Crystal Structure of Type II Peptide Deformylase from Staphylococcus aureus*

The first crystal structure of Class II peptide deformylase has been determined. The enzyme from *Staphylococcus aureus* has been overexpressed and purified in *Escherichia coli* and the structure determined by x-ray crystallography to 1.9 Å resolution. The purified iron-enriched form of *S. aureus* peptide deformylase enzyme retained high activity over many months. In contrast, the iron-enriched form of the *E. coli* enzyme is very labile. Comparison of the two structures details many differences; however, there is no structural explanation for the dramatic activity differences we observed. The protein structure of the *S. aureus* enzyme reveals a fold similar, but not identical to, the well characterized *E. coli* enzyme. The most striking deviation of the *S. aureus* from the *E. coli* structure is the unique conformation of the C-terminal amino acids. The distinctive C-terminal helix of the latter is replaced by a strand in *S. aureus* which wraps around the enzyme, terminating near the active site. Although there are no differences at the amino acid level near the active site metal ion, significant changes are noted in the peptide binding cleft which may play a role in the design of general peptide deformylase inhibitors.

Antibiotics that target cell wall biosynthesis, protein synthesis, or DNA replication in bacteria have been the mainstay for treatment of bacterial infections for decades. However, in recent times antibiotic-resistant bacteria have become a major health threat (1). This unmet medical need has prompted a new interest in the development of novel, potent, and orally available antibiotics. Among the many potential approaches to new antibiotics include those that target novel, bacteria-specific, biosynthetic pathways. Peptide deformylase is such a target. Inhibition of this enzyme would result in blockage of essential protein processing and has been shown to be bacteriostatic (2–3).

In eubacteria, as well as mitochondria and chloroplasts, the initiation of protein synthesis normally requires an N-formylated methionine residue (4–6). The special initiation tRNA, tRNA<sup>Met</sup>, is charged with methionine by the methionyl-tRNA synthetase (EC 6.1.10), which adds a methionine to either of the methionine tRNAs with the consumption of ATP. The formyl group is added to the charged tRNA<sup>Met</sup> from 10-formyltetrahydrofolate by methionine-tRNA<sup>Met</sup> formyltransferase (EC 2.1.2.9). The f-Met-tRNA<sup>Met</sup> is transferred to the ribosome where it is the preferred initiator of protein synthesis (7). Mature proteins do not retain N-formylmethionine. In fact, a heterogeneous population of amino acids is normally found at the N terminus of mature proteins: alanine, glycine, serine, threonine, or methionine. Larger amino acids are rarely found (6, 8, 9). This observation suggests that multiple catabolic processing might occur after or in concert with protein synthesis. This processing may be important for protein turnover (10), conservation of methionine, or it may be required for the activity of some enzymes (11–13). All known N-terminal peptidases cannot use formylated peptides as substrates (14). Thus, removal of the formyl group is an essential first step in N-terminal processing. The formyl group is removed from the peptide N terminus by a metalloenzyme, peptide deformylase (EC 3.5.1.27) (15–17). The processed protein is released for possible further processing by methionine aminopeptidase (EC 3.4.11.18). Because the formylation/deformylation cycle is unique to eubacteria and does not occur in cytosolic eukaryotic or Archaeabacteria protein synthesis (4), peptide deformylase is an attractive target for the design of new antibiotics.

Previous analysis of peptide deformylase gene sequences revealed that the predicted protein coding regions have a significant sequence similarity and three characteristic stretches of highly conserved amino acids: motif 1 (GΦΦΦAAXQ), motif 2 (EGΦΦS), and motif 3 (HEΦΦDH), where Φ is a hydrophobic amino acid (Fig. 1). The Cys of motif 2 and the two His residues of motif 3 are involved in active site metal ion binding (18). The fourth ligand of the tetrahedrally coordinated metal ion is a water molecule that is held in place by the Glu of motif 3. Subsequent analysis of diverse peptide deformylase sequences suggested that the enzyme family could be divided into two classes (19). Peptide deformylase enzyme from Gram-negative organisms represented by *Escherichia coli* (Class I) have ~30% sequence identity with each other; whereas many Gram-positive organisms express a structural variant that includes three major insertions (20, 21) relative to the *E. coli* sequence, some additional single amino acid insertions, and a distinct C terminus (Fig. 1). The overall sequence identity among Class II genes appears to be lower (15%) than Class I, and about half of these identities occur in or near the Class II-specific insertions. The first major insertion occurs after Tyr<sup>29</sup> (*E. coli*) and consists of 12 amino acids with high sequence similarity. In this

