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The N-terminal domain of elastin-binding protein of *Staphylococcus aureus* changes its secondary structure in a membrane-mimetic environment

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Elastin-binding protein of *Staphylococcus aureus* (EbpS) has been identified as an adhesin that can bind to soluble elastin or tropoelastin. However, the structure and exact function of EbpS remain to be elucidated. To gain insight into the molecular characteristics of EbpS, we investigated the physical properties of its N-terminal extracellular domain in various environments. CD spectroscopy showed that this protein was soluble and unstructured under aqueous conditions. Non-native secondary structures, however, were induced by several alcohols that provided membrane-mimetic environments. These changes may have some correlation with the function of this protein.

Key words: CD spectrum, elastin-binding protein of *Staphylococcus aureus* (EbpS), membrane-mimetic condition, secondary structure, structural change.

Abbreviations: DSC, differential scanning calorimetry; EbpS, elastin-binding protein of *Staphylococcus aureus*; ECM, extracellular matrix; MSCRAMMs, microbial surface components recognizing adhesive matrix molecules; TFE, 2,2,2-trifluoroethanol.

Infection with pathogenic bacteria is initiated by the colonization of host tissues with bacterial cells. Bacterial pathogens usually have many cell wall-associated proteins that recognize the mammalian extracellular matrix (ECM); these proteins are called microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) (*1*,*2*). The binding of these proteins to the ECM allows bacteria to adhere to, and colonize the host tissue.

Elastin is a major component of the ECM, and its primary physiological role is to give tissues and organs the property of reversible elasticity (*3*). Thus elastin and elastic fibres are present in abundance in tissues that require elasticity, such as the lung, skin and major blood vessels.

Staphylococcus aureus, which is a major cause of hospital- and community-acquired infections (*4*), can infect these elastin-rich tissues. One of the integral membrane proteins, elastin-binding protein of *S. aureus* (EbpS), has been identified as an adhesin to elastin (*5–8*). However, the protein that promotes the binding of *S. aureus* cells to soluble elastin peptides and tropoelastin was later reported to be not EbpS but fibronectin-binding proteins A and B (*9*). As a result, the biological function of EbpS remains to be clarified. To acquire the clue of the exact function of EbpS, we elucidated the molecular characteristics of N-terminal extracellular domain (1–204 residues) of EbpS (nEbpS).

For experiments, polyhistidine-tagged nEbpS was prepared with *Escherichia coli*. The gene encoding the protein was amplified by polymerase chain reaction from *S. aureus Mu50* genomic DNA (*10*). The recombinant protein was expressed by *E. coli* strain BL21 (DE3), and then purified with nickel chelate affinity chromatography and subsequent gel permeation chromatography. Detail protocol is described in Supplementary data. Purified protein was concentrated to 35 mg/ml with a centrifugal filter device. Concentration of the protein was decided by absorbance at 280 nm with a molar absorption coefficient of 4440 l/mol cm.

It is known that nEbpS migrates as a 45-kDa protein in SDS-PAGE analysis although the predicted molecular mass of nEbpS is 23 kDa (*6*,*7*). Furthermore, although the reason for this is not yet understood, a similar aberrant migration in SDS-PAGE has been reported in some cell-surface proteins of Gram-positive bacteria (*11–14*). To investigate this behaviour more closely, SDS-PAGE analysis with various concentrations of nEbpS was performed (Fig. 1). Although, as reported, nEbpS migrated as a protein of about 45 kDa when the concentration was 0.5 mg/ml or less, the shapes of the bands were abnormal at protein concentrations higher than 1.25 mg/ml (Fig. 1). Subsequently, nEbpS that had first been concentrated sufficiently to present abnormal migration behaviour and then diluted to 0.5 mg/ml was analysed by SDS-PAGE. Its behaviour was identical to that of low-concentration nEbpS that had not been subjected to high-level concentration (data not shown). It is therefore plausible that nEbpS can reversibly change its structure into several different ones, depending on its concentration. This feature was also confirmed by gel permeation chromatography. Concentrated nEbpS had several peaks on gel permeation chromatography in contrast to diluted nEbpS, which had single peak

