Experimental report

11/06/2017

Proposal: 8-04-715 **Council:** 4/2014

Title: Direct observation of domain motion in EcoO109I.

Research area: Biology

This proposal is a new proposal

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Samples: D2O
DNA
EcoO109I

Instrument	Requested days	Allocated days	From	To
IN15 Standard	7	7	08/07/2016	15/07/2016

Abstract:

EcoO109I is a type II restriction endonuclease, which provides a defense mechanism by degrading foreign DNA and crystallography studies suggested a possibility of an existence of domain motion, which assists for capturing DNA.

To prove the existence of domain motions in EcoO109I we combined MD simulation and small-angle X-ray scattering (MD-SAXS). It was found that SAXS profile in solution clearly showed the deviation from that in the crystal, implying that SAXS profile gives us the averaged structure in motion. We then performed MD simulation and calculated the time-averaged SAXS profile and SAXS profile from MD could describe the experimental one. Further MD simulation studies revealed a large fluctuation of radius of gyration with a period of ~ 50ns due to domain motion, which might be related to enzyme reaction.

In order to observe the domain motion in this protein directly we propose to perform NSE study on EcoO109I and we will also verify whether or not such observed domain motion is really relevant to its mechanism of enzyme reaction by comparing to MD results.

Through the various experimental and theoretical approaches, it was recognized that proteins are highly dynamical objects. Especially, large-scale movements of domains in multi-domain proteins are considered to be responsible for regulating their function. Hence, revealing the mechanism of domain motion in proteins is indispensable for understating the origin of their intrinsic function.

EcoO109I is classified into a type II R RestrictionEndonucleases (REases), which provides a defensemechanism by degrading the foreign DNA. Preliminary studies on EcoO109I offered us two interesting features. One is that the inter-domain space clarified X-ray crystallography (refer to Fig. 1) was smaller than the size of DNA. The second point is that solution structure was different from that expected from crystal structure. These experimental observations motivated us to consider that the domain motion must be existed for EcoO109I in solution state. To verify such an idea, we have performed MD-simulation and small-angle X-ray scattering (MD-SAXS) studies on this protein and have captured the possibility for existence of domain motion in solution state. However, we have not grasped the experimental proof of domain motion of EcoO109I directly. We then performed neutron spin echo studies on EcoO109I in order to observe domain motion in EcoO109I.

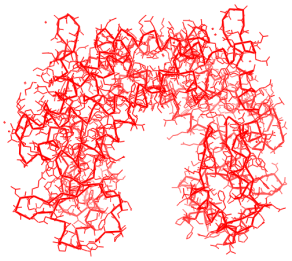

Fig. 1 Quaternary structure of EcoO109I.

We have prepared EcoO109I at the concentration of 10 mg/ml dissolved in D_2O buffer. Preliminary SAXS and SANS studied have revealed the absence of inter-particle interference at this concentration. NSE measurements were performed with IN15 using the combination of wavelengths of 6.3, 8, 10 and 12Å. To avoid the onset of nonspecific aggregation, temperature was fixed to 15 degree C. Fig. 2 shows the intermediate scattering function ($I(Q, t)/I(Q, t=0)$) obtained from EcoO109I at the concentration of 10 mg/ml. As an initial step for data analysis, cumulant expansion with $I(Q, t)/I(Q, t=0)=\exp(-D(Q)Q^2t+1/2K_2t2)$ where $D(Q)$ and K_2 corresponds to Q dependence of diffusion constant and second cumulant values, respectively. Results of fits with above equation were given by red solid curves in Fig. 2 and it

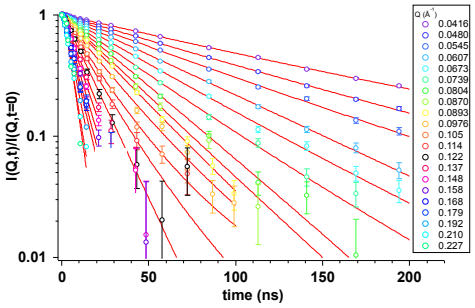

Fig. 2 Intermediate scattering function ($I(Q, t)/I(Q, t=0)$) and results of fit with cumulant expansion.

can be clearly seen that this function describes experimentally observed $I(Q, t)/I(Q, t=0)$ nicely. We then plotted Q dependence of $D(Q)$ in Fig. 3. At low Q region, $D(Q)$ was constant within experimental error. It should be noted that $D(Q)$ at low Q region coincided with translational diffusion constant evaluated from dynamic light scattering (DLS). On the other hand, clear modulation of Q was observed at the Q region above 0.10 $Å^{-1}$. Especially, broad peak was observed at around 0.13 $Å^{-1}$. It is known that Q dependence of $D(Q)$ was not observable from translational diffusion. Hence, the clear modulation of Q observed above 0.10 $Å^{-1}$ must be originated from rotational diffusion and domain motion (or internal motion), which is the main concern in this proposed work. However, it is difficult to separate the contribution from rotational diffusion and domain motion in this protein from the Q dependence of $D(Q)$ derived from NSE measurements. For the proper interpretation of NSE results, we are on the progress of performing all-atom MD simulation on EcoO109I. With the results from MD simulation (or trajectory), we will proceed the detailed analysis of underlying dynamics in this protein.

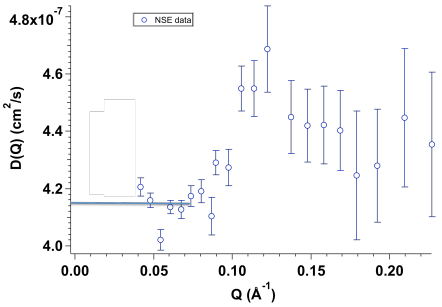

Fig. 3 Q dependence of $D(Q)$ and dotted line corresponds to translational diffusion coefficient from DLS.