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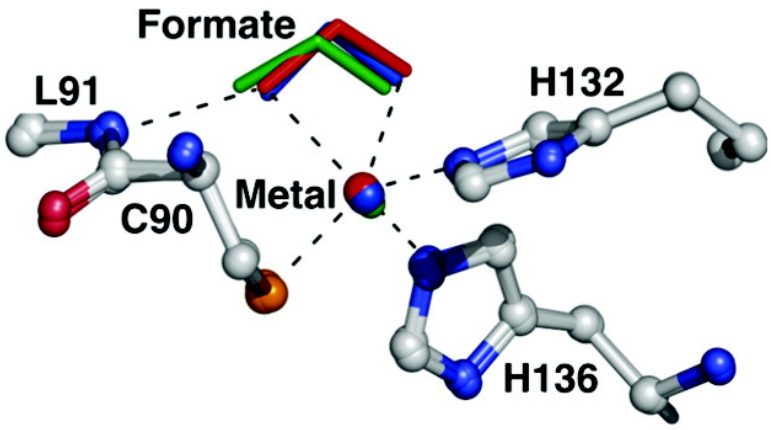

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Structures of *E. coli* Peptide Deformylase Bound to Formate: Insight into the Preference for Fe^{2+} over Zn^{2+} as the Active Site Metal

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While nearly all metalloproteases use zinc as their catalytic metal, one prominent exception is *E. coli* peptide deformylase (PDF), an enzyme that catalyzes the deformylation of nascent peptides generated during protein synthesis.[1] On the basis of the presence of a classical HEXXH zinc-binding motif,[2] *E. coli* PDF was originally thought to be a zinc enzyme. Subsequent studies demonstrated that the active site metal in wild-type PDF is iron,[3] making PDF the first iron metalloamidase. Given the rarity of iron-dependent metalloproteases, there has been great interest in elucidating the basis for PDF's distinct metal usage.

Attempts to clarify these differences have focused on analysis of its biochemical and structural properties. *E. coli* PDF's assignment as an iron enzyme was derived from comparisons of the catalytic activities of various metal-bound forms of PDF. In particular, the Fe^{2+}-bound PDF (Fe−PDF) has a 100-fold higher activity than the Zn^{2+}-bound form (Zn−PDF).[4,5] While alternate Ni^{2+}- (Ni−PDF) and Co^{2+}-bound forms (Co−PDF) were isolated and shown to exhibit nearly the same activity as that of Fe−PDF,[6] only Fe−PDF shares a key property of the as-isolated wild-type enzyme, that is, a rapid oxygen-dependent inactivation due to oxidation of Cys90, the third ligand to the metal.

In addition to these distinct metal-dependent activities, the Fe and Zn forms of *E. coli* PDF exhibited significant global differences. Denaturation profiles with guanidinium hydrochloride (GnHCl) revealed that Zn−PDF is intrinsically more stable than Fe−PDF as it requires 1.5 times higher GnHCl concentration to induce unfolding at room temperature.[3] Even more striking was the fact that Zn−PDF could be separated from Fe−PDF by hydrophobic interaction chromatography.[3]

Previous structural studies of PDF, however, have yielded no clear insight into the basis of these differences.[7−10] A comparison of the crystal structures of Zn−, Fe−, and Ni−PDF bound to Met-Ala-Ser by Becker et al.[9] revealed no differences in the overall fold and only a small difference at the active site, a slightly smaller tetrahedral volume at the zinc metal center. On the basis of the absence of clear structural differences, it was proposed that the tighter binding of Zn^{2+} rather than Fe^{2+} could hinder the metal's ability to alternate between tetrahedral and five-coordinate transition states. One of the limitations of this structural comparison, however, was that the structures contained bound water and, thus, did not allow for a direct examination of how the formyl group of a substrate interacts with the metal ion.

While our subsequent structures of Zn− and Co−PDF bound to the transition state analogue, (*S*)-2-*O*-(*H*-phosphonoxy)-L-caproyl-L-leucyl-*p*-nitroanilide (PCLNA), provided for such a comparison, here again, no structural differences were observed.[11] In both Co− and Zn−PDF/PCLNA complex structures, the *H*-phosphonoxy group was observed to bind in a monodentate fashion, with one oxygen bound to the metal and the other hydrogen bonded to the backbone NH of Leu91. As phosphate−oxygen bond lengths are longer than carbon−oxygen bonds, however, subtle differences of the binding of the true transition state could have been masked.

Due to the absence of metal-dependent differences in previous PDF structures, a satisfactory explanation for PDF's metal-dependent catalytic activity has been lacking. Herein, we report the high-resolution structures of Fe, Co, and Zn forms of *E. coli* PDF in complex with its deformylation product, formate. Comparison of these structures reveals a clear difference in the mode of formate binding to the metal that provides a rationale for understanding PDF's metal-dependent deformylation activity differences and potentially its metal-dependent global properties.