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The atomic coordinates and structure factors (code 1LMH) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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region the Mycoplasma genes predict an insertion of only 11 amino acids (22). The second characteristic insertion is of variable length and occurs after Asn55. A third insertion of three amino acids follows Ser51 and extends the sequence preceding the conserved motif 2. The last major difference between the two classes is the C terminus. In the Class II enzymes this sequence is more hydrophobic and nearly always shorter than the E. coli version. Class I peptide deformylase from the Gram-negative organism, E. coli, has been well characterized both in terms of enzymology and structure. Efforts to purify the enzyme were hampered for 3 decades by the rapid loss of activity upon purification. This lability was traced to the Fe2+ metal cofactor (23) and has been overcome by a combination of protein overexpression, elimination of oxygen, and the use of alternative metal cofactors. The Zn2+ active site metal ions. The Zn2+ expression, elimination of oxygen, and the use of alternative metal ions. The Zn2+ expression, elimination of oxygen, and the use of alternative metal ions. The Zn2+ expression, elimination of oxygen, and the use of alternative metal ions. The Zn2+ expression, elimination of oxygen, and the use of alternative metal ions. The Zn2+ expression, elimination of oxygen, and the use of alternative metal ions.

The amino acid sequence was identified from Human Genome Science (contig no. 168951) and cloned directly from S. aureus RN4220 genomic DNA to complete the open reading frame. The gene from strain RN4220 has the same peptide deformylase sequence as strain N315 (Fig. 1) with the exception of N171D. The peptide deformylase gene was cloned by PCR into a vector containing the tac promoter and expressed in E. coli. The plasmid containing the peptide deformylase insert was purified and used to transform a competent strain of E. coli JM109. The cDNA clone used for structural studies contained two mutations (R127K;H186Q). The correct amino acid sequence was expressed and purified in subsequent experiments, including biochemical experiments discussed herein. There was no effect of the R127K mutation on Km or Kcat of the mutant enzyme (data not shown), and the H186Q mutation is outside the open reading frame of the deformylase gene.

The peptide deformylase proteins were expressed using Luria broth and a C-terminal hexahistidine tag. The peptide deformylase proteins were expressed using Luria broth and a C-terminal hexahistidine tag. The peptide deformylase proteins were expressed using Luria broth and a C-terminal hexahistidine tag. The peptide deformylase proteins were expressed using Luria broth and a C-terminal hexahistidine tag. The peptide deformylase proteins were expressed using Luria broth and a C-terminal hexahistidine tag.

The open reading frame of E. coli peptide deformylase was amplified using PCR from an E. coli genomic library. Restriction enzyme BglII sites were engineered onto both ends, and the resulting insert was ligated into the BglII site of the E. coli expression vector pQE60 (Qiagen). Both constructs express peptide deformylase followed by a C-terminal hexahistidine tag. The peptide deformylase proteins were expressed using Luria broth with ampicillin (100 μg/ml) in both the seed and production media. Luria broth was prepared using 10 g of Bacto-tryptone, 5 g of Bacto yeast, and 5 g of NaCl added per liter of deionized water. The pH of the media was adjusted to 7.5 with KOH before sterilization. The Luria broth was autoclaved for 20 min in 100-ml volumes in 500-ml wide 1 The abbreviations used are: PCLNA, (S)-2-O-(H-phosphonoxyl)-l-caproyl-l-leucyl-p-nitroanilide (PCLNA); Ni-NTA, nickel-nitrilotriacetic acid; HPLC, high performance liquid chromatography; MAD, multiple-wavelength anomalous diffraction.

In this report we present the x-ray structure of Class II peptide deformylase from the Gram-positive pathogen Staphylococcus aureus at 1.9 Å resolution. Comparison of this structure with the prototypic Class I enzyme from E. coli reveals minor structural variation arising from the insertions as well as specific, active site differences that may impact the design of pathogen-specific peptide deformylase inhibitors.

EXPERIMENTAL PROCEDURES

Cloning and Expression—The S. aureus peptide deformylase sequence was identified from Human Genome Science (contig no. 168951) and cloned directly from S. aureus RN4220 genomic DNA to complete the open reading frame. The gene from strain RN4220 has the same peptide deformylase sequence as strain N315 (Fig. 1) with the exception of N171D. The peptide deformylase gene was cloned by PCR into a vector containing the tac promoter and expressed in E. coli. The plasmid containing the peptide deformylase insert was purified and used to transform a competent strain of E. coli JM109. The cDNA clone used for structural studies contained two mutations (R127K;H186Q). The correct amino acid sequence was expressed and purified in subsequent experiments, including biochemical experiments discussed herein. There was no effect of the R127K mutation on Km or Kcat of the mutant enzyme (data not shown), and the H186Q mutation is outside the open reading frame of the deformylase gene.

