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Structural reconstruction of the catalytic center of *Li*PDF through multiple scattering calculation with MXAN

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Abstract

Peptide deformylase (PDF, EC 3.5.1.27) is essential for the normal growth of eubacterium but not for mammalians. Recently, PDF has been studied as a target for new antibiotics. In this paper, X-ray absorption spectroscopy was employed to determine the local structure around the zinc ion of PDF from *Leptospira Interrogans* in dry powder, because it is very difficult to obtain the crystallized sample of *Li*PDF. We performed X-ray absorption near edge structure (XANES) calculation and reconstructed successfully the local geometry of the active center, and the results from calculations show that a water molecule (Wat1) has moved towards the zinc ion and lies in the distance range to coordinate with the zinc ion weakly. In addition, the sensitivity of theoretical spectra to the different ligand bodies was evaluated in terms of goodness-of-fit.

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1. Introduction

Peptide deformylase (PDF; EC 3.5.1.27), as a new member of the zinc metalloproteases superfamily, is an enzyme responsible for the removal of a formyl group from nascent polypeptides (Adams, 1968; Livingston and Leder, 1969). This deformylation process is unique for bacteria and does not exist in mammalian protein synthesis, so that PDFs have been extensively studied as a new target of structure-mechanism-based antibiotics. Therefore, an improved understanding of the catalytic mechanism is the pre-requisite for the development of new drugs.

PDF utilizes a metal ion to affect the hydrolysis of an amide bond. In the native state of this enzyme structure, the metal ion is tetrahedrally coordinated by two histidines, a cysteine, and a water molecule. Peptide

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deformylase from a real pathogenic bacterium *Leptospira Interrogans* (*Li*PDF), is the first bacterial zinc PDF with high activity. Up to date, although the structure of the main intermediate product during *Li*PDF's catalytic cycle has been obtained by protein crystallography (PDB entry code: 1Y6H, in which a formate instead of a water coordinates to the zinc ion in the catalytic center), no structural data of *Li*PDF in native state is available due to the great difficulty to obtain the crystallized sample of *Li*PDF.

X-ray absorption spectroscopy (XAS) is a unique tool to determine the local structure of metal-containing protein at ultra-high resolution and can provide subtle structural variation information precisely (Hasnain and Hodgson, 1999; Hasnain and Strange, 2003). Here we report a preliminary study using XAS technique to detect the fine structure of *Li*PDF catalytic center, expecting an improved understanding of the catalytic mechanism.

2. Experiment

*Li*PDF has been over expressed in *E. coli* and purified according to the protocol previously reported (Zhou and Gong, 2004). Expression was performed in 1 l LB medium (100 μM $ZnCl_2$ added) and incubated at 37 °C. When OD600 reached 0.6, the cultures were induced by the addition of a final concentration of 800 μM IPTG at 37 °C for additional 4 h. The cells from 1 l of culture were harvested in 20 ml of cold (4 °C) buffer A (50 mM Hepes pH 7.5, 10 mM NaCl, 100 μg/ml phenylmethanesulfonyl fluoride, 100 μM $ZnCl_2$) and then lysed by sonication while keeping on ice. The clear supernatant was collected by centrifugation and applied to a DEAE-sepharose column equilibrated with 200 ml buffer B (50 mM Tris-HCl pH 8.0, 10 mM NaCl, 100 μM $ZnCl_2$). The column was eluted with 250 ml of buffer B plus a linear gradient of 10–500 mM NaCl. The fractions with PDF activity were concentrated and applied to a Superdex G-75 column pre-equilibrated with Buffer C (50 mM Tris-HCl pH 8.0, 50 mM NaCl, 100 μM $ZnCl_2$). For XAS studies, the purified protein was dialyzed against pure water and then was lyophilized to dry powder. No formate salts were added during expression and purification process.

X-ray absorption spectra at the Zn *K* edge were collected in "fluorescence yield" (FY) mode, at the X-ray absorption station (Beam line 1W1B) of Beijing synchrotron radiation facility (BSRF). The storage ring was operating at the typical energy of 2.2 GeV with the current decreasing approximately from 135 to 80 mA during a time span of 8 h. To suppress unwanted harmonics, a detuning of 30% was performed between the two crystals of the monochromator. The incident beam intensity was monitored and recorded using ionization chamber filled by 25% argon-doped nitrogen mixture and the fluorescence signal was collected and recorded by a fluorescence ionization chamber filled by argon gas.

3. Results and discussion

The theoretical ab initio calculation of the XANES region is of great interest for structural studies of biological systems. In fact, despite the fact that the magnitude of the scattering amplitudes from light elements (which biological systems are mostly made of) severely limits the energy range of the available experimental data, the precise form of the spectrum in the low energy region is extremely sensitive to a variety of structural details that are often crucial for our understanding of the subtle relations between structure and function. In this context, a wealth of information has been obtained by several XAS studies on active sites in metalloproteins (Hasnain and Hodgson, 1999; Mijovilovich and Meyer-Klaucke, 2003).

