Quantitative investigation of the local structure around cobalt ion in two different peptide deformylase by XANES spectroscopy

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Abstract. The local atomic environment of a cobalt ion bound to the foldings of the active site of LiPDF (Co-LiPDF) and EcPDF (Co-EcPDF) in a pH 8.0 buffer solution has been determined using X-ray absorption near-edge spectroscopy (XANES) combined with \textit{ab initio} calculation. Both active metal sites have been perfectly reconstructed using suitable initial structure modes. Results show that the nearest water (Wat1), which can be deprotonated and turned into hydroxyl, locates at 1.91 Å from the metal centre in Co-LiPDF while a high enzymatic activity occurs at 2.15 Å in the Co-EcPDF. The stronger water-bonding of cobalt makes the distance between water and Glu144 (wat1-Glu144) longer. Such lengthening weakens the polarization capability of Glu144 to wat1 being responsible of the lower enzymatic activity of Co-LiPDF respect to Co-EcPDF.

1. Introduction

Peptide deformylase (PDF, EC 3.5.1.27) is an important metalloprotein in particular because of its role in drug design target researches since the deformylating apparently does not occur in eucaryotic cells [1-4]. In eubacterium, during the elongation, the formyl group of the polypeptide chain is removed hydrolytically by the PDFs, apparently universally conserved and essential for bacterial survival [5].

A previous work has shown that the activity of \textit{Escherichia coli} PDF (EcPDF) is strongly affected by changes of the metal ion. On the basis of the presence of a classical HEXXH zinc-binding motif, PDF was originally thought to be a zinc enzyme. Later studies demonstrated that the active site metal...
in wild-type PDF is iron. Actually, Fe$^{2+}$ ion in EcPDF is easily oxidized to Fe$^{3+}$ so that EcPDF loses its activity. EcPDF with Co$^{2+}$ replacing Fe$^{2+}$ shows a similar high catalytic activity with the advantage of an improved stability while Zn-EcPDF shows a decreased activity [6]. In contrast to EcPDF, Co$^{2+}$-bound Leptospira interrogans PDF (Co-LiPDF) is much less active than Zn-LiPDF [7]. Little is known about chemical and physical mechanisms responsible for the difference in the enzymatic activity of Co-LiPDF and Co-EcPDF in particular because of the lack of structural information around the metal sites of these proteins, largely due to the difficulty to obtain crystallized PDF sample.

X-ray absorption spectroscopy on biological systems (BioXAS) is a short-range probe sensitive to variations in the local structure and its use has brought considerable contributions to the reconstruction of the local environment of specific atoms in many biological systems [8-12]. With XAS biological samples can be studied in a variety of forms, including oriented single protein crystals, disoriented micro-crystals, frozen or room-temperature solutions and proteins inserted in membranes. Here, we present Co K-edge XAS spectra of Co-LiPDF and Co-EcPDF in a pH 8.0 buffer solution. Combining ab initio multiple scattering calculations, we applied the MXAN package to analyze data and extracted subtle local structure information around metal sites for LiPDF and EcPDF, especially the position of bonded water molecules.

2. Materials and methods

E.coli BL21(DE3) cells carrying plasmid pET-22b-def [6] and plasmid pET22b-LiPDF [7] were expressed, respectively. Cells were harvested by centrifugation and resuspended in 25 ml of buffer A (50 mM Tris-HCl 7.5, 10 mM NaCl). After sonication, cell debris was removed by centrifugation. The supernatant (25 ml) was applied onto a HiTrapTM 5 ml Q HP (Amersham Pharmacia Biotech) column equilibrated with buffer B (50 mM Tris-HCl pH 8.5, 10 mM NaCl). The column was eluted with 250 ml of buffer B plus a linear gradient of 10mM - 1M NaCl (AKTA purifier 900, Amersham Pharmacia Biotech). The fractions with LiPDF and EcPDF activity were concentrated and applied to a SuperdexTM 75 column (16/60, Amersham Pharmacia Biotech) pre-equilibrated with buffer C (50 mM Tris-HCl pH 8.5, 50 mM NaCl). The sample homogeneity was monitored by SDS-PAGE and the protein concentration was determined by Braford assay with BSA as the standard.

To get samples of Co-PDF, we first obtained 30 mg of metal-free PDF protein by adding 15 ml of 0.1 M EDTA to 0.5 ml of 60 mg/ml protein for 2 hours at 277 K. The additional EDTA was then removed by direct exchange dialysis and concentrated centrifugal tube. The procedure was repeated twice by direct exchange dialysis (dilute 100*100 times) and concentrated centrifugal tube (dilute 15*15 times). Metal-free PDF (0.5 ml, 20 mg/ml) was incubated with 1.2 mM CoCl$_2$ 60 ml for 2 hours at 277 K and then concentrated to 20 mg/ml. The purified Co-LiPDF and Co-EcPDF were dialyzed against 50 mM Tris-HCl pH 8.0. The sample homogeneity was monitored by SDS-PAGE. Finally, all PDF concentration was about 60 mg/ml and the Co$^{2+}$ concentration ~200 ppm. The protein concentration was determined by Braford assay with BSA as the standard [6].

