Identification of relative arrangement of domain in multi-domain protein as studied by small-angle neutron scattering

M. Sugiyama, R. Inoue, K. Morishima

Institute for Integrated Radiation and Nuclear Science, Kyoto University

From theoretical and computational approaches, it is revealed that domain motion in multi-domain protein (MP) is closely coupled to the regulation of biological function. Therefore, elucidation of the mechanism of domain motion is mandentory for clarifying the mechanism of multidomain protein function. Ubiquitin (Ub) is a very small protein consisting of 76 amino acid residues and is involved in the regulation of various cellular phenomena such as DNA repair, transcriptional regulation, and so on. The C-terminus of Ub is linked to another Ub by an enzymatic reaction through seven lysine residues (K) to form polyUb. We especially focused on the linearly linked tri-ubiquitin (l-triub). To investigate the solution structure of l-triub, we firstly performed small-angle X-ray scattering measuement of l-triub (refer to Fig. 1).

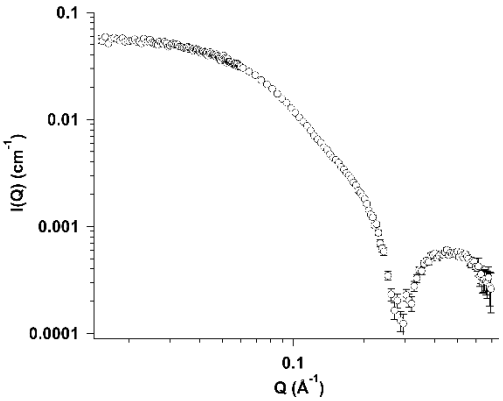

Fig.1. SAXS profile of l-triub.

In order to reproduce SAXS profile, we also performed all-atom molecular dynamics (MD) simulation of l-triub. Although several MD trajectories reprodued SAXS profile, it is not possible to judge which MD trajectory is appropriate for reproducing experimental result. To overcome current situation, we try to obatin extra scattering curve that is usable for selecting appropriate MD trajectory. For this purpose, we prepared special l-triub comprised of two hydrogenated domains and one 75% deuterated domain (segment-deuterated l-triub). When this specially prepared l-triub is dissolved in 100% D_2O, only two hydogenated domains are visible with small-angle neutron scattering (SANS). Namely, SANS measurement of segment-deuterated l-triub serve as an extra scattering curve that is usable for selecting appropriate MD trajectory. Figure 2 shows a SANS profile from a segment deuterted l-tirub consisting hydrogenated domains at two edges and 75% deuterated domain at the center (Fig.2). SANS profile from segment deuterted l-tirub is significantly different from SAXS profile from l-triub. It supports the effectiveness of combination of SANS and segment deuteration technique for studying the domain motion of MDP. We are on the progeree of analysis of this SANS profile with the aid of all-atom MD simulation.

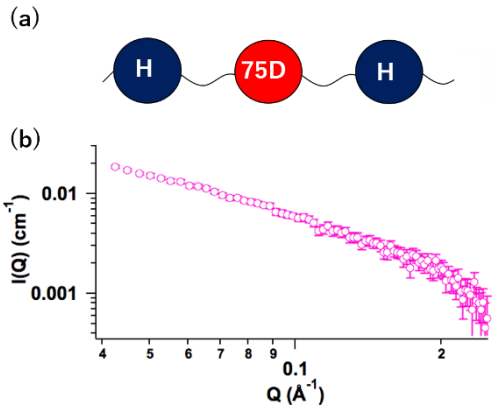

Fig.2. (a) Schematic view of segment deuterted l-tirub. (b) SANS profile of segment deuterted l-tirub.