In order to extract detailed structure information around the zinc ion site, a new approach implemented by a code called MXAN (Minuit XANes) was introduced (Della Longa et al., 2001; Benfatto and Wu, 2003), which is capable of yielding a quantitative analysis for the spectrum from the absorption edge up to 200 eV, through fitting the reconstructed plot from ab initio calculation to the experiment data by adjusting the input structural model. This approach has the power of distinguishing the contributions between water molecular and oxygen atoms (Benfatto et al., 1997; D'Angelo et al., 2002). This method takes into account multiple-scattering (MS) events in a rigorous way through the evaluation of the scattering path operator (Natoli and Benfatto, 1986; Wu et al., 1996), and its effectiveness has been successfully tested in a number of interesting situations (Della Longa et al., 2001; Benfatto et al., 2003).

The best fitting result was reached by moving the neighboring atoms of the initial structure model by MXAN, Fig. 1 presents a comparison of the fitted curve with the experimental data. We have even introduced this method based on the *Ec*PDF crystal structure (PDB code: 1BS5) for initial model (Li et al., 2005) and in this paper present the improved best fitting which was based on the refined crystal structure of *Li*PDF (PDB code: 1Y6H). The cluster used in the fitting contains all the 50 atoms within 6 Å from zinc ion. As we can see in Fig. 1, all the main absorption features were reproduced very well, and consequently, the structure of the catalytic center of *Li*PDF has been reconstructed successfully. The difference in the two main peaks (experiment and best fitting) maybe coming from the choice of Muffin-Tin approximation, but the main structure information

are all from the region behind 10 eV after the E_0. Some refined critical distances are listed in Table 1, and for comparison, those data of *E. coli* PDF as the initial model are also listed there. According to the results of calculation, the structure of the catalytic center of *Li*PDF in native state has been plotted in Fig. 2.

The crystal structure of *Li*PDF has been reported (Zhou and Gong, 2004). However, this crystal was obtained with 4 M formate; so it is the product of *Li*PDF catalysis, in which a formate group binds with the zinc ion. Since the sample used for XAS was dialyzed against pure water, the metal center structure of *Li*PDF determined by XAS is of the native state, in which a water molecule Wat0 coordinates to the zinc ion, instead of a formate. Therefore, we substitute the formate group

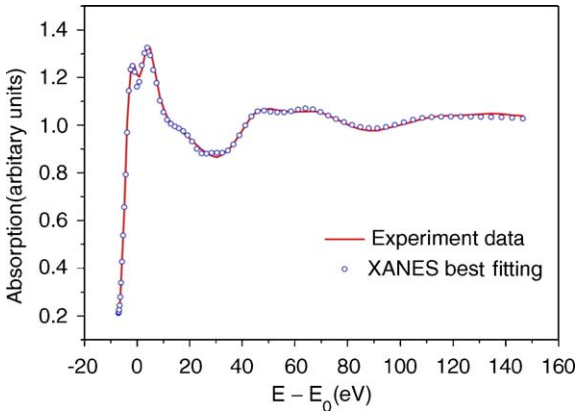

Fig. 1. Comparison between the best-fit result and the experimental data for dry powder sample of *Li*PDF. The theoretical result is obtained by moving all the neighboring atoms independently.

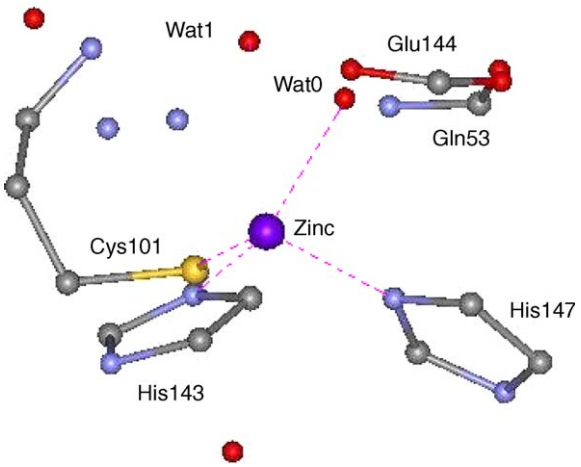

Fig. 2. Local structure of the catalytic center in *Li*PDF. The catalytic center calculated from *Li*PDF XANES fitting with carbon in gray, nitrogen in livid, oxygen in red and sulfur in yellow respectively. The coordinations of the zinc ion (in purple) are indicated in pink dash line. A water molecule (Wat0) coordinates with the zinc ion, and another water molecule (Wat1) lies at a distance within the range to coordinate with the metal ion weakly.

