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3D fine structure of copper binding site in C-terminal of human prion and its comparison with rabbit prion

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Abstract: Prion protein is considered to be a copper-related protein, and the copper ion induces the secondary structure changes of prion proteins, making them aggregate and deposit in the central nervous system, and causing transmissible spongiform encephalopathies (TSE). The three-dimensional fine structure of C-terminal high-affinity copper binding site was determined in recombinant human prion 91-231 ($HuPrP^{91-231}$), which lacks the octapeptide repeat region, using X-ray absorption near-edge structure (XANES) combined with the *ab initio* calculations in the framework of the multiple-scattering theory. Results show that amino acid residues have closer distances from the copper ion compared with those of the rabbit prion. This might be the reason why different species have different immunities to TSE.

Key words: HuPrP; copper; XANES; MXAN; RaPrP

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人朊蛋白C端铜结合位点三维精细结构以及与兔朊蛋白的对比

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摘要:朊蛋白是铜结合蛋白,而铜离子介导了朊蛋白的二级结构变化,使其聚沉在中枢神经系统中,导致传染

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性海绵状脑病. 重组人朊蛋白 91-231 截去了八肽重复区域,利用 X 射线吸收谱近边结构解析出了 C 端高亲和性的铜结合位点的三维精细结构. 结果显示与兔朊蛋白相比人朊蛋白中氨基酸与铜结合更紧密. 本研究可能揭示了不同物种对传染性海绵状脑病具有不同抗性的原因.

关键词:人朊蛋白;铜;X 射线吸收谱;MXAN;兔朊蛋白

0 Introduction

Prion protein (PrP), the GPI membrane-anchored protein, exists in two distinct forms, the cellular form (PrP^{C}) and the scrapie form (PrP^{Sc}), and they have the same covalent sequence but different secondary conformations[1]. PrP^{Sc} is able to catalyze the conformation change of normal PrP^{C} and convert it into the infectious isoform. Deposition of PrP^{Sc} in the central nervous system is considered as the key event in the development of transmissible spongiform encephalopaties (TSE)[2]. Although the normal physiological function is unknown, PrP has high selectivity and affinity to copper(Ⅱ) ions[3]. Copper(Ⅱ) ions do not change the conformation directly, but destabilize the native fold of PrP^{C} and make the transition more favorable[4]. There are five copper binding sites in a full-length PrP, four of which are located in the octapeptide repeat domain within residues 60-91 in N-terminal, and the fifth has the highest affinity, which contains residues 91-111 in C-terminal[5]. It is reported that the conformation changes occurred at the C-terminal domain[6]. Therefore, we are more interested in the fifth copper binding site.

Most mammalian and avian species, including man, are sensitive to TSE. The human forms of prion diseases contain Creutzfeldt-Jakob disease and Kuru[1]. However, the rabbit is one of the few mammalian species reported to be resistant to TSE on account of the conformation stability of rabbit prion (RaPrP) itself[7]. It may be interesting and significant to compare the different structures of copper-binding sites between HuPrP and RaPrP, and to provide an essential insight into the molecular mechanism of the immunity to TSE.

In this work, we used the XANES technique to determine the three-dimensional (3D) fine structure of the fifth copper-binding site in the recombinant protein $HuPrP^{91-231}$, which lacks the octapeptide repeat domain in N-terminal. XANES is sensitive to variations in the detailed structure information around selected atoms in biological systems[8-9]. We measured the Cu *K*-edge X-ray absorption spectra of $HuPrP^{91-231}$, and reconstructed the 3D local structures around metal ions using the MXAN, a new procedure to fit the XANES in the framework of the multiple-scattering theory.

1 Material and methods

1.1 Protein expression and purification

The plasmid pET30a-$HuPrP^{91-231}$ was transformed into *E. coli* BL21(DE3) cells and the recombinant protein was expressed, purified and refolded as described in Ref. [10]. $HuPrP^{91-231}$ was dialysed in 20 mmol/L HAc/NaAc buffer with pH 5.5. The concentration of the protein was determined by UV absorption at 280 nm.

1.2 XANES measurement and analysis

The Cu *K*-edge X-ray absorption spectra were collected at the X-ray Absorption Fine Structure Station (Beamline 14W) of Shanghai Synchrotron Radiation Facility (SSRF) in the fluorescence mode at room temperature. The storage ring operated at a typical energy of 3.5 GeV with the current ranging from 300 to 200 mA during a time span of 12 h. A Si[311] double crystal monochromator and a focusing mirror with a cut-off energy of 22.5 keV were used throughout the experiment. Energy calibration was carried out with a copper foil. The incident beam intensity was monitored using an ionization chamber filled by a 25% argon-doped nitrogen mixture and the fluorescence signals were

collected by a Lytle detector filled with argon gas.

