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SAXS study of EbpS, a cell-wall associated protein of *Staphylococcus aureus*

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Introduction

Staphylococcus aureus (*S. aureus*) is a pathogenic bacterium responsible for most nosocomial infections occurring in hospitals worldwide [1]. *S. aureus* produces a number of proteins on the cell surface that are involved in infection, but whose precise functions and mechanism of action are still largely unknown.

Elastin binding protein of *S. aureus* (EbpS) is a cell-wall associated protein initially identified as the element required for bacterial attachment to human elastin. However, other reports have shown that adhesion of *S. aureus* to host cells is largely the result of interactions with other classes of adhesion proteins, such as fibronectin binding proteins. Because *ebps* gene is very well conserved and unique to *S. aureus*, we hypothesized that its protein product EbpS should have some crucial function beyond cellular adhesion.

Previously we showed that extracellular N-terminal region of EbpS (EbpS-N), which is thought to possess the functional domain of full-length EbpS, exhibits intrinsically disordered structure in aqueous solution [2]. We also demonstrated that transferring EbpS-N from aqueous solution to water/alcohol mixtures induced appearance of regular secondary-structure elements, suggesting that EbpS-N is able to adopt several different structures and exert its function(s) in an environment-depended manner [2]. In this report we describe recent progress in the structural study of both EbpS-N2 (slightly longer fragment than EbpS-N) and full-length EbpS using small-angle X-ray scattering (SAXS).

Experimental

SAXS experiments were conducted at beamline BL-10C of the Photon Factory. A wavelength of 1.488 Å was used, and scattering patterns were recorded in a R-AXIS7 image plate. EbpS contructs were expressed in *E. coli* and purified by chromatographic methods. Protein was kept in a solution containing 20 mM Tris-HCl, 300 mM NaCl, and 0.1% n-dodecyl maltoside at pH 8.0. Protein concentration was adjusted to 7.5 mg/ml. Data collection was set to 2 minutes. Curves were first analyzed by Kratky plot. Distance distribution function P(r) was calculated with the program GNOM. Electron density maps were constructed from the P(r) function using the program DAMMIN.

Results and Discussion

Kratky plots of EbpS-N2 calculated from the scattering profile monotonically increased against scattering angle. Such type of plot profile is a classical feature of disordered proteins, thus corroborating our previous observations using circular dichroism [2].

We next examined the structure of full-length EbpS using SAXS. First, we analyzed the Kratky plot calculated from the scattering profile of full-length EbpS. Values of the function $Q^2J(Q)$ gradually increased with the wide angle region, although this increase did not follow the monotonic increase observed with the N-terminal region. We concluded that full-length EbpS is partially folded. In addition, we analyzed the distance distribution function P(r) and constructed a model of the electron density map. Both P(r) function and models of electron density indicated that full-length EbpS forms hollowed disk-shaped particles.

We also examined the structure of full-length EbpS in the presence of divalent cation Zn^{2+}, which we found could be related to the biological function of EbpS. Details of the effect of Zn^{2+} will be reported in a manuscript now under progress.

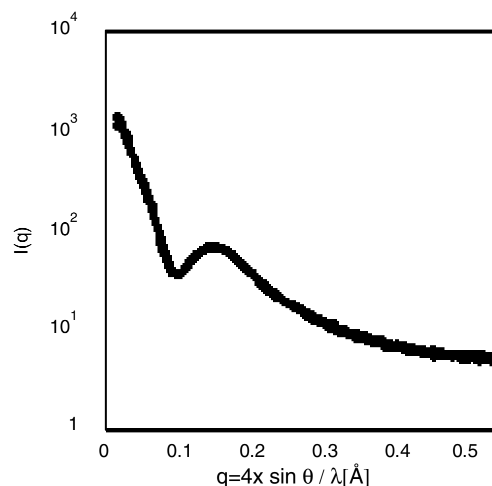

Fig. 1. SAXS profile of full-length EbpS.

References

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