The peptide deformylase proteins were expressed using Luria broth with ampicillin (100 μg/liter) in both the seed and production media. Luria broth was prepared using 10 g of Bacto-tryptone, 5 g of Bacto yeast, and 5 g of NaCl added per liter of deionized water. The pH of the media was adjusted to 7.5 with KOH before sterilization. The Luria broth was autoclaved for 20 min in 100-ml volumes in 500-ml wide 1 The abbreviations used are: PCLNA, (S)-2-O-(H-phosphonoxyl)-l-caproyl-l-leucyl-p-nitroanilide (PCLNA); Ni-NTA, nickel-nitrilotriacetic acid; HPLC, high performance liquid chromatography; MAD, multiple-wavelength anomalous diffraction.

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mouth fermentation flasks. Ampicillin was filter sterilized and added just before inoculation. The 100-ml seed stock fermentations were carried out in 500-ml wide mouth flasks and were inoculated from agar cultures and incubated overnight at 37 °C with agitation at 200 rpm. The seed fermentations were used to inoculate at 2% the 100-ml product fermentations that were also carried out in 500-ml wide mouth flasks. These fermentations were incubated with agitation at 200 rpm for slightly longer than 2 h and were then induced (A450 nm reached 0.6). Isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 0.4 mM. The induced fermentations were continued for an additional 3.5 h until the A reached 3.0. Multiple fermentations produced a final harvest of 4 liters of deionized water: 39.44 g of MgCl2. The trace metal solution contained per liter 0.01 mM FeSO4·7H2O, 5.58 g of MnSO4·H2O, 1.11 g of FeCl3·6H2O, 0.48 g of NaNO3, 0.25 g of CaCl2·2H2O, 0.33 g of NaCl, 1 g of ascorbic acid at 100 mg, 5 mg, and 0.3 mliter of deionized water, respectively. Multiple shake flasks were used to attain the desired fermentation volume. Because JM109 is not a methionine auxotroph, incorporation of selenomethionine was accomplished through down-regulation of methionine biosynthesis just prior to induction (45). The culture was grown in 500-ml wide mouth fermentation flasks at 37 °C with an agitation rate of 200 rpm until the A450 nm reached 3.7. Isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 120 mM. The growth of the culture was continued for an additional 3 h when the A reached about 2. Cells were then harvested by centrifugation and stored at −80 °C.

Purification—Cell paste from a 2-liter E. coli fermentation expressing S. aureus peptide deformylase was lysed in 50 ml Tris-HCl, pH 8.0, with lysozyme dissolved at 1 mg/ml. The suspension was set on ice for 10 min, and large strand DNA was broken by repeatedly shearing with a syringe and 19-gauge needle. Cell extract was collected and centrifuged at 20,500 rpm for 40–45 min at 5 °C. Ni-NTA resin from Qiagen was equilibrated in lysis buffer (without lysozyme) and stirred into the cell extract. The resin suspension was poured into a column, washed extensively with lysis buffer, and peptide deformylase was eluted with 50 ml Tris-HCl, pH 8.0, buffer containing 200 mM imidazole. The eluate from the nickel column was concentrated by ultrafiltration with an Amicon stirred cell. Sodium nitrate at room temperature did not give signal in the mass spectra (data not shown) in the crystallized protein. Atomic absorption spectroscopy confirmed the presence of iron and zinc using protein prepared with unsupplemented Luria broth.

Cryoprotection—S. aureus Peptide Deformylase—After purification the protein was stored in a buffer containing 25 mM Tris-HCl, pH 8.0, and 50 mM NaCl at a concentration in excess of 50 mg/ml. The protein concentration had (dilution with 25 mM Tris-HCl, pH 8.0). This protein could be frozen immediately in 50-μl aliquots for later experiments. Cryostabilization experiments began with commercially available, random sparse matrix screens (50). Drops of 1 μl of protein and 1 μl of well solutions were set up in hanging drop vapor diffusion experiments at room temperature. Crystals grew in 1 week with well condition 6 of the Hampton Crystal Screen. Follow-up grid screens were set up to optimize the crystallization condition. After optimization crystals could be routinely grown with seeding from sitting drop vapor diffusion experiments by mixing 4 μl of peptide deformylase and 4 μl of reservoir solution (17–27% polyethylene glycol 4000, 200 mM MgCl2, and 100 mM Tris-HCl, pH 8.5). Crystals were successfully stabilized and slowly transferred into a cryopreservation solution containing 25% polyethylene glycol 4000, 100 mM Tris-HCl, pH 8.5, 100 mM MgCl2, and 25% glycerol. Crystals were frozen in liquid nitrogen for cryogenic data collection. The crystals belonged to the space group C2221, with one molecule in the asymmetric unit. The unit cell parameters are a = 94.113, b = 121.873, c = 47.579 Å. Identical crystals were grown with selenomethionine peptide deformylase enzyme.

