μSR study of the electron transfer mechanism of a blue copper protein, Pseudoazurin

A. D. Pant,[*1,*2,*3] T. Yamaguchi,[*1] K. Ishida,[*3] and T. Kohzuma[*1]

Electron transfer (ET) is an important phenomenon to understand biological processes such as photosynthesis and respiration systems. The study of ET in protein using positive muon was initiated by K. Nagamine's group with the cytochrome c protein.[1] However, the electron transfer path/mechanism is not clearly understood yet. We aim to perform a systematic study to establish the application of muons to life science.

Since biological systems are complex in geometry and dynamic, we started studying the ET mechanism in a small and single active-site blue copper protein, rather than large systems. Copper proteins that contain single- or multi-copper sites in their prosthetic group work in a wide range of ET processes and biological energy conversion cycles.[2] The copper centers in these proteins play a main role in ET. However, there remain several obscure points in the ET pathway;[3] that is, there is no clear mechanism to understand the electron transfer. Blue copper protein (type: T1Cu) contains only a single copper-ion binding site that serves as an electron mediator between large proteins. Pseudoazurin (PAz), a member of T1Cu, functions in the electron transfer processes in several microorganisms. With the application of the muon spin rotation and relaxation (μSR) method, we propose to probe the electron transfer mechanism in PAz, in which electron transfer occurs only from/to the copper center. The μSR study on PAz will provide novel insight to understand the muon method for studies in life science.

In order to understand the ET mechanism and functions of copper proteins, we will perform μSR measurements in reduced pseudoazurin (PAzCu1+, diamagnetic) and oxidized pseudoazurin (PAzCu2+, paramagnetic) (Fig. 1) using surface muons (~4 MeV). For reference, a similar set of measurements will also be performed in buffer solution and pure water. The samples are prepared and purified (solution, concentration ~10 mM, volume ~700 μL) in Kohzuma laboratory, Ibaraki University, Mito. The solution is filled in a sample cell (silicone rubber ring, ϕ20 mm, $t \sim 2$ mm, polyvinylidene chloride film ~15 μm on both sides of the ring), which is masked by silver ring (ϕ18 mm, t 0.3 mm) so that the incident muon beam can be prevented from stopping in the rubber. The blue oxidized PAz changes to the reduced form using ascorbic acid. Indium sealing is used in a helium glove bag environment. The measurements will be performed at a transverse field of 2.3 G, zero field, and a longitudinal field at different temperature ranges (20 K–300 K).

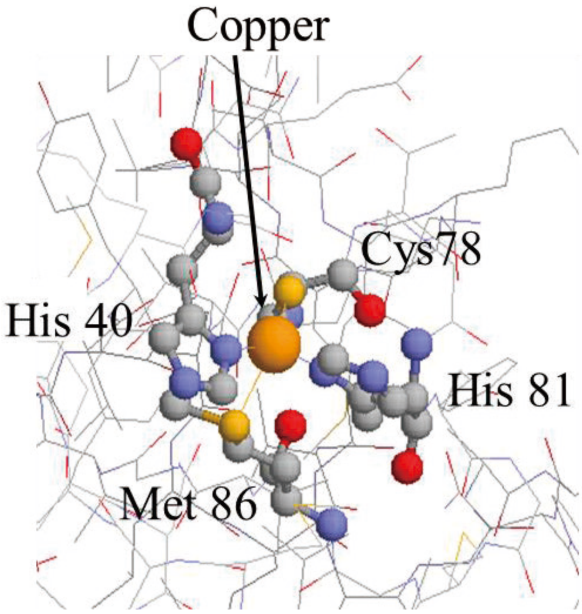

Fig. 1. Structure of pseudoazurin (PDB: 1BQK) with the coordinate scheme around Cu(II). Copper is an active site for electron transfer.

When a surface muon beam (~MeV) is incident on the sample solution, the beam energy is transferred to the solution until the end of the radiation track. A large fraction of incident muons will be found in the diamagnetic muon form and some in the muonium form (bound state of muon and an electron, Mu = μ^+e^-). If low-energy muons are stopped around the copper center of reduced PAz, there is a possibility of electron transfer from the copper center to the muon to form Mu. The Mu formed by the former (in solution) and latter (by electron transfer) cases will be distinguished by their frequencies. Interactions will exist between muons and electrons as well as muons and nuclei. These behaviors are expected to be reflected in the time spectra. The relaxation of Mu depends on its interaction with the environment. For example, a small amount of oxygen will cause the fast relaxation of Mu.[4] The formation of Mu at different sites in the sample will provide information about the electron transfer mechanism.

*1 Institute of Quantum Beam Science, Ibaraki University
*2 IMSS, KEK
*3 Meson Science Laboratory, RIKEN Nishina Center

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