Elucidation of tri-ubiquitin dynamics concerning about status of interaction interfaces

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Ubiquitin is a small protein with 76 residues and regulates many functions in a cell: proteolysis, DNA-repair, post-translational modification, signal transduction and so on. The ubiquitin has a hydrophobic interface, and through the interface, the ubiquitin makes connection with a protein having ubiquitin-interaction motif. The functions depend upon which Lys-residue are used for the connection. When Lys48 is used for the connection, this poly-ubiquitin (K48 poly-ubiquitin) works as a degrading index of protein in a proteasome-ubiquitin system.

We investigated the structure and dynamics of K48 di-ubiquitin (K48-di-Ub) with NMR [1]. K48-di-Ubs are in an equilibrium and/or has transit dynamics between open and closed forms in solution. In the closed form, the activity of K48-di-Ub becomes low due to store of the interaction interface and, therefore, this conformational change regulates the ubiquitin functions. The aim of the NSE experiment is to reveal an inner domain motions between Ub domains in K48-tri-Ub in the closed and open forms. The equilibrium between the two conformations is dependent on temperature; the closed and open ones are dominant in the higher (42 degrees) and lower (10 degrees) temperature.

We measured NSE of K48-tri-Ub at the two temperature of 10 and 42 degrees at about 10 mg/ml. An incident neutron wavelength of 6 and 8 angstroms was used to cover fourier times up to 20 ns in a q-range from 0.06 to 0.22 inverse angstroms. Intermediate scattering functions for both states were successfully obtained. The effective diffusion constants (Deff) were obtained by exponential fittings at each q. Deff should include translational and rotational diffusion as well as internal domain motions. The experimentally obtained Deffs are consistent with the MD simulation derived ones at 10 degrees (see Fig.). Based on these results, the contribution of the internal motions can be estimated. The analysis on the temperature dependence of the internal dynamics is on progress. We are trying to analyze the NSE data combined with computational analysis to observe the functional domain motions of K48-tri-Ub.

Reference:
[1] T. Hirano, M. Yagi-Utsumi, K. Kato, et al., J. Biol. Chem., 286, 37496 (2011).

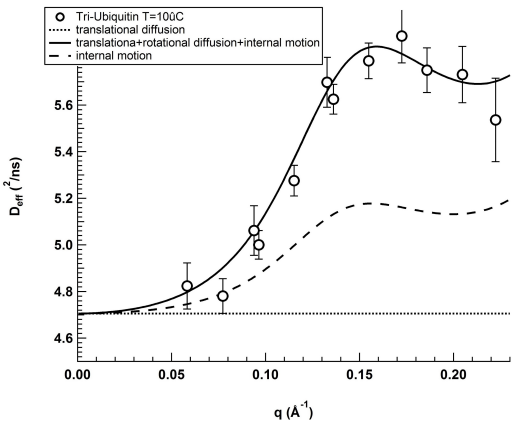

Fig. 1. Q-dependence of effective diffusion constants (Deff) from NSE experiment and MD simulation (translational diffusion, translational diffusion + rotational diffusion + internal motion, and internal motion).