Experiments were performed upon samples in solution kept in a 1.2-mm-thick Teflon spacer cell sealed by Kapton films. The samples contained about 1.76 mmol/L HuPrP$^{91-231}$ in the 20 mM HAc/NaAc buffer, pH 5.5. A solution of $CuAc_2$ was added with a mole ratio $c_{Cu^{2+}} : c_{HuPrP} = 0.8 : 1$, certifying that binding occurred at a single high-affinity site. To ensure the liability of the experiment, each sample was measured twice in sequence under the same experimental conditions.

We quantitatively analyzed the XANES spectrum of HuPrP to extract the geometrical information using the package MXAN. The software is based on the full potential calculation in the framework of the multiple scattering theory and able to fit the XANES spectrum from the absorption edge up to 200 eV via varying the configurations of the coordinated atoms or groups around the absorbing metal ions[11-12]. The convergent cluster for calculations contains about 40 atoms with a radius of 7 Å (1 Å=0.1 nm).

2 Results and discussion

It is an initial and necessary step to choose a reasonable starting cluster for MXAN fits, though the final result of the geometrical structure from the XANES fit does not depend on the input of the starting model. In previous researches, a typical initial cluster was chosen from the data of crystallographic or NMR structures. Due to the flexible nature of the loop region HuPrP$^{91-122}$, there are no such high resolution data. So here the initial cluster should be constructed according to the knowledge reported before. As mentioned in Ref. [13], the first coordination shell amino acid residues of the fifth copper-binding site may be His 96, His 111, Gln 98 and Met 109. In our previous work[14], we also reported the same coordination residues on the copper-binding site of the RaPrP$^{90-121}$. And then theoretical XANES spectra at Cu *K*-edge for the HuPrP were calculated as functions of the cluster size, as shown in Fig. 1. It is found that the experimental features were reproduced well in the calculated spectrum when the cluster contained 20 atoms with a radium of 4.4 Å. When the cluster was further up to 7.6 Å, no new features appeared in the calculated spectrum. That means the theoretical spectrum was made convergent with this initial cluster.

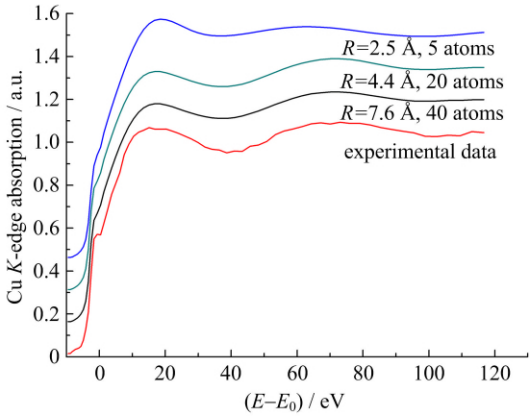

Fig. 1 Experiment data (bottom line) and calculated spectra as functions of the cluster size (upper lines)

As a result, with a radius of about 7 Å, the structure of copper-binding site in RaPrP$^{90-121}$ was chosen as the initial cluster, shown in Fig. 2. A comparison between experimental data and calculated spectrum using this initial cluster is shown in Fig. 3. Fig. 3 exhibits that the calculation spectrum reproduced all of the features of the experimental one, although with some little discrepancies presented both in the positions and intensities of the features. It suggests that the chosen cluster is appropriate but the geometrical configuration of the cluster should be rearranged.

Fig. 4 shows the best calculation spectrum, compared with experimental data of Cu-HuPrP$^{91-231}$. An excellent agreement was achieved, both in the positions and the intensities of the features. Finally, we provided an optimal cluster with correct atom types and their appropriate positions around the Cu sites in the Cu-HuPrP protein. The key structural parameters extracted from the XANES fit are summarized in Tab. 1. The sketch of the corresponding cluster is shown in Fig. 5, in contrast to that of RaPrP$^{90-228}$[14].