The different metalated forms of PDF were crystallized as described previously (Supporting Information). Phases were determined by the molecular replacement method using our original 2.9 Å Zn−PDF[8] structure as the starting model. The structures of Fe−, Co−, and Zn−PDF in complex with formate were determined to 1.85, 1.30, and 1.76 Å resolution, respectively (Table S1). Clear 5σ F_O-F_C density for the formate ion was observed in each of the three structures (Figure S3).

Unlike the structures of Fe− and Co−PDF, refinement of the Zn−PDF structure proved to be unexpectedly challenging. The initial model would not refine below an *R* factor of 29%, while the average thermal factors ($B = 46.25$ Å^2) were much higher than those for Co− ($B = 24.00$ Å^2) and Fe−PDF ($B = 23.90$ Å^2). Additionally, difference density (Figure S1) was found near the active site that persisted even after fitting of the zinc and formate ions. On the basis of the location of this density, we hypothesized that it might correspond to a second partially occupied Zn^{2+} ion bridged by Cys90. Refinement suggested an occupancy of 5% for this second Zn^{2+} site. This model was attractive since the presence of a second metal might lead to structural perturbation of the protein and inhibit its activity.

Consistent with this hypothesis, treatment of Zn−PDF with 300 mM EDTA led to a 1.2-fold increase in its catalytic activity (Figure S2). This increase was in the range expected for removal of the partially occupied site, but still much lower than the 100-fold higher activity exhibited by Fe−, Co−, and Ni−PDF, thus precluding the role of the second Zn^{2+} ion as being the source of the lower Zn−PDF activity compared to that of Fe−PDF.

Subsequently, Zn−PDF crystals were soaked with EDTA and then used for data collection. The resulting structure solved to 1.76 Å refined normally with $R = 17.30\%$, $R_{free} = 21.50\%$, and the *B* factor for the metal of 14.66 Å^2. In support of its correct assignment, the extra density attributed to the second Zn^{2+} ion was no longer present in this EDTA-soaked Zn−PDF structure.

As observed in previous structures,[9] the overall folds of Fe−, Co−, and Zn−PDF were nearly identical, with root mean square deviations (rmsd's) between the main-chain atoms of the Fe−PDF structure and those for Co− and Zn−PDF being 0.17 and 0.16 Å, respectively. In each structure, the active site metal was coordinated

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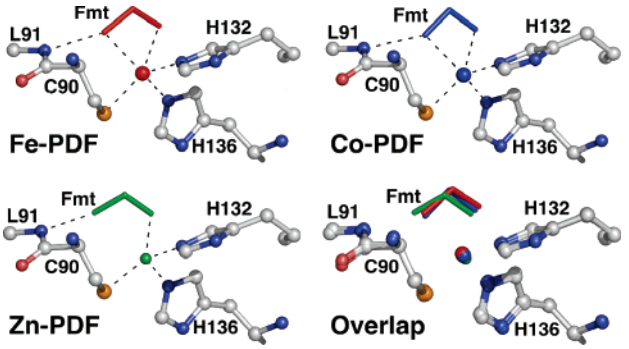

Figure 1. Comparison of the active sites of Fe−PDF, Co−PDF, and Zn−PDF bound to formate. In each case, formate and the corresponding metal ion have the same color. Note the bidentate binding of formate in Fe− and Co−PDF and its monodentate-binding mode in Zn−PDF.

by Cys90 and His132 and His136 from the conserved HEXXH motif. While in our original 2.9 Å resolution structure,[8] a water molecule was believed to be coordinated to the metal, the electron density of the current higher-resolution structures suggests that this ligand is better modeled as formate.

In all three structures, the bound formate is stabilized by hydrogen-bonding interactions with the backbone NH of Leu91 and side chains of Gln50 and Glu133. A key difference, however, is the mode of formate binding to the metal. In Fe−PDF and Co−PDF, formate binds to the metal in a bidentate fashion, with both formate oxygens situated within coordinating distance of the metal (Fe^{2+}···O(Fmt) bond lengths = 2.44 and 2.30 ± 0.17 Å (Figure 1)). In contrast, in the Zn−PDF structure, formate binds to Zn^{2+} in a monodentate fashion. The formate oxygen, O1, is bound to the metal at a distance of 2.09 ± 0.17 Å, while the second formate oxygen, O2, is positioned at a distance of 2.88 Å, which is too far to be coordinated to the metal. Consistent with this observation, monodentate binding of formate in the structure of Zn−PDF from *Leptospira interrogans*[12] (Zn^{2+}···O(Fmt) bond lengths = 2.31 and 2.58−2.85 Å) and a synthetic Zn−PDF−formate model by Goldberg and co-workers[13] (1.98 and 2.59 Å) have also been observed.

