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Solution structure of MBP-fused MDV1 for determining coiled-coil orientation

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1 Introduction

A coiled-coil domain (CCD) consists of two or more α-helices that are twisted around each other to form a super-helical structure. Coiled-coils (CCs) have a heptad repeat pattern $(a\text{-}b\text{-}c\text{-}d\text{-}e\text{-}f\text{-}g)_n$, where the *a* and *d* positions are usually occupied by hydrophobic residues [1-2]. CCs are easily detected by prediction programs or servers but they are very versatile structural domains that can adopt many different structures. They can form different oligomeric complexes, and the helix orientation can either be parallel or antiparallel. Because of these characteristics, CCs typically participate in diverse protein-protein interactions that are involved in many biological functions and components. To understand the detailed molecular functions of CC-containing proteins, it is absolutely necessary to determine the relative orientation of each coil because the functional domain outside a parallel CCD dimer must be in close proximity, but that outside an antiparallel CCD is located further away. So far, many programs or servers have been developed to determine the oligomeric state of CCDs but none of these programs provide adequate discrimination between parallel and antiparallel orientations. To overcome the above limitations, we have developed a new biochemical technique using a fusion tag, which is an appropriately spaced and oriented molecule that also aids in enhancing the solubility of CC proteins, to assess the orientation of the CC dimer [3].

2 Experiment

The gene fragment of yeast MDV1 (mitochondrial division protein 1; 231-300) was ligated into a modified pMAL vector with an N-terminal hexa-histidine sequence. The overexpressed fusion protein was loaded to a column containing amylose resin and collected by gravity flow followed by anion exchange and size exclusion chromatography. The concentration of MBP (Maltose Binding Protein)-MDV1 was 9.8 mg/ml. Scattering data of this protein was collected at BL-10C. The scattering images from the proteins were reduced to 2D data by circular integration. Preliminary analysis of these 2D data using PRIMUS (ATSAS program suite) provided the radius of gyration (R_g), Porod volume, and experimental molecular weight.

3 Results

CCD of MDV1 was observed as a clear antiparallel dimer [4]. To confirm the directionality of the CCs of MDV1 in solution, we used SAXS (small angle X-ray scattering). The theoretical scattering curve calculated using DAMMIF fits well with the scattering intensity of fusion protein (Fig. 1A). This fusion protein has a P(r) function with a two peaks and D_{max} is about 150 (Fig. 1B). The experimental scattering data and the pattern calculated from the atomic co-ordinates match well using the program CRYSOL (Fig. 1C). These results clearly showed that it has a dumbbell-shaped envelop (Fig. 1D). The crystal structure was fitted into the SAXS envelop.

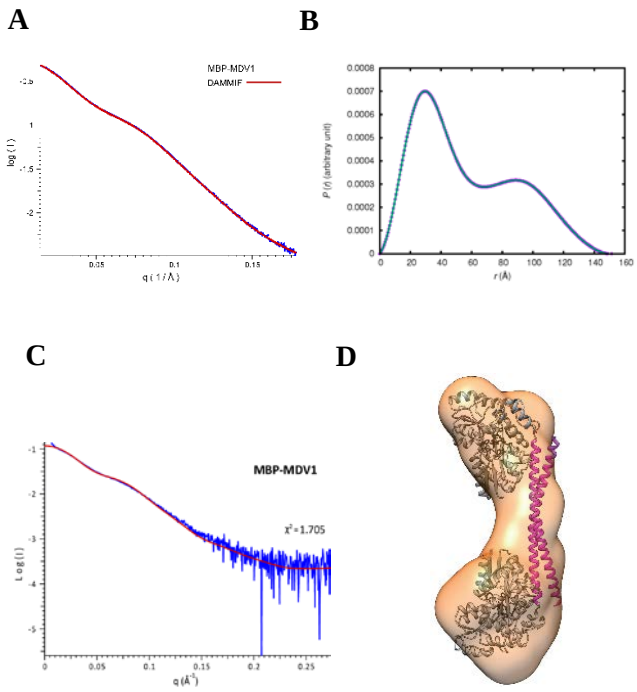

Fig. 1: SAXS curves of MBP-MDV1. Scattering curve calculated with the DAMMIF (A) and distance distribution function, P(r) function (B). Comparison of I(q) calculated by CRYSOL from the atom structure (C). Molecular envelop fitted the high-resolution crystal structure (D).

References

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