Structure Determination—The x-ray data for the MAD phasing of peptide deformylase were collected at the Advanced Photon Source on beamline 17ID operated by the Industrial Macromolecular Crystallography Association and consisted of three separate wavelength experiments centered about the selenium kappa edge (low, 1.0323 Å; edge, 0.97939 Å, inflection point; high, 0.97925 Å, peak of the absorption edge). The appropriate x-ray wavelength was selected using the 111 reflection from a cryocooled Si monochromator crystal. This beam was

The final aminopeptidase-coupled assay solution contained 50 mM NaHepes, 10 mM NaCl, pH 7.0, 0.1% potassium casein (Sigma), 100 μM formyl-Met-Leu-p-nitroanilide, 5 mg of peptide deformylase protein (iron form) or 100 mg of peptide deformylase protein (zinc form), and V. proteolytica aminopeptidase. Assays were conducted in a 96-well V-bottom plate system (SpectraMax 250 reader) using an absorbance of 340 nm. Time points were taken every 20 s over a 30-min time course at 25 °C. The final formate dehydrogenase-coupled assay solution contained 50 mM NaHepes, 10 mM NaCl, pH 7.0, 0.1% gelatin (enzyme immunoassay grade, Bio-Rad), 250 μM formyl-Met-Ala-Ser, 20–80 ng of peptide deformylase protein, 3.125 mM NAD+ and, 5–50 μg of formate dehydrogenase from Pseudomonas oxalaticus. Assays were conducted in a 96-well plate system (SpectraMax 250 reader) using an absorbance of 340 nm. Time points were taken every 20 s over a 30-min time course at 25 °C. In some cases we also used a third direct C-18 HPLC assay that lacked the coupling enzymes. For the HPLC assays, we followed the generation of the product Met-Leu-p-nitroanilide described in the reaction above, using UV absorbance at 315 nm.

Comparison of the Stability of the Iron-enriched S. aureus Peptide Deformylase with Iron-enriched E. coli Peptide Deformylase—Expression of the iron form of S. aureus or E. coli peptide deformylase in E. coli (both JM109) was completed after the preculture of the cells in 1.6 mM FeCl3 in Luria broth. Incubation was allowed to proceed for 1 h prior to the addition of 0.3 mM isopropyl-β-D-thiogalactopyranoside. Induction was allowed to proceed at 37 °C. Isolated cells were extracted in the presence of 10 μg/ml catalase. The extracts were subjected to Ni-NTA chromatography for the purification of the C-terminally hexahistidine-tagged versions of both the E. coli and S. aureus enzymes. The purified proteins (concentrated to at least 1 mg/ml by Amicon ultrafiltration) were then stored in 10 μg/ml (minimum concentration) catalase. To examine the stability of the iron-enriched forms of the peptide deformylase proteins, dilutions of the enzymes were made in the presence or absence of catalase (at 100 μg/ml) followed by enzymatic assays. Assays were run using formyl-Met-Leu-p-nitroanilide as substrate, coupled to the Vibrio aminopeptidase. Similarly, the metal forms of S. aureus peptide deformylase were prepared by supplementation of E. coli K12 with 1.6 mM FeCl3, 640 μM ZnCl2, or control media followed by purification by Ni-NTA chromatography as described above (in the absence of catalase) (26, 29). The peptide deformylase from each experiment (iron-enriched, zinc-enriched, or no supplementation) was then assayed as described above.

Metal Ion Assocation—X-ray fluorescence experiments were carried out at the Advanced Photon Source beamline 17ID near the kappa edge for iron (1.743 Å), nickel (1.488 Å), and zinc (1.284 Å). The characterization of the presence of each metal ion (data not shown) in the crystallized protein. Atomic absorption spectroscopy confirmed the presence of iron and zinc using protein prepared with unsupplemented Luria broth.

Cryostabilization of S. aureus Peptide Deformylase—After purification the protein was stored in a buffer containing 25 mM Tris-HCl, pH 8.0, and 50 mM NaCl at a concentration in excess of 50 mg/ml. The concentration was adjusted to 37 °C. Isolated cells from each culture were frozen immediately in 50-μl aliquots for later experiments. Cryostabilization experiments began with commercially available, random sparse matrix screens (50). Drops of 1 μl of protein and 1 μl of well solutions were set up in hanging drop vapor diffusion experiments at room temperature. Crystals grew in 1 week with well condition 6 of the Hampton Crystal Screen. Follow-up grid screens were set up to optimize the crystallization condition. After optimization crystals could be routinely grown with seeding from sitting drop vapor diffusion experiments by mixing 4 μl of peptide deformylase and 4 μl of reservoir solution (17–27% polyethylene glycol 4000, 200 mM MgCl2, and 100 mM Tris-HCl, pH 8.5). Crystals were successfully stabilized and slowly transferred into a cryopreservation solution containing 25% polyethylene glycol 4000, 100 mM Tris-HCl, pH 8.5, 100 mM MgCl2, and 25% glycerol. Crystals were frozen in liquid nitrogen for cryogenic data collection. The crystals belonged to the space group C2221, with one molecule in the asymmetric unit. The unit cell parameters are a = 94.113, b = 121.873, c = 47.579 Å. Identical crystals were grown with selenomethionine peptide deformylase enzyme.
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RESULTS AND DISCUSSION

