). Moreover, a comparison of the monomer structures with and without bound PEG shows no significant differences. In fact, the r.m.s. deviation between corresponding $C_\alpha$ atoms is 0.25 Å (residues 66–68 and 161–168 are omitted from the comparison), which is within the expected coordinate errors of the models. The monomer structure is apparently rigid and structurally insensitive to the binding of PEG and to different environments in the crystal.

Active Site—Sequence alignment of deformylases from other eubacteria reveals three conserved regions, $G^48XG\times\times\times\ xQ$, $E^88\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\times\time...
FIG. 5. Interaction scheme between the catalytic Ni\(^{2+}\) ion, water molecules W1, W2, W3, and amino acid residues in the active site of peptide deformylase. With the exception of Leu-6, all residues shown in the figure are well conserved. Bonds and bond angles between the Ni\(^{2+}\) ion and its ligands are shown. Dashed lines indicate hydrogen bonds with distances between donor and acceptor atom given in Å. The second proton of W1 and the distance to the side chain of Gln-50 are in brackets to indicate the absence of a hydrogen bond.

For PEG-1000 concentrations up to 10 mM, identical \(k_{\text{cat}}\) values of 4.7 ± 0.2 s\(^{-1}\) were found, whereas the apparent \(K_M\) values increased linearly from 4.1 mM (without PEG-1000) to 12.5 mM (with 10 mM PEG-1000), yielding a calculated \(K_F\) value of 6 mM. The observed pattern shows that inhibition by PEG-1000 is competitive with respect to formyl-Met.

**DISCUSSION**

Since its discovery about 30 years ago PDF is known to rapidly lose its activity, which makes its purification difficult (2, 23). Even in recent work with the recombinant enzyme, specific activities have been reported that are off by orders of magnitude (6, 24). Meanwhile, it has been shown that PDF is a Fe\(^{2+}\) enzyme (11, 12) instead of a zinc metalloprotease as previously believed (8). Whereas the Zn\(^{2+}\) form is nearly inactive (11, 12), we routinely observe 1200 units/mg specific activity for PDF-Fe\(^{2+}\) and 900 units/mg for PDF-Ni\(^{2+}\) (11).

The three structures now known appear to be generally similar although significant differences remain that could well be of importance for understanding the enzyme mechanism. The two zinc structures (8, 13) have been compared previously (13), and the following discussion focuses on the differences between the zinc and nickel structures determined by x-ray analysis. These differences include Pro-9, which is in a cis-conformation as well as two of the three \(\alpha_1\) helices, Glu-11–Arg-14 and Phe-142–Tyr-145, which have been modeled as \(\alpha\)-helices \(\alpha_1\) and \(\alpha_2\) in the zinc structure (13). Other structural differences are found in the region Glu-87–Leu-99, which includes the \(\beta\)-ribbon. In the active site region the fourth metal ligand corresponding to W1 seems to be displaced, and the important water molecule W2 appears to be absent from the zinc structure. Also, the side chain of Gln-50 is more involved in stabilizing W2 rather than W1, and the amide hydrogens of Leu-91 and Ala-47 are found at distances 5 and 4.6 Å from W1, respectively, which is too far for a hydrogen bond as reported previously (13).

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intricate network of hydrogen bonds involving conserved residues of the enzyme family. The side chain of His-132 forms a hydrogen bond with the side chain of Gln-88, which itself is fixed by Arg-102 and indirectly by Asp-135. His-136 is held in place by Leu-13 mediated by a water molecule W3. The fourth ligand W1 is fixed by hydrogen bonds with the side chain oxygens of Glu-133 and a water molecule W2. The side chain of Gln-50, although it comes close to W1, is oriented in such a way that it cannot form a reasonable hydrogen bond with W1. In fact, there is no accepting group for the second proton of W1 in our structure, which leads us to speculate that it could well be a hydroxyl anion instead of a water molecule. The side chain of the conserved Gln-50 forms hydrogen bonds donating protons to water W2 and to the hydroxyl group of Ser-92. Interestingly, water W2, the side chain amide of Gln-50, and the main chain amide of Leu-91 are found in the arrangement required for the tetrahedral transition state, if W2 were replaced by the carboxyl oxygen of the formyl group. The amides of Gln-50 and Leu-91 could well serve as an anion trap to compensate the negative charge developing at the formyl oxygen.

The PEG molecule fits into a pocket formed by residues Gly-43, Ile-44, Ile-86, Gln-88–Leu-91, side chain of Arg-97, Cys-129, His-132, Gln-133, and the Ni\(^{2+}\) ion. The interactions are predominately hydrophobic except for the non-conserved Arg-97, which forms a hydrogen bond with the PEG molecule. The close proximity of the PEG molecule to the Ni\(^{2+}\) ion and to the conserved residues suggests that it might overlap with the enzyme’s substrate/product binding site. For clarification, inhibition studies were carried out as specified under “Experimental Procedures.”
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