Solution structure of protein under high pressure

R. Inoue, N. Aizawa, M. Sugiyama

Institute for Integrated Radiation and Nuclear Science, Kyoto University

To maintain vital activities, it is essential for proteins, which is one of the main components in living systme, to possess a folded structure (folded state). However, when proteins are significantly deviated from the so-called physiological conditions of normal temperature and pressure, they tend to shift from the folded state to the unfolded state, and eventually lose their biological functions. Hence, it has long been believed that under high-pressure environments, including deep-sea environments, organisms cannot exist at all because they cannot maintain the fold state necessary to express their functions. Contrary to this expectation, a wide variety of organisms have been reported at depths of 8400 m (84 MPa). However, for a long time, there has been no clear answer as to why life could exist at such high pressures. One of the interesting materials, which are significantly included in marine organisms at deep sea, is trimethlyoxide (TMAO). Although deteiled mechanim is still far from understainding, it is considered that this TMAO play a main role for the preservance of protein under high pressure. As a first apparoch, we investigated the solution structure of protein under high pressure by small-angle neutron scattering (SANS). The sample used in this work was alpha glucosidase (gsj) at the concentration of 3.6 mg/mL dissoved in D_2O buffer. SANS measurements were performed with SANS-U instrument installed at JRR-3, Tokai. To mimic the pressure at deep sea, we selected 100 MPa for the high pressure measurement. As a reference, we also measured the solution strcuture of gsj at ambient pressure. To monitoer the time depedence of structural change, one exposure time was set to 30 min. Fig.1 shows the frame dependence of radius of gyration (R_g), which was calculated from Guinier approximation, at amibient pressure. The calculated R_g value was almost constant regardless of frame and such calculated R_g value

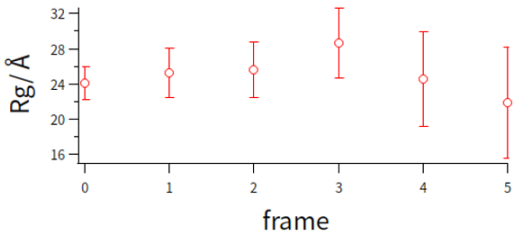

Fig.1. Frame dependence of R_g of gsj solution at ambient pressure.

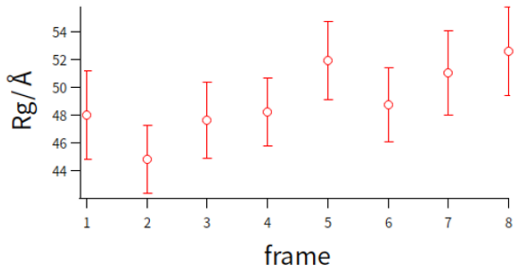

Fig.2. Frame dependence of R_g of gsj solution at 100 MPa.

was the same as that evaluated from small-angle X-ray scattering within experimental error. As a next step, we applied 100 MPa to the gsj solution. Fig. 2 shows the frame dependence of at 100 MPa and R_g value increased to double of that at ambient pressure. This finding means that Gsj was denatured by applying high pressure without existence of TMAO. To figure out the contribution of TMAO to the satbaility of protein at high pressure, we are also planing to perform high pressure SANS measurement of gsj with the coexistence of TMAO. In addition, we are also planning to prepare an inner-cell, which is fitted to solution scattering in near future.