Cu(I) Modified Metallothionein with Copper X-ray Absorption Spectroscopy

Meiyi Li (李美儀)[1], I-Jui Hsu (許益瑞)[2], U-Ser Jeng (鄭有舜)[3], Yew-Chung Sermon Wu (吳耀銓)[1], Jyh-Fu Lee (李志甫)[3], and Yu Wang (王瑜)[4]

[1]Department of Materials Science and Engineering, National Chiao Tung University, Hsinchu, Taiwan
[2]Department of Molecular Science and Engineering, National Taipei University of Technology, Taipei, Taiwan
[3]National Synchrotron Radiation Research Center, Hsinchu, Taiwan
[4]Department of Chemistry, National Taiwan University, Taipei, Taiwan

Metallothioneins (MTs) is a cysteine-rich protein that has highly affinity with various metal ions[1]. Engineering MT with specific metal ions is believed one of candidates for molecular sensor or devices[2]. This report describes the possible structures characterized by x-ray absorption spectroscopy (XAS) of Cu modified MTs. The XAS experiments of Cu modified MTs are carried out in fluorescence mode with a 13-element solid state detector at BL17C. Figure 1 shows rich local structural features of the copper ions in the Cu-modified MTs after refolding process. The spectrum of Cu(II)-MT shows only one main shoulder peak at 8985.9 eV; whereas the spectrum of Cu(I)-MT exhibits the characteristic shoulder peak of Cu^{I} at 8981.8 eV [3] together with the main peak at 8985.4 eV. The differences between the XANES profiles of the two Cu-modified MTs can be better resolved with the first derivative profiles $d\mu/dE$ shown in the inset of Fig. 1.

Guided by the XANES result and the structural information from Cambridge crystallographic data center (CCDC)[4] on 4- and 5-coordinated Cu complexes, we furthermore fit the EXAFS data of the Cu(I)- MT. The best fitted results are displayed in Fig. 2 with the fitted $k^3\chi(k)$ spectra shown in the insets. The fitted parameters imply that Cu^{I} is mainly coordinated by four ligands of Cu-O/N and Cu-S(Cys) bonds, with two averaged distances of 1.96(1) Å and 2.49(3) Å, respectively. The Cu-O/N bonding is attributed to O/N atoms of nearby amino acids. Likely, some of the oxygen atoms belonging to carbonyl groups (C=O) of the amino acids may furthermore act as linkers for Cu ions, resulting in the observed Cu-Cu distance of 3.35(1) Å and a bond angle 117° of Cu-O-Cu. Consequently, accompanied backscattering from the C atoms of the amino acids, at a distance of 2.79(5) Å, can be observed. Based on the average distance 1.23 Å of C=O [5], the corresponding bond angle of Cu-O-C is estimated to be ~121°.

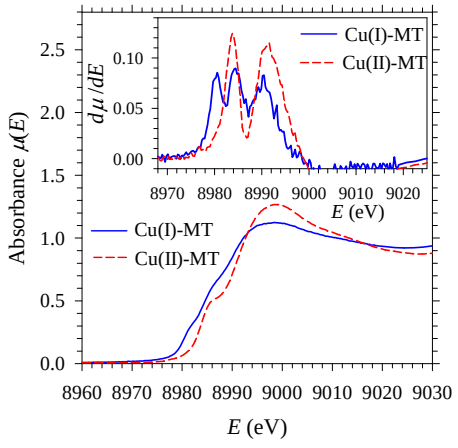

Fig. 1

In summary, the possible structure of Cu(I)-MT is depicted in Fig. 3. Three copper clusters bridged with S(Cys) is formed. Each copper cluster neighbored with oxygen atoms belonging to carbonyl groups (C=O)[6]. The possible structure is similar dimmerized MT as shown in NMR solution state structure[7]. MTs are easy to dimerize due to 20-cysteine existence.

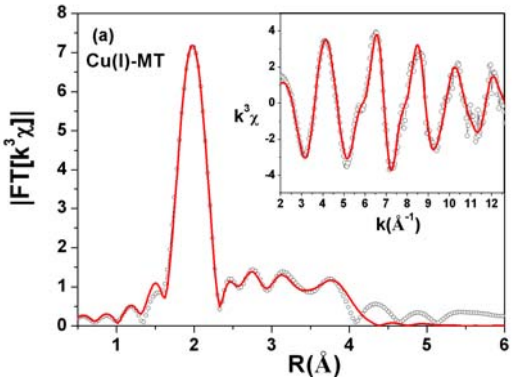

Fig. 2

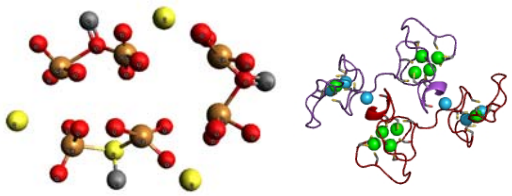

Fig. 3

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