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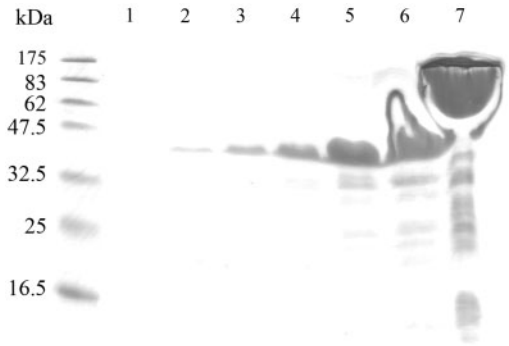

Fig. 1. **SDS-PAGE was performed with nEbpS at various concentrations.** A 15% polyacrylamide gel was used as a separation gel. The sample buffer used was 62.5 mM Tris, 2.3% SDS 8% and glycerol; and the running buffer was 24.8 mM Tris, 192 mM glycine and 1% SDS. Migrated protein was visualized with Coomassie Brilliant Blue R-250. Concentrations of nEbpS are: lane 1, 0.025 mg/ml; lane 2, 0.125 mg/ml; lane 3, 0.25 mg/ml; lane 4, 0.5 mg/ml; lane 5, 1.25 mg/ml; lane 6, 2.5 mg/ml; lane 7, 7.5 mg/ml.

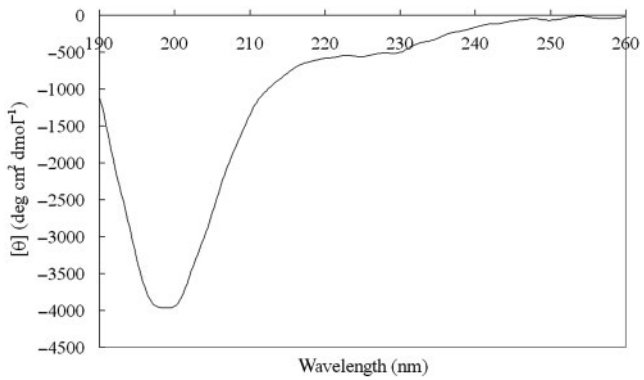

Fig. 2. **CD spectrum of nEbpS in 10 mM sodium phosphate buffer.** CD measurements were carried out in a quartz cell with an optical path length of 1 mm. The spectra were averaged over four scans collected at 0.1 nm intervals, with a 1 nm bandwidth, 2 s response time and 20 nm/min scan speed. All measurements were performed at 25°C. The sample used was 0.1 mg/ml nEbpS in 10 mM sodium phosphate buffer pH 8.0. The sample was incubated at 4°C overnight before measurement.

(data not shown). This result suggests that the increase in nEbpS concentration leads to multimer formation at a concentration enough to oligomerize due to intermolecular interaction. This feature can be observed in some other cell surface proteins from *S. aureus* (MN, YT, and KT; unpublished data), suggesting that it reflects an essential and universal molecular property for existence at the surfaces of cells in which the environment is vertiginously changed.

To acquire the structural feature of nEbpS, we investigated the secondary structure of the nEbpS by using CD. Figure 2 shows the CD spectrum of nEbpS in 10 mM sodium phosphate buffer (pH 8.0). The spectrum had a large negative band at around 200 nm, representing proteins with disordered structures. Incubation for

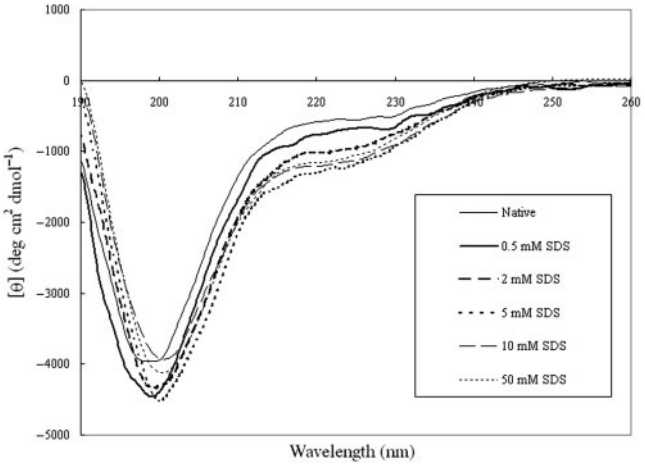

Fig. 3. **CD spectra of nEbpS in sodium phosphate buffer containing SDS at various concentrations.** The measurement parameters were same as Fig. 2 except that samples were incubated at room temperature overnight before measurements.