Comparison of the Stability of Iron-enriched S. aureus and E. coli Peptide Deformylase—We have found that the expression of S. aureus peptide deformylase containing a C-terminal hexahistidine tag fusion provides a facile method for purification of this protein. During the course of this work, we also found that the enzymatic activity of the purified form of the iron-enriched protein was stable, in sharp contrast to the instability reported for the Fe
\(^{2+}\)
form of E. coli peptide deformylase (25). To validate this finding we expressed the iron form of S. aureus or E. coli peptide deformylase in E. coli after the preincubation of the cells in 1.6 mM FeCl\(_3\) in Luria broth as described under “Experimental Procedures.” After induction with isopropyl-\(\beta\)-\(D\)-galactopyranoside, isolated cells were extracted in the presence of 10 \(\mu\)g/ml catalase, to act as an oxygen scavenger and to prevent the inactivation of the iron form of each peptide deformylase. The C-terminally hexahistidine-tagged versions of the E. coli and S. aureus peptide deformylase enzymes were purified by Ni-NTA chromatography and then were stored in 10 \(\mu\)g/ml catalase. To examine the stability of the iron-enriched forms of the peptide deformylase proteins, dilutions of the enzymes were made in the presence or absence of 100 \(\mu\)g/ml catalase, followed by enzymatic assays under standard conditions as described above. Assays were run using formyl-Met-Leu-Glu-Arg (formyl-Met-LA) as substrate.

Similarly, the metal forms of S. aureus peptide deformylase were prepared by supplementation of E. coli K12 with 1.6 mM FeCl\(_3\), 640 \(\mu\)M ZnCl\(_2\) or control media, followed by purification by Ni-NTA chromatography as described above (in the absence of catalase) (26, 29). The peptide deformylase from each experiment (iron-enriched, zinc-enriched, or no supplementation) was then assayed. The addition of zinc lowers the specific activity of the peptide deformylase, whereas the addition of iron increases the specific activity by 3–6-fold (average) compared with control media (Fig. 3). These data support the notion that the S. aureus, but not E. coli, peptide deformylase enzyme is capable of forming a stable metal complex with iron, whereas the zinc metal complex with S. aureus peptide de-

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**Table I**

| Data collection and phasing statistics (MAD data set) | \(\lambda = 1.03321\) Å | \(\lambda = 0.97939\) Å | \(\lambda = 0.97928\) Å |
|--------------------------------------------------------|------------------------|------------------------|------------------------|
| Resolution                                             | 1.9 Å                  | 1.9 Å                  | 1.9 Å                  |
| Average redundancy                                     | 4.5                    | 4.5                    | 4.5                    |
| No. of unique reflections                             | 21,519                 | 21,904                 | 22,008                 |
| % completeness                                        | 97.5%                  | 99.3%                  | 99.7%                  |
| \(R_{	ext{sym}}\)                                      | 0.042                  | 0.065                  | 0.071                  |
| \(R_{	ext{sym}}\) (1.96–1.89 shell)                   | 0.152                  | 0.168                  | 0.186                  |
| \(R_{	ext{calcruc}}\) anomalous                       | 0.98 (19,178 refs.)    | 0.64 (18,418 refs.)    | 0.58 (18,679 refs.)    |
| Phasing power                                          |                        |                        |                        |
| Centrics                                               | 0.70                    | 1.83                   | 2.15                   |
| Acentrics                                              | 0.80                    | 2.10                   | 2.15                   |
| Mean FOM\(^a\)                                         | Overall                | Centrics               | Acentrics              |
| Before solvent flattening                              | 0.714 (21,048 refs.)   | 0.627 (2,014 refs.)   | 0.724 (19,034 refs.)   |
| After solvent flattening                               | 0.788                  |                        |                        |

\(a\) \(R_{	ext{sym}} = \sum_{i} |I_{i} - <I>| / \sum |I_{i}|\), where \(I\) is the intensity of an individual reflection from a symmetry-related group of reflections and \(<I>\) is the mean intensity.

\(^b\) refs., reflections.

\(^c\) FOM, figure of merit.

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\(^3\) J. B. Moon, unpublished data.

\(^4\) B. C. Finzel, unpublished data.
formylase behaves similarly to that described for the Zn$^{2+}$ form of the E. coli enzyme.