This difference in formate binding is important because it provides the first clues into the origins of the lower catalytic activity of *E. coli* Zn−PDF. In catalytic schemes for the mechanism of PDF, the crucial step is nucleophilic addition of a metal-bound hydroxyl on the carbonyl of the N-terminal formyl of the peptide substrate. A critical aspect of this mechanism is that the metal serves both as an electron-withdrawing group to help deprotonate the metal-bound water and as a Lewis acid to activate the bound carbonyl substrate. The differential binding of formate in these PDF structures suggests it is easier for both the hydroxyl and formyl−carbonyl to bind the metal in Fe−PDF and Co−PDF than in Zn−PDF. On the basis of the structure of the Zn−PDF−formate complex, it would be predicted that the formyl−carbonyl is only activated by its hydrogen bonds to the protein side chains of Leu91 and Gln50. Without Lewis activation by the metal ion, one would expect that Zn−PDF should be less active, which is exactly what is observed. Thus, the different modes of formate binding observed in these structures provide a reasonable explanation for *E. coli* PDF's metal-dependent activity differences.

The new question is "Why does formate bind differently in *E. coli* Zn−PDF than in Co− and Fe−PDF"? While currently unclear, one possibility is a stronger preference of zinc for a tetrahedral geometry.[2] To understand this argument, consider that the formyl−carbonyl oxygen can adopt either an orientation that forms optimal hydrogen bonds with Leu91 and Gln50 or one with suboptimal hydrogen-bonding interactions, but with an additional coordinate bond to the metal. In this model, the orientation adopted would be linked to the strength of the metal−carbonyl bond. If this interaction with the metal ion is weakened, as observed in Zn−PDF, the formate oxygen would be expected to adopt an orientation that promotes its stronger interaction with Leu91 and Gln50. Consistent with this hypothesis, the Zn−PDF structure exhibits the shortest hydrogen bonds between its formate ion and Leu91 and Gln50.

This model, however, is partially challenged by the recent discovery of deformylases whose Zn forms are almost as active as their Fe homologues.[14] If the origin of *E. coli* PDF's metal-dependent activity is due to zinc's geometric preference, then specific interactions must be present in these activated Zn−PDFs that help to either activate the formyl group or stabilize its binding to the zinc metal. Biomimetic studies could greatly aid in addressing this possibility.

A second explanation for this formate binding difference is that *E. coli* Zn−PDF exhibits enhanced flexibility relative to that of Co− and Fe−PDF. Support for this hypothesis comes from Zn−PDF's ability to bind a second metal ion, its higher stability (increased entropic stabilization), and its different behavior on hydrophobic interaction columns. This greater flexibility would allow Leu91 and Gln50 to form more optimal interactions with the formyl−carbonyl, thereby weakening its interaction with the metal. While more difficult, a comparison of the dynamics of highly active Zn−PDFs with that of *E. coli* by NMR may ultimately be required to test this hypothesis.

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Supporting Information Available: PDF−EDTA activity data and the details of the crystallographic structure determinations, including data collection and refinement statistics, the active site geometries, and formate omit maps. The coordinates have been deposited in the protein databank (PDB IDs: 1XEN, 1XEO, and 1XEM). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Meinnel, T.; Mechulam, Y.; Blanquet, S. *Biochimie* **1993**, *75*, 1061.
(2) Lipscomb, W. N.; Straeter, N. *Chem. Rev.* **1996**, *96*, 2375.
(3) Rajagopalan, P. T. R.; Yu, X. C.; Pei, D. *J. Am. Chem. Soc.* **1997**, *119*, 12418.
(4) Rajagopalan, P. T. R.; Datta, A.; Pei, D. *Biochemistry* **1997**, *36*, 13910.
(5) Ragusa, S.; Blanquet, S.; Meinnel, T. *J. Mol. Biol.* **1998**, *280*, 515.
(6) Groche, D.; Becker, A.; Schlichting, I.; Kabsch, W.; Schultz, S.; Wagner, A. F. V. *Biochem. Biophys. Res. Commun.* **1998**, *246*, 342.
(7) Meinnel, T.; Blanquet, S.; Dardel, F. *J. Mol. Biol.* **1996**, *262*, 375.
(8) Chan, M. K.; Gong, W.; Rajagopalan, P. T. R.; Hao, B.; Tsai, C. M.; Pei, D. *Biochemistry* **1997**, *36*, 13904.
(9) Becker, A.; Schlichting, I.; Kabsch, W.; Groche, D.; Schultz, S.; Wagner, A. F. V. *Nat. Struct. Biol.* **1998**, *5*, 1053.
(10) Dardel, F.; Ragusa, S.; Lazennec, C.; Blanquet, S.; Meinnel, T. *J. Mol. Biol.* **1998**, *280*, 501.
(11) Hao, B.; Gong, W.; Rajagopalan, P. T. R.; Zhou, Y.; Pei, D.; Chan, M. K. *Biochemistry* **1999**, *38*, 4712.
(12) Zhou, Z.; Song, X.; Li, Y.; Gong, W. *J. Mol. Biol.* **2004**, *339*, 207.
(13) Chang, S.; Sommer, R. D.; Rheingold, A. L.; Goldberg, D. P. *Chem. Commun.* **2001**, *22*, 2396.
(14) Serero, A.; Giglione, C.; Meinnel, T. *J. Mol. Biol.* **2001**, *314*, 695.

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