Table 1
Some critical distances in structures

	Atom pair	Bond length (Å)	Atom pair	Bond angle (deg)
*Li*PDF XAS structure	Zn-Cys101SG	2.27	His143-Zn-His147	109.5
	Zn-His143NE2	2.07	His143-Zn-Wat0	102.4
	Zn-His147NE2	2.01	His147-Zn-Wat0	90.6
	Zn-Wat0OH2	2.38	His143-Zn-Cys101	110.1
	Zn-Wat1OH2	2.72	His147-Zn-Cys101	99.7
			Wat0-Zn-Cys101	139.9
Former XAS structure	Zn-Cys101SG	2.21	His143-Zn-His147	112.1
	Zn-His143NE2	2.08	His143-Zn-Wat0	99.5
	Zn-His147NE2	2.08	His147-Zn-Wat0	88.8
	Zn-Wat0OH2	2.25	His143-Zn-Cys101	118.7
	Zn-Wat1OH2	2.78	His147-Zn-Cys101	105.4
			Wat0-Zn-Cys101	133.5
E. coli PDF crystal structure	Zn-Cys90SG	2.13	His132-Zn-His136	107.4
	Zn-His132NE2	2.02	His132-Zn-Wat0	100.5
	Zn-His136NE2	2.16	His136-Zn-Wat0	93.4
	Zn-Wat0OH2	2.25	His132-Zn-Cys90	117.6
	Zn-Wat1OH2	3.14	His136-Zn-Cys90	102.2
			Wat0-Zn-Cys90	131.2

The upper is the theoretical result calculated by MXAN. For comparison, the middle is former XAS structure based on *Ec*PDF for initial model, and the catalytic center of the *Ec*PDF as the initial structure of MXAN fitting is also listed in the lowest one.

by Wat0 which was similar to the catalytic center of *Ec*PDF. As shown in Table 1, the critical distances of the first coordinating shell atoms in this structure are very close to those in the initial model, demonstrating the conservation of catalytic centers of different PDFs (Meinnel and Blanquet, 1995). However, unexpectedly, in refined structure, another conserved water molecule Wat1 (from a conserved three-molecule water chain near the zinc ion) that stabilizes the active center in the crystal structure of *Li*PDF-formate, was observed to move towards the zinc ion in this structure, so that it lies in the distance range to coordinate with the metal ion weakly. The function of this water molecule in the catalytic mechanism still needs to be studied further.

To evaluate the sensitivity of the theoretical spectra to the coordinating distances, the four nearest ligand bodies were moved independently along radial direction, and the residual square, which describes the goodness-of-fit between theoretical spectrum and experimental data, was calculated every time. The results were plotted in Fig. 3. Apparently, every plot reached the minimum when all the ligands locate the best-fitted position, and any shift of any one ligand body from this position would reduce the goodness-of-fit, corresponding to the increase of residual square. However, the move towards to the absorption center of His143 and His147 increased the residual square very sharply, which can be attributed to the great interaction of the five-member ring with other atoms (see Fig. 2). When both the Histidine run away, their influences behaved similarly with the impact of Cys101, for there was no great interaction among different ligand bodies. Wat0 molecule, had a smaller contribution to the residual square increase than other three ligand bodies when moving either close to or far away from the central atom, because there is just one oxygen atom in this ligand body.

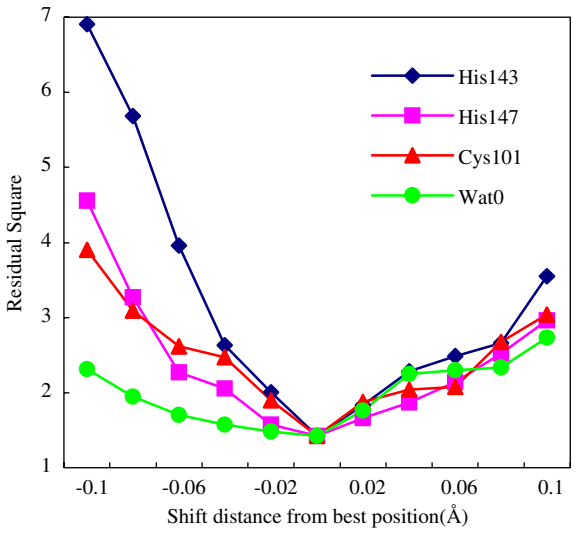

Fig. 3. Changes of goodness-of-fit along with the shift of all the nearest ligand bodies from the best-fitted positions. Every plot was obtained by moving one ligand atom only when fixed others at original location.

4. Conclusion

We have shown that the catalytic center structure of *Li*PDF can be refined quantitatively by a new analysis procedure of MXAN. Full MS analysis of the Zn *K*-edge XANES spectrum, taking into account a cluster including 50 atoms lying up to 6 Å from the central zinc atom, reconstructed detailed structural information around the zinc site successfully. Unexpectedly, we have found that the water molecule Wat1 moves towards the zinc ion in this structure, lying in the distance range to coordinate with the metal ion weakly. If this movement is necessary for the activity of PDF, there is a need for further studies on *Li*PDF containing different metal ions. Different ligand bodies contribute to the goodness-of-fit differently, which can be demonstrated by moving them independently and evaluating the sensitivity of the residual square to their movement.

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