Structure of S. aureus Peptide Deformylase—The Class II peptide deformylase structure is composed mostly of $\beta$-sheet with helical regions near the N terminus (7 to 4 and 27 to 53) and from residues 147 to 161. The N-terminal helical segments form a knot-like cluster (see Fig. 5, right) whereas the $\beta$-sheet regions are confined to the C-terminal two-thirds of the protein. The other helical region forms the core of the structure and is also involved in catalysis. The conserved motif 3 is found on this central helix and is involved in the coordination of the active site metal ion (Fig. 4, a and b) (33, 61, 62). The $\beta$-sheet regions surround the centrally located helix and help create the shallow active site cavity. The $\beta$-sheet-rich section is composed of three $\beta$-sheet elements: an N-terminal antiparallel three-stranded $\beta$-sheet, a central antiparallel three-stranded $\beta$-sheet, and a C-terminal mixed $\beta$-sheet. The C terminus of the protein

| Table II: Final refinement statistics and quality of model for peptide deformylase using the remote wavelength (1.0332 Å) data set |
|---------------------------------|-----------------|
| Refinement |
| Resolution range (working + test completeness, %) | 10–1.9 (94.8) |
| Reflections in working set (completeness, %) | 18,819 (85.4) |
| Reflections in test set (completeness, %) | 2,067 (9.4) |
| $R_{free}^a$ | 0.180/0.212 |
| Estimated error of $R_{free}$ | 0.005 |
| Sigma cutoff of 0.00 and bulk solvent modeling applied |
| Highest bin 2.02–1.90 (working + test completeness, %) | 3,074 (85.5) |
| Reflections in working set highest bin | 2,764 |
| Reflections in test set highest bin | 310 |
| $R_{free}$ in highest bin | 0.192/0.231 |
| Estimated error of highest bin $R_{free}$ | 0.013 |
| Coordinate error (Å) from Luzzati plot | 0.19 |
| Coordinate error (Å) from Sigma A | 0.08 |
| Coordinate error (Å) from cross-validated Luzzati plot | 0.22 |
| Coordinate error (Å) from cross-validated Sigma A | 0.10 |
| No. of protein atoms (includes 6 seleno-methionine residues) | 1,452 |
| No. of solvent molecules | 427 |
| No. of zinc ions | 1 |
| Rms deviation from target restraints. $^b$ |
| Bond lengths (Å) | 0.004 |
| Bond angles (°) | 1.3 |
| Dihedral angles (°) | 22.9 |
| Improper angles (°) | 0.71 |
| Average B factors (Å$^2$) |
| All protein atoms | 14.46 |
| All main chain atoms | 13.93 |
| All side chain atoms | 14.79 |
| All solvent molecules | 33.18 |
| Zinc ion | 21.17 |
| Ramachandran plot statistics (%) |
| Most favored regions | 93.8 |
| Additionally allowed regions | 6.2 |
| Generously allowed regions | 0 |
| Disallowed regions | 0 |
| $^a$ R and $R_{free} = \langle \Sigma \left| F_{obs} - F_{calc} \right| \rangle / \Sigma F_{obs}$. |
| $^b$ Rms, root mean square. |

**Fig. 2.** The S. aureus peptide deformylase is stable as the iron-enriched form whether or not catalase is added as an oxygen scavenger, whereas the instability of the E. coli protein is amplified if catalase is not added during the assay.

**Fig. 3.** The activity of purified S. aureus peptide deformylase is influenced by the addition of metal ions to the media. The specific activity of the enzyme is enhanced by iron and diminished by zinc.
forms a last short strand of mixed β-sheet and is poised at the mouth of the active site (Fig. 5).

**Comparison of Class I and Class II Peptide Deformylase—** With the availability of numerous *E. coli* peptide deformylase x-ray and NMR structures, we have carried out a detailed comparison between the Class I and Class II enzymes. The root mean standard deviation between 134 common main chain atoms). This superposition is the basis of the structure-based alignment of the protein sequences for selected members of both classes (Fig. 1). The strictly conserved residues (in boldface) across both classes are limited to the conserved motifs with three exceptions: Arg124 (S. aureus), Asn92, and Val122. This valine is not conserved across a previous 34-organism alignment and will not be considered further (20). Arg124 makes a salt bridge with Glu109 from conserved motif 2 in both the *E. coli* and *S. aureus* crystal structures (Fig. 4b). The hydrophobic portions of the glutamic acid side chain contribute to the surface of the formyl-methionine substrate binding pocket. The side chain of Asn92 makes a set of hydrogen bonds to both the main chain amide and carbonyl of Leu22. This interaction presumably stabilizes the tertiary structure and is also present in both crystal structures. The conserved residues of motif 1 form part of the wall of the active site crevice and provide loci for hydrogen bonding of peptide substrates (32). Motifs 2 and 3 contribute the protein side chains of His154, His158, and Cys111 which coordinate the active site metal ion (Fig. 4b).