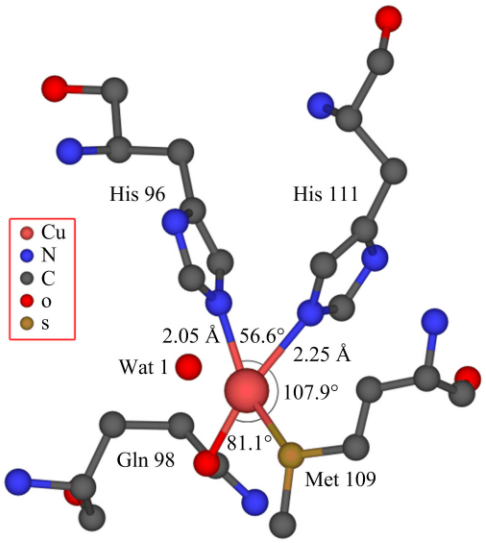

Fig. 2　Initial cluster of HuPrP constructed for multiple scattering calculations

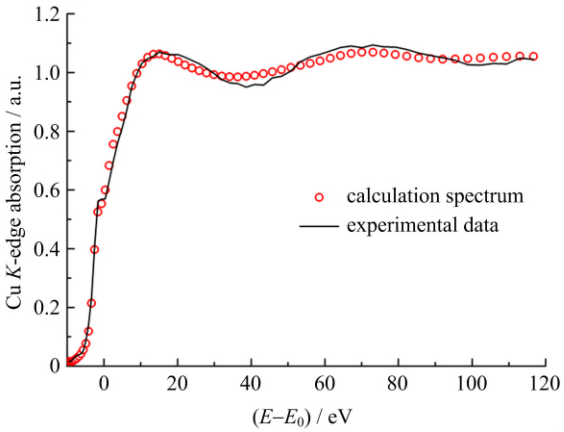

Fig. 3　Comparison between the calculated spectrum using the initial cluster and the experimental one

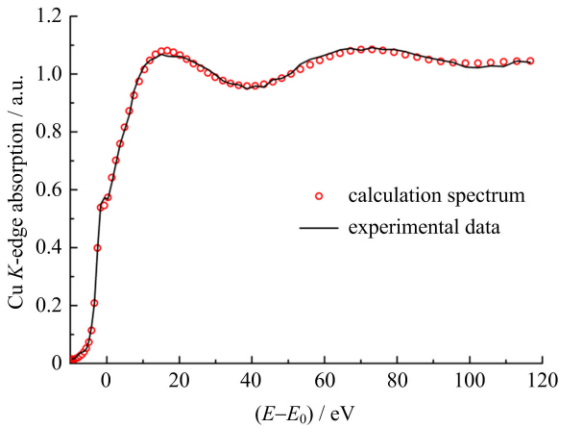

Fig. 4　Comparison between the best-fit XANES spectrum at Cu *K*-edge and the experimental data in the HuPrP

Tab. 1　Comparison of the geometrical parameters around the Cu site between HuPrP and RaPrP

atom pairs	distance/Å	
	HuPrP	RaPrP
Cu—N(His 96)	**1.96±0.02**	**2.05±0.02**
Cu—N(His 111)	**1.98±0.02**	**2.25±0.02**
Cu—O(Glu 98)	1.95±0.02	1.91±0.03
Cu—S(Met 109)	2.28±0.03	2.27±0.03
Cu—O(Wat 1)	3.23±0.04	3.15±0.06

【Note】 The parameters of HuPrP is from the XANES fit and that of RaPrP are from Ref. [14]. The latter is also extracted from the XANES by MXAN.

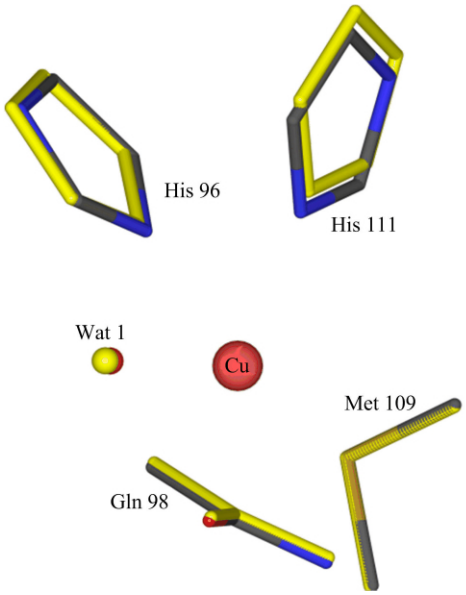

Fig. 5　The coordination of the copper ion (pink ball), RaPrP is shown in yellow and HuPrP is shown in color

It is important and interesting to compare the copper-binding site structures of HuPrP$^{91-231}$ with those of RaPrP$^{90-238}$[14], which are also extracted from the XANES by MXAN. It is noticed that both of them are penta-coordination, in the shape of distorted square-pyramid, and share the same coordinate amino acid residues. But a significant difference is the bond lengths between histidine residues and copper ions. The bond lengths in HuPrP are shorter, showing a stronger interaction between metal ions and proteins, which makes it more favourable to be the misfolded isoform. This is a structural proof of the detailed molecular mechanism of the conformation conversion of prion proteins introduced by copper ions.

3 Conclusion

In summary, we investigated the local fine structure around the copper binding site in $HuPrP^{91-231}$ by the XANES spectroscopy combined with *ab initio* full multiple scattering calculations and provided an exact 3D structure in the loop region. Compared with previous work we have done on the RaPrP, the HuPrP shows a stronger interaction with copper ions, and is converted into a greater conformation change. This work provides valuable insights into the underlying molecular mechanism of immunity to TSE.

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