30 min at 70°C did not change the CD spectrum of nEbpS (data not shown). As with heat treatment, acidic [Sodium acetate buffer (pH 5.6)] or alkaline [*N*-cyclohexyl-3-aminopropanesulfonic acid buffer 0pH 10.4)] conditions did not change the CD spectrum of nEbpS (data not shown). Additionally, nEbpS in 10 mM sodium phosphate buffer, sodium acetate buffer, or *N*-cyclohexyl-3-aminopropanesulfonic acid buffer provided no major peaks during heating by differential scanning calorimetry (DSC) by capillary Valerian Plotnikov (VP) -DSC (MicroCal, Northampton, MA, USA), suggesting that nEbpS does not have a rigid tertiary structure under aqueous conditions (data not shown). From these results, we concluded that nEbpS adopts disordered structures, and its CD spectrum did not change with changes in temperature or pH. Although little is known about the behaviour of such proteins with disordered structures, the functional importance of disordered structures has been discussed (*15–20*). It has been proposed that the increased intrinsic plasticity represents an important prerequisite for effective molecular recognition in cell-adhesion proteins or, proteins involved in cellular signalling and regulation, and so on (*15–20*). Similar to these proteins, nEbpS might utilize its disordered structure to bind to targets *in vivo*.

To address the molecular characteristic of nEbpS, we examined the secondary structure of nEbpS in the presence of SDS using CD spectroscopy. It is known that a submicellar concentration of SDS tends to induce β-strand conformation, whereas an α-helical structure is more likely to be induced at or above micellar concentrations (*21–23*). Figure 3 shows the CD spectra of nEbpS in the presence of SDS at various concentrations. Although between 220 nm and 200 nm, the bands in SDS buffer were slightly more negative than the bands in buffer that did not contain SDS, the overall shapes of the spectra were similar, indicating that no marked structural change in nEbpS was induced by the presence of SDS.

Recently, a water and alcohol mixture has been suggested as a model system for studying the local decrease in dielectric constant near the membrane surface or in the inner region of the membrane on the structure of proteins; many proteins transform their secondary conformations in these locations (*21–25*). Due to this feature, we examined the CD spectra of nEbpS in methanol, ethanol, or trifluoroethanol (TFE) at various concentrations (Fig. 4). In the case of methanol (Fig. 4A), the spectrum changed to that of a typical α-helical protein with increasing methanol concentration up to 70%, at which concentration there were two negative peaks at 222 and 208 nm and one positive peak at about 190 nm. However, a further increase in the concentration of methanol to 100% resulted in a spectral change to that of a β-strand–like shape, *i.e.* a negative band at about 218 nm and a positive band at about 195 nm. According to k2d program (http://www.embl-heidelberg.de/~andrade/k2d.html), the ratio of α-helix, β-strand and random structure of nEbpS in 100% methanol are estimated to be 8%, 44% and 48%, respectively. As with methanol, TFE and ethanol both induced changes in the spectra of nEbpS to α-helix-like shapes, but no change to a β-strand-like shape occurred, even in 100% solution of each alcohol (Figs 4B and C). Thus the spectrum of nEbpS switched from disordered to an α-helix-like pattern depending on the alcohol concentration. According to k2d program, the α-helix ratios of nEbpS in Ethanol or TFE are estimated to be 10% and 23%, respectively. The α-helix-like spectra induced by the alcohols could not be changed even after incubation of 30 min at 70°C (data not shown), suggesting that the induced α-helical structure was rigid and stable. A change in the solvent from alcohol to aqueous led to complete recovery of the unstructured state: *i.e.* the CD spectrum of nEbpS changed from an α-helix- or β-strand-like shape to a disordered one (data not shown).