The active site metal ion is represented by the gray spheres. The prominent difference at the C terminus (red) is the lack of the *E. coli* helix in the *S. aureus* enzyme.

**Fig. 5.** Ribbon diagram of the *S. aureus* and *E. coli* peptide deformylase structures colored in reverse rainbow beginning with the N terminus (blue). The active site metal ion is represented by the gray spheres. The prominent difference at the C terminus (red) is the lack of the *E. coli* helix in the *S. aureus* enzyme.
helices form a Class II-specific helical cluster that precedes conserved motif 1. In the *E. coli* structure the N-terminal helix lacks the extending residues and is followed by a β-turn and motif 1. The conserved motif 1 sequence forms the third (and edge) strand of the N-terminal β-sheet. The insertion of residues Gly81-Gly83 in the *S. aureus* structure extends the turn between strands II and III of the N-terminal β-sheet. These turn residues project near the mouth of the active site depression in Class II peptide deformylase and can vary in length. The fifth insertion, Val100, is in the turn between strand I of the central antiparallel β-sheet and the central strand of the C-terminal mixed sheet. The sixth insertion occurs at the end of the central strand of the mixed sheet and includes Pro106 and Thr107. These residues are positioned at the opening of the active site crevice and may be important determinants of *S. aureus* specificity. The subsequent conserved residues (motif 2) form the other wall of the active site crevice. Residue Cys111 at the center of this sequence is one of the active site metal ligands. The conserved glutamic acid projects downward to form a part of the crevice wall and makes a conserved salt bridge with Arg124, which is found in the center of the first strand of the mixed β-sheet. The insertion of Ala119 results in a slight bulge of the connecting strand which precedes the first strand of the C-terminal mixed β-sheet. This seventh insertion,

**FIG. 6.** Stereo pair of the superimposed α-carbon backbone of the *S. aureus* peptide deformylase (gray) and the *E. coli* enzyme (green). The yellow spheres indicate the metal ions.

**FIG. 7.** Stereo pair of the superposition of the active site cavity of the *S. aureus* peptide deformylase structure (gray carbons) and the *E. coli* peptide deformylase structure (1bsj.pdb) (green atoms). Selected *S. aureus* residues from Table III are labeled in black. Arg27 from the *E. coli* enzyme is labeled in green. The yellow spheres are the metal ions.

| S. aureus | E. coli |
|----------|--------|
| Val59    | Ile44  |
| Ser57    | Glu42  |
| Arg56    | Glu41  |
| Asn117   | Arg9   |
| Ile150   | Ile128 |
| Thr107   | Glu87  |
| Pro106   | Leu86  |
| Leu105   | Leu125 |
| Tyr147   | Cys129 |

**TABLE III**

Specific sequence differences in the active site residues between *S. aureus* and *E. coli* peptide deformylase

*E. coli* residues are numbered according to Ref. 31.
the sixth insertion (Pro^{106}/Thr^{107}), and the C-terminal extension are all in close proximity and constitute a surface specific to \textit{S. aureus} peptide deformylase and Class II in general. The insertion after Asp^{134} is specific to \textit{Mycoplasma} and occurs in a surface loop (Fig. 1). From the simplest comparison of these two x-ray structures one is immediately struck by the obvious difference at the C terminus (Fig. 5). The \textit{E. coli} enzyme has a long protruding α-helix that abuts the protein surface behind the active site cavity. The C terminus of the \textit{S. aureus} enzyme does not contain an equivalent α-helix but wraps around to complete a short stretch of β-sheet, terminating near the opening of the active site cavity. This is the only major topological difference between the two structures (Fig. 6).

It follows from the low overall sequence identity between \textit{E. coli} and \textit{S. aureus} peptide deformylase that the lining of the active site cavity would not be identical. This expectation is in fact borne out by the present structure (Fig. 7). Analysis of the active site cavity suggests that nine residue changes are found in the crevice and the annulus about the active site depression (Table III). Some particularly interesting differences include the structural replacement of Arg^{97} (\textit{E. coli}) for Arg^{56} (\textit{S. aureus}) where an arginine side chain is retained in a similar three-dimensional position, but the side of the cavity from which it projects is altered. Leu^{125} (\textit{E. coli}) forms part of the hydrophobic surface that binds the peptide substrate methionine side chain. This residue is replaced with Tyr^{147} in \textit{S. aureus}, and the side chain hydroxyl is positioned near the mouth of the active site cavity. In addition, a number of subtle hydrophobic-hydrophobic changes are observed as are a number of polar-polar changes.

