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STUDY ON THE LOCAL STRUCTURES OF COMMD6 AND 7 BOUND TO Cu(II) BY EXAFS/XANES MEASUREMENTS

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Introduction

COMMD6 and COMMD7 belong to COMMD (COpper Metabolism gene *MURR1* Domain) family and, in human, the family consists of 10 members. COMMD6 and COMMD7 have 85 and 200 amino acid residues, respectively. In the COMMD family, COMMD1 has been studied the best and it is related to copper metabolism[1], regulation of transcription factor NF-κB, and so on. So COMMD6 and COMMD7 are considered to be important for copper metabolism, and our group aimed to clarify the mechanism of their binding to Cu.

Their structural information is not reported because of their structural novelty. Therefore we attempted to measure EXAFS/XAFS spectra of COMMD6-Cu(II) and COMMD7-Cu(II), and we analyzed the data.

Experimental

COMMD6 and COMMD7 were dissolved in Tris buffer (20 mM Tris, 100 mM NaCl, pH7.5). We prepared three COMMD6 samples (concentration of protein: 1.1mM, 1.4mM, 1.8mM) and three COMMD7 samples(concentration of protein: 0.5mM, 1.0mM, 1.5mM), added Cu(II)(final concentration: 20mM), respectively. Cu K-edge X-ray absorption measurements were performed at BL-12C of Photon Factory equipped with a Si(111) double crystal monochromator. X-ray absorption spectra were recorded in the fluorescence mode using a 19 element solid state detector. The protein solutions were sealed in PTFE sample cells which have Kapton windows and frozen by liquid nitrogen prior to the measurements. The samples were kept at low temperature using a cryocooler during the measurements.

Results and Discussion

The data are shown in Fig.1 (EXAFS data of COMMD6 and COMMD7) and Fig.2 (XANES data of COMMD6 and COMMD7). The numeric values of each graph are concentrations of protein. There were differences of shape between COMMD6 and COMMD7, especially in EXAFS data; the number of main peaks was one (COMMD6) and two (COMMD7), respectively. It suggested that COMMD7 bound Cu(II) more strongly than COMMD6. This corresponds to the fact that COMMD7 has more amino acid residues than COMMD6. More detailed analysis of the data including EXAFS curve fitting and multiple scattering XANES calculation is now under way.

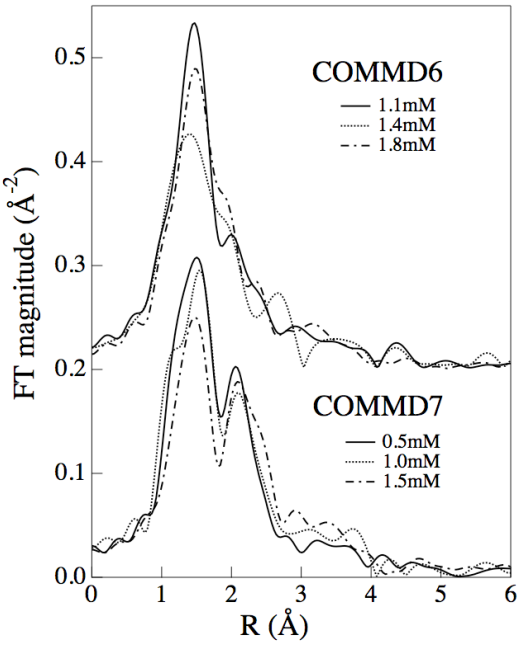

Fig.1: Fourier transformed EXAFS spectra of COMMD6 and COMMD7

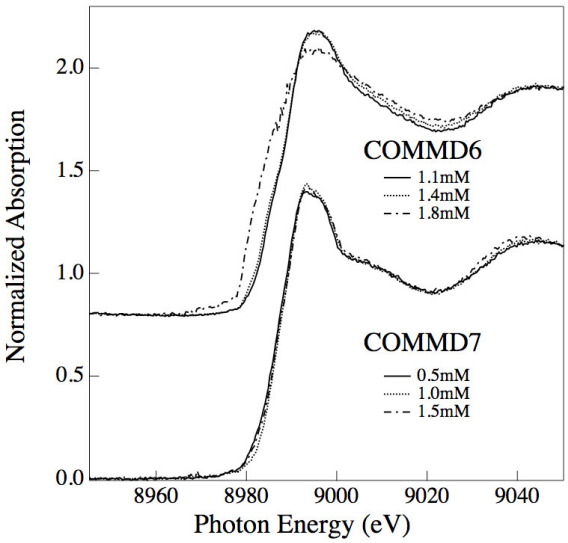

Fig.2: XANES spectra of COMMD6 and COMMD7

References

[1] S. Narindrasorasak et al., Biochemistry 46,3116(2007)

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