Dynamics of multi-domain protein ER-60 revealed by small angle X-ray scattering data and molecular dynamics simulations

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Small-angle X-ray scattering (SAXS) profile of a biomolecule reflects its meso- and nano- scale structure. Since the profile is contributed by all molecules in solution, the SAXS is a powerful method to study structural ensemble of the protein. We are establishing methods to elucidate structural ensemble of proteins at near-atomic resolution by combining SAXS and molecular dynamics simulations.

In this study we focused on structure and dynamics of multi-domain protein ER-60. ER-60 is a member of Protein disulfide isomerase family, which promote correct protein folding via isomerization of disulfide bonds. The ER-60 is composed four domains, **a**, **b**, **b'**, and **a'**. Both **a** and **a'** domain have active Cys-Gly-His-Cys (CGHC) motif [1]. In each CGHC motif, two cysteines take either S-S (oxidized) or -SH (reduced) states. We have obtained SAXS profiles of ER-60 with both all CGHC oxidized (oxidized ER-60) and CGHC reduced (reduced ER-60). Our SAXS profiles did not match known crystal structure, and the SAXS profiles of the two states were slightly different from each other [2].

To investigate behaviour of ER-60 in solution, we performed multi-scale molecular dynamics simulations. First, fluctuation of each domain was examined by atomistic MD simulations. The fluctuation around active motif differed between oxidized and reduced ER-60, but no significant difference was seen in the other regions. It suggests that the difference of SAXS profile between two states is not due to the difference of intra-domain dynamics.

Second, motion of full-length ER-60 was examined by coarse-grained molecular dynamics (CGMD) simulations with CG Martini model [3], where each amino acid is represented by one to six particles. We have successfully obtained simulation trajectory which reproduce our SAXS profile. From the simulation trajectory, we analysed inter-domain interface and frequency of binding/dissociation of each pair of the four domains.

Third, structural difference between oxidized ER-60 and reduced ER-60 was studied by coarser CGMD simulations with AICG2+ model [4], which enable extensive structural-sampling. We compared simulation snapshots which reproduce SAXS profile of oxidized ER-60 with simulation snapshots which reproduce that of reduced ER-60. Our simulation showed that the difference of two SAXS profiles reflect the difference in position of **a'** domain [2].

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