Solution structure of intrinsically disordered region of Hef

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For long time, it is believed that three-dimensional ordered structure is essential for the development of protein's function. Contrary to such common perception, Wright and Dyson [1] reported that some proteins intrinsically or natively lack three-dimensional ordered structure even under their functionally active state. At present, these proteins are known as intrinsically disordered protein (IDP) and play biologically significant roles especially for Eukaryotes. Both high contents of hydrophilic and charged residues in its constituent amino acid sequence render the destabilization of ordered structures, leading to highly flexible structure which drastically change with time. Because of its highly flexible structure, the application of existing experimental method such as X-ray crystallography, Cryo-EM and so on is highly limited for determining its structure. To overcome such a situation, we determined to apply solution small-angle scattering methods for solving IDP's structure. In this work, we especially focused on the solution structure and dynamics of intrinsically disordered region (IDR) of Hef (helicase- associated endonuclease for fork-structured DNA) (Hef-IDR) [2] as an example of IDP. The solution small-angle scattering measurement was performed with SANS-U spectrometer installed at JRR-3, Tokai. The wavelength and sample-to detector distances used for present measurement were 7 Å, 4 m and 1 m, respectively. The Hef-IDR solution at the concentration of 5.0 mg/mL was prepared for this work. Figure 1 shows the Guinier plot of Hef-IDR solution and the radius of gyration (R_g) was calculated to 30.4 ± 1.2 Å. Considering its molecular weight (~11 kDa), the calculated R_g value is extremely high. In order to deny the possibility that such high Rg value is originated from the contamination of aggregates in Hef-IDR solution, we also performed analytical ultracentrifugation measurement on the same solution. Figure 2 indicates $c(s_{20,w})$ of

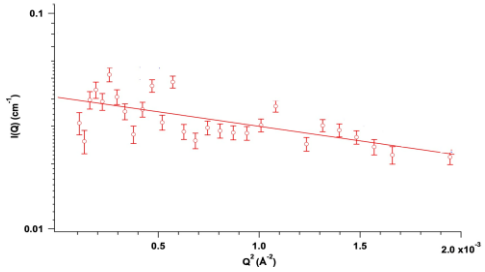

Fig. 1 Guinier plot of Hef-IDR at the concentration of 5.0 mg/mL (circle). The solid line corresponds to the result of fit with Guinier approximation.

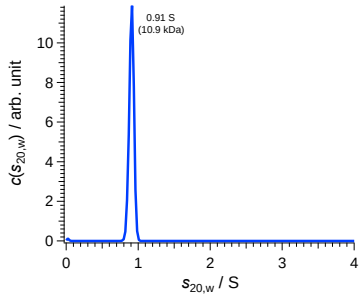

Fig. 2 $c(s_{20,w})$ of Hef-IDR solution.

Hef-IDR solution. It is evident that this Hef-IDR solution is dominated by monomeric Hef-IDR. In other words, quite high R_g value is originated from native monomeric Hef-IDR. To obtain candidate Hef-IDR's solution structure, we initiated to analyze the solution scattering profile with ensemble optimization method.

[1] H. J. Dyson, P. E. Wright P, Curr. Opin. Struct. Biol. **12**, 54 (2002).
[2] S. Ishino et al., J. Biol. Chem. **289**, 21627 (2014).