

Zn and Co XAFS Studies on the Core Site Models of Hydrolases

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Introduction

Active sites of metzincin-clan proteases possess a motif sequence represented by $\text{HEX}_2\text{HX}_3\text{GX}_2\text{H}$, where H and E denote His and Glu residues, respectively. In the course of our model studies using synthetic peptides having this type of amino acid sequence, we found that they show UV-vis. and resonance Raman spectra characteristic of μ -hydroxy- μ -peroxo dinuclear Co^{III} species, $\text{Co}^{\text{III}}-(\mu\text{-OH})(\mu\text{-O}_2)\text{-Co}^{\text{III}}$, when mixed with Co^{2+} in aqueous organic media, and that these species shows hydrolase activity stronger than that containing $\text{Zn}^{2+}/\text{HEX}_2\text{HX}_3\text{G-X}_2\text{H}$. In this study, we confirmed that $\text{Co}^{2+}/\text{HEX}_2\text{HX}_3\text{G-X}_2\text{H}=1/1$ generates a species having a $\text{Co}^{\text{III}}-(\mu\text{-OH})(\mu\text{-O}_2)\text{-Co}^{\text{III}}$ bridging structure when exposed to air.

Experimental

In the XAS experiments, a thirteen-residue peptide AHELTHKEGR KHY (**L**) was used as that having the motif sequence, $\text{HEX}_2\text{HX}_3\text{GX}_2\text{H}$. This peptide was synthesized in the solid phase by using Boc-chemistry. Detailed spectroscopic study on $\text{Co}^{\text{II}}/\text{AHELTHKEGR KHY} = 1/1$ in aqueous solutions indicated that two reactions are competing in the solution: rise and decay of a species which can be estimated as a $\text{Co}^{\text{III}}-(\mu\text{-OH})(\mu\text{-O}_2)\text{-Co}^{\text{III}}$ compound. The decay of this species led to unknown fragments. Based on these result, two powder samples, **1** and **2**, were prepared for XAFS experiments: $\text{Co}(\text{NO}_3)_2$ and **L** were mixed into an aqueous DMSO (1:1) solution under Ar. This solution was divided into two parts: One was exposed to air in a moment and then immediately frozen in liq N_2 . The other was frozen in liq N_2 without exposing to air. These frozen materials were lyophilized. The obtained powders were sealed into polyethylene pellets under Ar as samples **1** and **2**, respectively, in a well maintained glove box ($\text{O}_2 < 0.1$ ppm)

The Co K-edge XAFS spectra of **1** and **2**, as well as the other pellets of authentic samples, $[\text{Co}(\text{NH}_3)_6]^{3+}$, **3**; $[\text{Co}(\text{H}_2\text{O})_6]^{2+}$, **4**; $[(\text{NH}_3)_5\text{Co}-(\mu\text{-O}_2)\text{-Co}(\text{NH}_3)_5]^{5+}$, **5**; $[(\text{en})_2\text{Co}-(\mu\text{-O}_2)(\mu\text{-OH})\text{-Co}(\text{en})_2]^{4+}$, **6**, were observed at 295 K in transmission mode using the EXAFS facilities of the beam line BL-10B of Photon Factory in the National Institute of High Energy Physics (KEK). Si(311) channel-cut monochromator was used. Monochromator were calibrated using the 9660.0-eV K-edge Zn foil. Data were analyzed by using REX combined with FEFF8.0. The equation of motion (EM)

method [1] was employed to treat the effect of vibration modes in ligands.

Results and Discussion

Comparison the XANES spectra of **1** and **2** with **3** and **4** revealed that 1) sample **1**, which was exposed to air, has an intermediate threshold energy (E_0) between Co^{II} and Co^{III} , whereas sample **2** conserves the valence state of Co^{II} , and that sample **1** has an intrinsically similar line-shape to those of μ -oxo bridged species **5** and **6**. Considering the sampling process, sample **1** should be the mixture of Co^{II} species contained in sample **2** and $\text{Co}^{\text{III}}-(\mu\text{-OH})(\mu\text{-O}_2)\text{-Co}^{\text{III}}$ species. Deconvolution of the XANES spectrum of **1** indicated that the sample contained 28 % of Co^{II} species and 72 % of $\text{Co}^{\text{III}}-(\mu\text{-OH})(\mu\text{-O}_2)\text{-Co}^{\text{III}}$. EXAFS analysis for sample **1** was carried out on the intrinsic function extracted from the

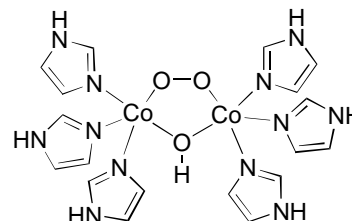


Fig. 1. Estimated core of $\text{Co}^{\text{II}}/\text{AHELTHKEGR KHY} = 1/1$ exposed to air (sample **1**)

mixed spectrum assuming this content.

EXAFS function of sample **1** was well explained by the structure given in Fig. 1. FEFF-EM simulation based on this structure gave bond lengths $\text{Co-O}(\text{O})= 1.87 \text{ \AA}$, $\text{Co-O}(\text{H})=1.93 \text{ \AA}$, $\text{Co-N}=1.94 \text{ \AA}$, and $\text{Co-Co}=3.29 \text{ \AA}$. Each imidazole group situates co-facial in the plane composed of Co ion and the imidazole plane; thus, imidazoles are not inclined from Co-N axes.

References

- [1] A. V. Poirarkova and J. J. Rehr, Phys. Rev. B **59**, 948 (1999).

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