The spectra of nEbpS under various conditions revealed that nEbpS could reversibly adopt a disordered structure under aqueous conditions, as well as two different non-native structures (*i.e.* α-helical- and β-sheet-like structures) in methanol, ethanol, or TFE. Although it was reported that the presence of SDS can induce non-native protein structures (*21–23*), the spectrum of nEbpS was not changed by the addition of SDS to the aqueous buffer. SDS is known to induce non-native structures in proteins by providing a hydrophobic environment (*21*,22). These results suggest that not only a hydrophobic environment, but also a low dielectric constant, is needed to induce the non-native structure of nEbpS.

Although alcohols often induce α-helix to polypeptide, very few proteins, which adopt disordered structure under aqueous condition and several different structures under membrane-mimetic conditions, have been reported (*25*). One of the proteins which have such feature is the α-synuclein. The α-synuclein is known to adopt disordered secondary structure under aqueous condition and two different non-native secondary structures in organic solvents, which is similar to that of the nEbpS, although nEbpS exists at cell surface and α-synuclein exists in various regions of brain (*24*,26,27). It was reported that α-synuclein aggregates into amyloid fibrils (*28*,29) and binds negatively charged phospholipid membranes and micelles *in vitro* (*28*,30,31). Further studies are now under progress for understanding the molecular characteristics of EbpS from these viewpoints.

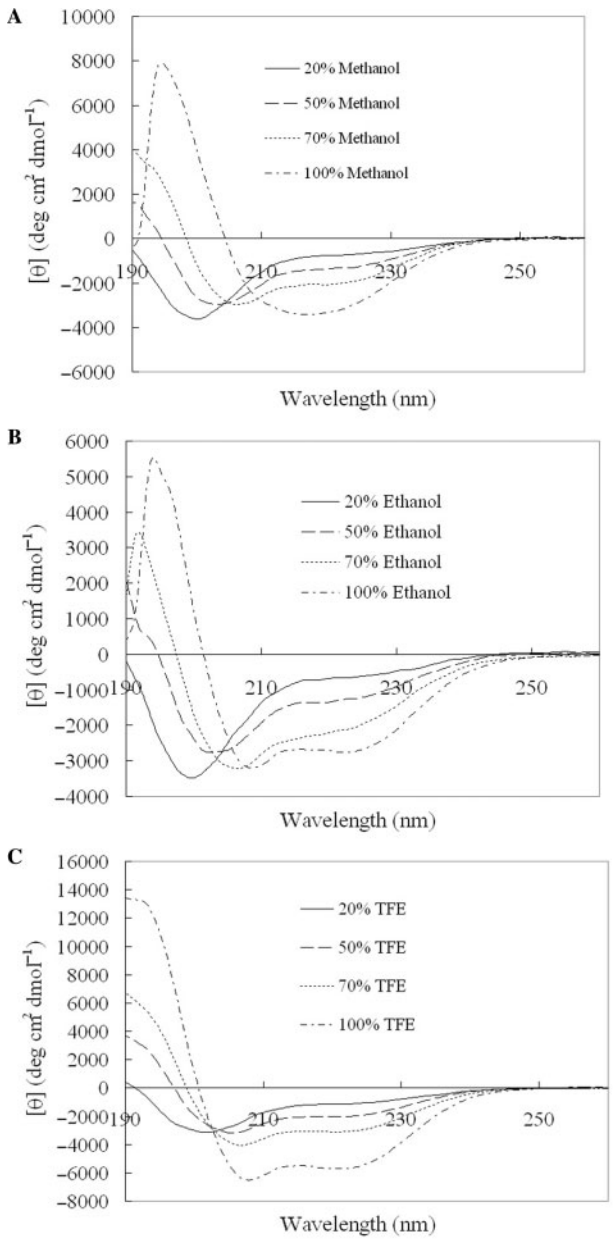

Fig. 4. **CD spectra of nEbpS in the presence of methanol (A), ethanol (B), or TFE (C) at various concentrations.** The measurement parameters were same as Fig. 2.

Supplementary data are available at JB online.

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