Our comparison of Class I and Class II peptide deformylase identifies the parameters that will influence the discovery of general inhibitors of this enzyme, as well as those that might be specific for Gram-positive or Gram-negative bacteria. The sequence and structural identity between enzymes in the immediate vicinity of the active site metal ion suggest that this is the determining locus for general inhibitors (Fig. 4b). Common modes of interaction are expected between various metal binding inhibitors across all bacteria (2, 3, 32, 69–73) and may extend to parasite peptide deformylase (19). In addition to direct metal chelation, general inhibitors may also be expected to exploit interactions with the conserved catalytic Gln^{65}, Gln^{109} (the side chain that projects above the metal from the back of the active site, Fig. 4a); and main chain interactions with Leu^{112} and Gly^{110}. Hydrogen bonds with different subsets of these conserved amino acids are observed in the published complexes (2, 32, 33). Although all peptide deformylase enzymes are expected to bind a methionine side chain optimally in the long and fairly narrow hydrophobic S1′ subsite (74), the lack of strict conservation of side chain residues that compose this subsite affords an opportunity for specificity. The left side of the pocket is composed of strictly conserved residues, Gln^{109}, Gly^{110}, and Cys^{111}. The remaining subsite surface is defined by variable residues: Val^{99} (Ile, \textit{E. coli}), Tyr^{147} (Leu), ile^{150} (Ile), Val^{151} (Cys), and Leu^{105} (Ile). Accommodation of bulkier hydrophobic groups in S1′ may be achieved more readily in certain peptide deformylase enzymes. It may also be possible to exploit electronic differences in this subsite, i.e., the free cysteine of the \textit{E. coli} enzyme is replaced by Val^{151} in the present structure (Fig. 7).

Because of more prevalent differences in the S2′, S3′, and S4′ subsites between Class I and Class II, designing generality into inhibitors becomes increasingly difficult. The \textit{S. aureus} enzyme S2′ subsite differs from the \textit{E. coli} enzyme in several important ways. The path of the main chain is altered to narrow the subsite at Ser^{57} and to project the backbone carbonyl of this residue more directly into the subsite pocket. Arg^{56} forms a lid across the back of the S2′ subsite further restricting its size. In the product complex bound to \textit{E. coli} peptide deformylase (33), the product makes several key interactions. These include a hydrogen bond between the peptide backbone carbonyl of the product and the side chain of Arg^{97}. Equivalent interactions have been seen in other complexes including thermolysin (68). The possibility for this hydrogen bond is lost in \textit{S. aureus} peptide deformylase because Arg^{56} is replaced by Asn^{117}. The shorter asparagine residue cannot reach to influence substrate binding. In addition, Arg^{56} of \textit{S. aureus} cannot replace the loss of Arg^{167}. In \textit{S. aureus}, Arg^{56} projects from the other side of the binding pocket (Fig. 7) and places the guanidine group a few angstroms distal in S2′ and inappropriately positioned for substrate hydrogen bonding. Several differences can also be noted in the region around S3′ when the two enzymes are compared. There is an insertion at Pro^{106} which changes the shape of the subsite. The Thr^{107} (Glu, \textit{E. coli}) introduces a different polar group that may interact with substrates or inhibitors on the left side of the pocket. In \textit{S. aureus} the Tyr^{147} hydroxyl on the right side of the pocket introduces a new hydrogen bonding group into the binding cleft. Superposition of the product complex (33) with the present structure places the new hydrogen bond donor/acceptor within 3 Å of the product peptide backbone nitrogen. A similar tyrosine hydroxyl hydrogen bond has been observed in human aspartic proteases (75, 76). The S4′ subsite is completely exposed to solvent and does not form a distinct pocket. However, protein surface residues in this region derive from the Class II-specific insertion of variable length and may modify the path by which peptide substrates (or inhibitors) approach the active site.

The comparison of \textit{E. coli} and \textit{S. aureus} peptide deformylase reveals the structural differences between Class I and II peptide deformylase enzymes but fails to explain the stability differences we observe between the prototypic enzymes. Examination of the active site variation suggests that general inhibitors of peptide deformylase will possess specificity determinants found near the metal and in S1′. However, compounds with realistic drug-like molecular masses on the order of 400–500 Da would most certainly reach into the more divergent S2′ and S3′ regions. Thus, careful consideration of structural variation across classes will be required for the design of general inhibitors. Conversely, specificity to \textit{S. aureus} over other bacteria may be introduced by incorporation of specific hydrogen-bonding groups into inhibitors that exploit the S2′ and S3′ subsites. This structure determination is the essential first step toward a program of structure-based antibiotic discovery centered about a pathologically important target.

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Crystal Structure of Type II Peptide Deformylase from *Staphylococcus aureus*

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