Analysis of flexible structure of multi-domain protein by SANS using segment deuteration technique.

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Some proteins, which are categorized as multidomain proteins (MDP), are comprised of multiple domains and intervening intrinsically disordered regions (IDR). MDP regulates the functions by changing the structure in solution. Therefore, the analysis of its flexible structure is essential for understanding the functions. However, the flexibility makes them difficult to analyse the structure by even the application of state-of-the-art experimental methods such as crystallography or cryo-electron microscopy. As an example of MDP, we focused on biologically significant protein Hef (helicase-associated endonuclease for fork-structured DNA) from hyperthermophilic archaeon. Hef consists of an N-terminal helicase domain, a C-terminal nuclease domain, and an IDR connecting the two domains [1, 2]. It is considered that the two domains work cooperatively to unwind and cleave the damaged fork structured DNA. However, it remains unclear how its structural flexibility contributes to cooperative work of the two domains. To overcome current situation, we first analysed flexible structure of Hef in solution as an ensemble-averaged structure based on small angle X-ray scattering (SAXS) data. Using ensemble optimization method (EOM) [3], we succeeded in reproducing the SAXS profile with an ensemble of six representative structures (Fig.1). However, since the flexible structural ensemble was calculated from only one SAXS profile, it is still questionable whether the calculated ensemble averaged protein reflects the structural population of Hef in solution correctly or not. Namely, additional experiments are mandatory for verifying this result. For this purpose, we developed a new technique that enables to selectively observe the scattering from a specific region by small angle neutron scattering (SANS). It should be noted that 75% deuterated protein can be scatteringly invisible in 100% D₂O solvent with SANS, whereas the scattering from the non-deuterated protein can be observed [4,5]. Therefore, if we prepare Hef composed of 75% deuterated region and non-deuterated region (segmentally deuterated Hef), it is possible to selectively observe the scattering from the non-deuterated region (Fig.2). In this presentation, we will report the preparation of segmentally deuterated protein samples, SANS measurement and preliminary structural analysis based on SANS data.

![Figure 1. Ensemble structure analysis based on SAXS data.](image1)

![Figure 2. The schematic view of preparation of segmentally deuterated Hef.](image2)