Structural Analysis of Intrinsically Partially Folded EspB from Enterohaemorrhagic *E. coli* O157:H7 by Combination of CD and SAXS in Conjunction with Protein Dissection Technique

EspB is one of the major intrinsically disordered pathogens from enterohaemorrhagic *Escherichia coli*, which promotes actin reorganization by activating α-catenin from the host cell. EspB assumes a partially folded structure under native conditions. We analyzed the structural properties of EspB by a combination of various spectroscopic techniques including circular dichroism and small-angle X-ray scattering in conjunction with protein dissection. The results suggested that EspB assumes partially folded α-helical segments around G41–Q70 whereas other regions, particularly around the C-terminal half, are almost entirely extended. Furthermore, titration studies by fluorescence anisotropy indicated that the α-helical segments directly interact with α-catenin.

Infection by enterohaemorrhagic *Escherichia coli* (EHEC), O157:H7, can cause various severe symptoms including abdominal cramps, diarrhea and hemolytic uremic syndrome (HUS) [1]. Particularly for young children, HUS can develop into acute renal failure and in the worst case, lead to death.

EHEC produces various virulence factors including Shiga-like toxins as well as proteins that induce morphological rearrangement of host cells [1]. It is therefore important to clarify the structural property of these virulence factors in order to understand the detailed mechanism of EHEC infection.

EspB is one of the virulence factors of EHEC which promote the rearrangement of actin cytoskeleton. EspB is directly injected into host cytosol through the type III secretion system (T3SS) of EHEC and binds to the C-terminal vinculin homology domain of α-catenin in host cytosol. It has been indicated that the binding of EspB to α-catenin induces some conformational rearrangement of α-catenin and enhances the ability of α-catenin to promote actin bundling [2]. Previous studies on the structural property of EspB by various spectroscopic techniques such as circular dichroism (CD), intrinsic tyrosine fluorescence and multi-angle light scattering indicated that this protein assumes a partially folded conformation with an amount of α-helical structures but with well-ordered specific tertiary contacts when in the isolated state [3]. Thus, EspB is one of the intrinsically disordered proteins with some partially folded structure. This conformational uniqueness is considered to be required for efficient secretion through the narrow tube of T3SS. Further analysis clarified that many T3SS-dependent pathogenic proteins tend to be classified into "*intrinsically disordered protein*" (IDP) which cannot form a specific well-defined structure by itself unlike the general globular proteins.

Recent developments in modern high-resolution techniques of structural biology enable us to routinely solve the structures of globular or membrane proteins. However, protein crystallization is basically improbable for such a flexible molecule. Line broadening phenomena in solution NMR are observed for EspB probably due to the slow conformational exchange within the ensemble of partially folded structures [3].

To overcome these problems, we employed a combinatorial approach using various low-resolution techniques such as small-angle X-ray scattering (SAXS) and CD spectroscopy in conjunction with a protein dissection strategy [4]. SAXS analysis revealed that this protein possesses highly expanded conformation with a radius of gyration (R_g) of 67.8 Å. Surprisingly, this value is similar to that of an ideal random coil (62.0 Å) or completely unfolded protein [Fig. 1(a)]. However, CD indicates the presence of α-helical structures [Fig. 1(b)]. These results suggested that the protein possesses highly unstructured regions together with partially folded α-helical segments.

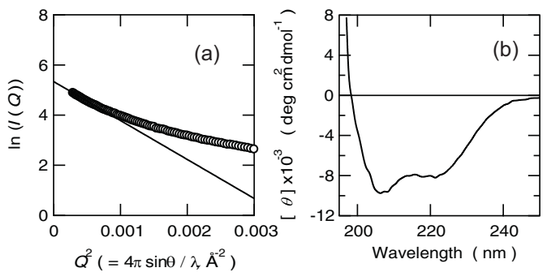

Figure 1: Conformational properties of EspB analyzed by SAXS (a) and CD (b). (a) Guinier plot. The slope of this plot corresponds to R_g^2 /3. (b) Far-UV CD spectrum of EspB. The minimum at 222 nm indicates the presence of α-helical structures.

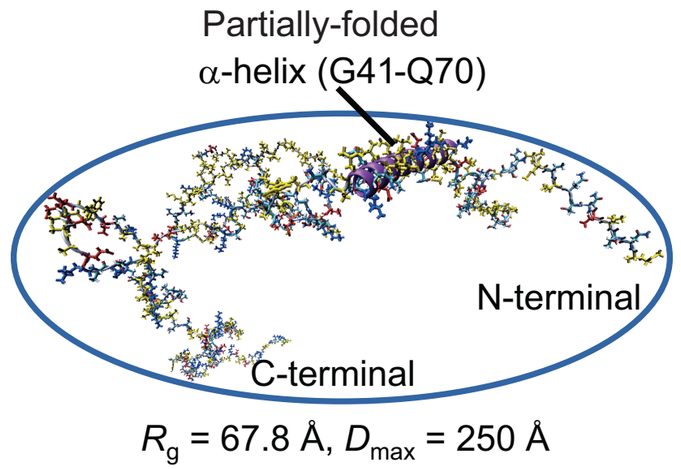

Figure 2: Structural model of EspB estimated from the results of SAXS and CD in combination with the protein dissection. R_g was obtained from the Guinier plot in Fig. 1. D_{max} corresponds to the maximum distance estimated by the analysis of probability function calculated from the scattering intensity. For more details of the analysis, see reference [4].

In order to identify the α-helical regions within the whole EspB, we employed the protein dissection technique. We analyzed far-UV CD spectra of various peptide fragments corresponding to several parts of EspB and found that the fragments which correspond to G31 to Q70 can be a core region of the α-helical segments. Based on these observations, we constructed structural models of EspB as illustrated in Fig. 2. Moreover, the α-helical fragments having the sequence of G31–Q70 of EspB had high affinity to the C-terminal vinculin homology domain of α-catenin. These results indicate that α-catenin binds to EspB through the preexisting less stable particular conformation in a manner of conformational selection [5].

To date, the structural properties of only a limited number of IDPs have been clarified but none of them assumes a partially folded structure. Our combinatorial approach using protein dissection with CD and SAXS successfully illustrated the structural property of one of such difficult targets. This approach was feasible because of the nature of IDP in which fewer nonlocal contacts between residues apart from each other in amino acid sequences are present. Although the resolution of our structural model is still low, further developments of this technique, e.g. in combination with molecular dynamics simulation, will be a powerful strategy to clarify the structural detail of IDPs with partially folded conformations.

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