Measurements at the Co K-edge were carried out at the beamline 1W1B of the Beijing Synchrotron Radiation Facility (BSRF) in the fluorescence yield (FY) mode at room temperature. The typical energy of the storage ring was 2.5 GeV and experiments were performed with a decreasing electron current from 250 mA to 160 mA. The incident beam intensity was monitored using an ionization chamber flowed by a 25% argon-doped nitrogen mixture while the fluorescence signal was collected by means of a Lytle detector flowed by argon gas. After XAFS measurement, we recycled the samples and measured again the activity of the samples. Data point out that proteins did not lose their activity. The behavior is probably associated to the high radiation resistance of LiPDF.
In order to extract structural/geometrical information around the metal site, we performed a quantitative analysis of the XANES spectrum using the MXAN code. This package is capable of performing a quantitative analysis of a XANES spectrum from the absorption edge up to 200 eV via comparison between experimental data and many theoretical calculations obtained by changing relevant geometrical parameters of the atomic site [13-14]. The X-ray absorption cross-sections have been calculated using the full multiple scattering (MS) scheme in the framework of the muffin-tin (MT) approximation for the shape of the potential [15-16]. In particular, the exchange and correlation part of the potential have been determined on the basis of the local density approximation of the self energy. Inelastic processes have been taken into account by a convolution with a broadening Lorentzian function having an energy-dependent width of the form \( \Gamma(E) = \Gamma_c + \Gamma_{\text{mfp}}(E) \). The constant part \( \Gamma_c \) includes the core-hole lifetime (1.8 eV) [17] and the experimental resolution (1.5 eV), while the energy-dependent term represents all intrinsic and extrinsic inelastic processes [18-19]. The muffin-tin radii were chosen according to the Norman criterion with a 15% overlap [20-21]. This method takes into account for MS events in a rigorous way through the evaluation of the scattering path operator [15, 22]. Its reliability has been successfully tested over the years in a number of different applications [13, 18, 23]. The spectrum is convergent when the cluster includes 39 atoms within a radius of 5.5 Å. Therefore the local structure of both Co-LiPDF and Co-EcPDF active centers can be properly represented by a cluster that contains 50 atoms within 6 Å from the metal ion.

3. Results and Discussion

In Fig.1, we reported the Co K-edge XANES spectrum of Co-EcPDF (black curve) and Co-LiPDF (red curve) in a buffer solution at pH 8.0. Spectra are characterized by four main features: P, A, B and C. As we know, XAS features in this region are mainly dominated by scattering events of neighbor atoms around the photoabsorber. An obvious pre-edge peak can be also observed in the spectrum due to unoccupied 3d states. The main peak A is due to the scattering among the nearest atoms. The presence of this structure confirms the atomic coordination of Co. The valley B is related to higher order MS pathways of the excited photoelectrons involving nearest neighboring atoms while the position of peak C is correlated to the distance of first-shell atoms. According to Natoli’s rule [reference] it can be seen that the subtle difference of peak C between the two XANES spectra indicates that the average distance of the first-shell atoms around Co in Co-EcPDF is longer than in Co-LiPDF.
In order to obtain accurate structural information around the metal site, based on the available crystallographic structure of Li-PDF (PDB code 1Y6H(B)) [24] and Ec-PDF (PDB code 1XEM) [25] we performed also a quantitative analysis of XANES spectra using the MXAN code. Fig. 2 shows the best fits (blue dots) of experimental (red curve) XANES spectra of Co-EcPDF (left) and Co-LiPDF (right) in a buffer solution at pH 8.0. As we can see, all features in the near-edge region of the Co K-edge have been successfully reproduced. The square residue values for these calculations are 1.362 and 0.453, respectively. Some refined critical distances of the best fits are summarized in Tables 1. Comparisons of the local structure obtained by best fits of the Co-EcPDF and the Co-LiPDF are plotted in Fig. 3.

Figure 3. A: the local structure of the best fit of Co-EcPDF; B: the local structure of the best fit of Co-LiPDF; C: comparison between the local structure of the best fit of Co-EcPDF (colored stick) and Co-LiPDF (thick yellow sticks) at pH 8.0.

In the catalytic process of Co-EcPDF, the most important step is associated to the proton of the catalytic water molecule (wat1) that is transferred to the amide at the N-terminus of the peptide with the help of Glu133 (Li-PDF: Glu144). The added positive charge makes possible to nitrogen leaving the group [25-26]. Our result shows a fundamental difference between the local structures around metal site of Co-EcPDF and Co-LiPDF: the position of the wat1. The Co-wat1 distance in Co-LiPDF is 1.91 Å, much shorter that of Co-EcPDF that is 2.15 Å. The stronger water-bonding of cobalt affects the distance between water and Glu144 (wat1-Glu144) that become longer (3.25 Å). The mechanism weakens also the polarization of Glu144/133 to wat1 and prevents the transfer of the proton of wat1 to the amide at the N-terminus. This is probably the main reason at the base of the lower enzymatic activity of Co-LiPDF respect to Co-EcPDF.

Table 1: Refined critical distances and error of the best fits of Co-LiPDF and Co-EcPDF
4. Conclusion

X-ray absorption spectroscopy (XAS) is a powerful tool to investigate the local coordination environment of specific atoms with sub-atomic resolution. In this work we investigated the active site of EcPDF and LiPDF by using XANES combined with ab initio multiple scattering calculations. All features in the near-edge region of the Co K-edge have been successfully reproduced and detailed structural information around the metal ion has been obtained. From the analysis, we show that the bond between the Co ion and the catalytic water molecule in Co-LiPDF is much shorter than in Co-EcPDF, explaining the lower enzymatic activity of the former. Further researches on PDF containing different metal ions and with different pHs solution can be considered to improve our understanding of pH-dependent and metal-dependent enzymatic activity providing relevant structural information for any further applications of these proteins, in particular for the specific design of new drugs.

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