Structural analysis of proteins in denatured states by small-angle neutron scattering

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Proteins are usually folded into definite structures and function. Whereas the “structural biology”, which determines these definite structures of proteins (and other biological macromolecules), enjoys huge success, it is not clear how linear polypeptide chains are folded into the complex definite structures. This “folding” problem remains elusive. Moreover, many proteins and regions within the proteins have been found to be “denatured” under the normal conditions. In such intrinsically disordered proteins or regions (IDP or IDPR), denaturation itself should be important for their functions. To elucidate the folding problem and the mechanisms how IDPs and IDPRs function, structural analysis of the proteins in the denatured states is required. Since these proteins are disordered, the distribution of the structures itself needs to be analyzed.

To analyze the structural distributions of the IDPs, small-angle neutron scattering (SANS), combined with protein deuteration, may be useful. Because the atomic scattering lengths of hydrogen (H) and deuterium (D) are very much different, H-labeling of certain amino-acid residues with H atoms in perdeuterated proteins produces a significant distribution in the scattering length density otherwise nearly homogeneous density. In this study, we prepared the perdeutrated (D-) proteins and the D-proteins containing the labeled amino-acid residues with H, and carried out the SANS measurements on these samples to test feasibility of such measurements.

We chose staphylococcal nuclease (SNase) as a model protein, because this protein is one of the typical globular proteins and the deletion mutant (Δ131Δ) of this protein is naturally denatured. SNase at pH 7.0 is in the globular state whereas Δ131Δ at pH 7.0 and pH 3.0 is in the molten-globule (denatured but compact) state and in the denatured state, respectively. We prepared the perdeuterated proteins of SNase and Δ131Δ, those containing the H-labeled leucine (Leu) residues, and the usual hydrogenated proteins as standards, in D_2O solutions. The measurements were carried out using the instrument, SANS-U, at the JRR-3 Research Reactor in Tokai, Ibaraki.

Figure 1 shows the obtained SANS curves. The curves of the globular state of SNase, and the molten-globule and the denatured states of Δ131Δ are shown to be different, confirming the different structures. The curves of the perdeuterated proteins and those containing the H-Leu are also shown to be different, indicating that the H-labeling shows observable effects. This indicates feasibility of the method. The detailed analysis employing model calculations are currently underway.

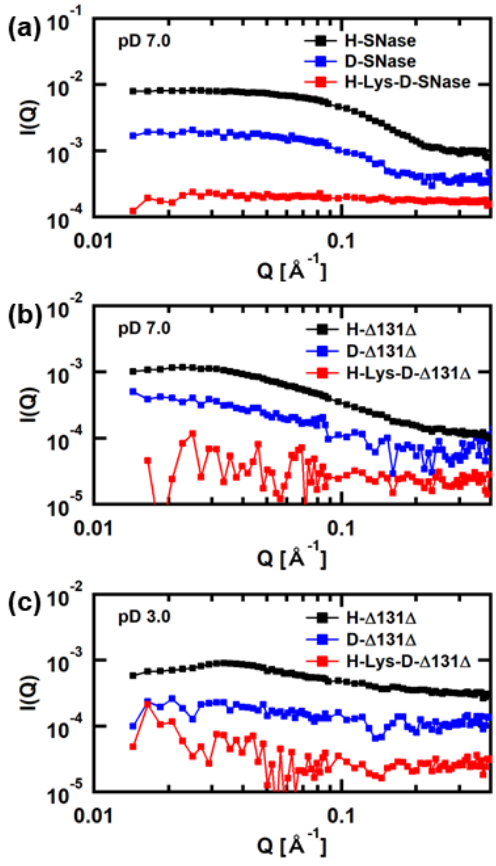

Fig. 1. Figure 1. Summary of the SANS curves of (a) SNase at pD 7.0, (b) the mutant Δ131Δat pD 7.0, and (c) the mutantΔ131Δ at pD 3.0.