Resonant X-ray Scattering and Absorption for the Global and Local Structures of Cu-modified Metallothioneins in Solution

Meiyi Li (李美儀)[1], Yu-Shan Huang (黃玉山)[2], U-Ser Jeng (鄭有舜)[2], I-Jui Hsu (許益瑞)[3], and Jyh-Fu Lee (李志甫)[2]

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

With Cd and Zn metal ions removed from the native rabbit-liver metallothionein upon unfolding, Cu-modified metallothioneins (Cu-MTs) were obtained during refolding in solutions containing CuI or CuII ions. X-ray absorption near-edge spectroscopic (XANES) results confirm the respectively assigned oxidation states of the copper ions in CuI-MT and CuII-MT. Global and local structures of the Cu-MTs were subsequently characterized by anomalous small angle X-ray scattering (ASAXS) and extended X-ray absorption fine structure (EXAFS). Energy-dependent ASAXS results indicate that the morphology of CuII-MT resembles that of the native MT whereas CuI-MT forms oligomers with a higher copper content. Both dummy residue simulation and model-shape fitting of the ASAXS data reveal consistently rod-like morphology for CuII-MT. Clearly identified Cu-S, Cu-O, and Cu-Cu contributions in the EXAFS analysis indicate that both CuI and CuII ions are bonded with O and S atoms of nearby amino acids in a four-coordination environment, forming metal clusters smaller than metal thiolate clusters in the native MT. It is demonstrated that a combination of resonant X-ray scattering and X-ray absorption can be particularly useful in revealing complementary global and local structures of metalloproteins due to the atom specific characteristics of the two techniques.

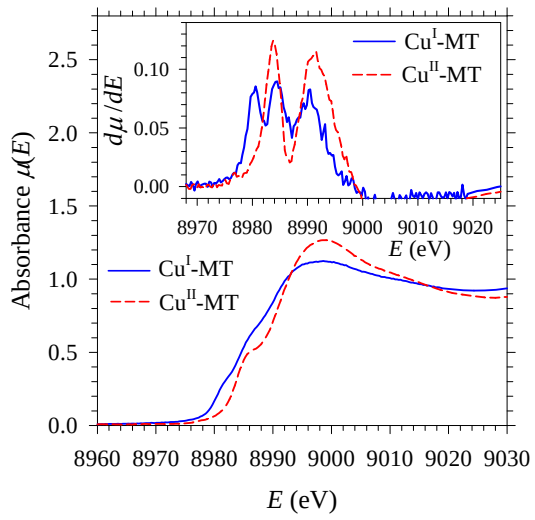

Fig. 1: XANES data of CuI-MT and CuII-MT. Shown in the inset are the corresponding d /dE profiles.

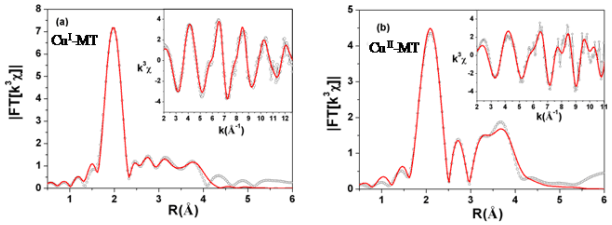

Fig. 2: Fourier transformed amplitudes FT[$k^3\chi(k)$] (open circles) of Cu^I-MT in (a) and Cu^{II}-MT in (b) are respectively fitted (solid curves) with the parameters summarized in Table 2. Insets show the respectively fitted $k^3\chi(k)$ data..

We have demonstrated that resonant X-ray scattering (ASAXS) and X-ray absorption make a unique combination in providing direct global morphology and local coordination geometry of the metal ions for metallothioneins in solution. The size, rod-like morphology, and copper content of the modified Cu-MTs are revealed by ASAXS, whereas the coordination geometries around copper ions are elucidated by XAS as in a four-coordinated mode bonded to O and S atoms of the amino acids and the small metal clusters. Both global morphology and local metal ligand bonding of the CuI-MT and CuII-MT are found to deviate from the native MT but each to a different extent. O

verall, the integrated structural information gives insights into the mechanism of the unfolding-refolding process for the production of Cu-MTs from the native ones. Same approach can be easily applied to other metalloproteins containing different metal ions due to the atom specific character of X-ray resonance scattering and X-ray absorption.

Reference

[1] M. Li, Y.-S. Huang, U. Jeng, I.-J. Hsu, Y. S. Wu, Y.-H. Lai, C.-H. Su, J.-F. Lee, Y. Wang, and C.-C. Chang, Biophyiscal J. **97**, 609 (2009).