The unfolding mechanism of Pseudoazurin determined by Small Angle Neutron Scattering and Molecular Dynamics simulation

([1]*Graduate School of Science and Engineering, Ibaraki University*, [2]*Frontier Research Center for Applied Atomic Sciences, Ibaraki University,* [3]*Australian Neutron Scattering Center, ANSTO*) ○Attila Taborosi,[1] Takahide Yamaguchi,[1,2] Kathleen Wood,[3] Andrew Whitten,[3] Seiji Mori,[1] Takamitsu Kohzuma,[1,2]

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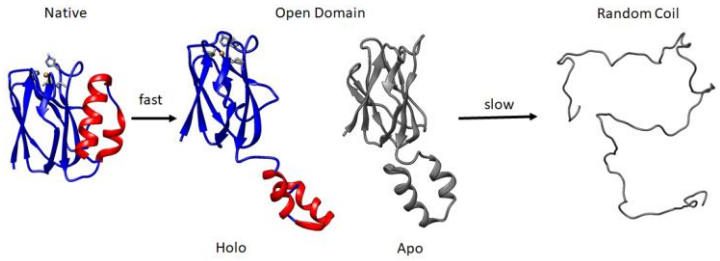
Pseudoazurin (PAz) is a blue copper protein, which function as an electron donor in denitrification process of certain bacteria. The native fold of PAz is composed by the β-barrel structure with two α-helices at the C-terminal, while the Cu ion exist at the surface of protein fold. It has been demonstrated through several of our studies[1-4] that the stability of PAz depends on the noncovalent weak interactions in the second coordination sphere at the active site. Furthermore, the CD and ESI-MS experiments identified the "folded-holo", "folded-apo" and "unfolded-apo' forms of PAz under the acidic pH condition. It was also found that the structure around at pH 3 seemed to be an intermediate state between folded and fully unfolded state.

Therefore, further investigation had been done on the unfolding mechanism of PAz WT and two Met16 variants (Phe16 and Ile16) with Small Angle Neutron Scattering (SANS) at ANSTO and Constant pH Molecular Dynamics (CpHMD) methods. The structure of each states were analyzed by SANS, especially the determination of the partially unfolded state at pD 3.0. The structure at pD 3.0 was "Open-Domain" form, where the α-helices is detached from β-barrel domain. The CpHMD simulation, verified by the experimental and theoretical R_g comparison, confirmed this Open-Domain form as the intermediate state of protein unfolding. Also, the CpHMD simulation revealed the reason of the dissociation of α-helices from the β-barrel as the starting step of the unfolding. The kinetic SANS experiment of unfolding process for WT, Met16Phe and Met16Ile verified the existence of Open Domain, and the difference in the rate constants approved the stability variation between WT and Met16 variants.



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