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# Crystallization and preliminary X-ray analysis of B-domain fragments of a *Staphylococcus aureus* collagen-binding protein

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Recombinant proteins of monomeric and dimeric B-domain repeats of a *Staphylococcus aureus* FDA 574 collagen-binding adhesin have been crystallized. The single repeat unit ($B_1$) was crystallized in a body-centered orthorhombic lattice with $a$ = 96.9, $b$ = 101.3, $c$ = 120.8 Å in either the $I222$ or $I2_12_12_1$ space group. These crystals diffracted to 2.5 Å resolution and the calculated $V_m$ values of 3.2 and 2.2 Å$^3$ Da$^{-1}$ suggest the possibility of a dimer or a trimer in the asymmetric unit. The two-repeat fragment ($B_1B_2$) crystallized in the orthorhombic space group $P2_12_12_1$ with cell dimensions $a$ = 42.4, $b$ = 79.4, $c$ = 130.4 Å and diffracted to 2.3 Å resolution. For this species, the calculated $V_m$ value of 2.2 Å$^3$ Da$^{-1}$ indicates the presence of a monomer in the asymmetric unit.

## 1. Introduction

The molecular mechanism of microbial adherence has attracted significant attention owing to its pivotal role in the pathogenic process. The binding of bacteria to adhesive extracellular matrix proteins such as fibrinogen (Hawiger *et al.*, 1982), fibronectin (Kuusela, 1978), vitronectin (Chhatawal *et al.*, 1987) and the collagens (Speziale *et al.*, 1986) has emerged as an important mechanism of host-tissue adherence. Few bacterial MSCRAMMS (microbial surface components recognizing adhesive matrix molecules) have been isolated and characterized. The *S. aureus* collagen MSCRAMM binds to a variety of collagens with a moderately high affinity (House-Pompeo *et al.*, 1994). A 135 kDa protein from this strain had been isolated, purified and identified as the collagen-binding MSCRAMM (Switalski *et al.*, 1989). Molecular characterization of the gene revealed the presence of a non-repetitive A domain and a repetitive B domain (Patti *et al.*, 1993; Fig. 1). Recently, we determined the crystal structure of CBD19, which represents the smallest sub-fragment of the A domain with measurable collagen-binding activity (Symersky *et al.*, 1997). Biochemical studies of the repeats of the B domain are in progress. In our analysis of the structural and functional relationships of this collagen adhesin, we initiated the crystal structure analysis of the $B_1$ and $B_1B_2$ fragments of the collagen-binding MSCRAMM. Various strains of *S. aureus* express collagen-binding MSCRAMMs in which the number of B-domain repeats ranges from one to four units (Gillaspy *et al.*, 1997). Further X-ray crystal structure analysis of the recombinants discussed here may reveal the association of these repeating domains in the architecture of this collagen adhesin.

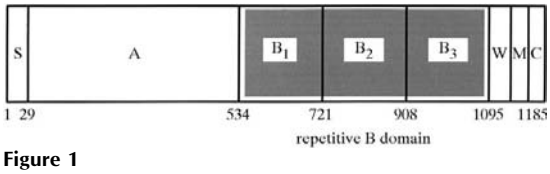


**Figure 1**
Schematic diagram of the *S. aureus* FDA 574 collagen-binding MSCRAMM. The putative signal peptide (S), non-repetitive binding domain (A), repeat domain ($B_1B_2B_3$), cell-wall domain (W), membrane-spanning domain (M) and charged carboxyl-terminal domain (C) are indicated.

## 2. Materials and methods

### 2.1. Expression and initial purification of recombinant proteins.

A series of expression plasmids were constructed using the vector pQE-30 (Qiagen Inc., Chatsworth, CA). Recombinant proteins expressed from this vector contain a N-terminal tail of six histidine residues. Expression constructs were produced when the corresponding *cna* gene fragments were amplified from *S. aureus* FDA 574 genomic DNA (Marmur, 1961) by the polymerase chain reaction (PCR) together with the flanking oligonucleotides. PCR and the subsequent cloning of the amplified gene product into the expression plasmid were performed using standard molecular-biology protocols (Ausubel *et al.*, 1991; Sambrook *et al.*, 1989). After cloning in *E. coli*, plasmids were isolated and subjected to automated DNA-sequencing analysis (Molecular Genetics Core Facility, UT-Houston Medical School) to confirm the expected open reading frame (ORF) as it corresponds to the *cna* gene

sequence. The published sequence (Patti *et al.*, 1992, 1993) is identical to the now-determined sequence except for the following: Ile665→Thr and Ile852→Thr.

For each recombinant, an 18 h bacterial culture [*E. coli* strain JM101, grown at 310 K in Luria Broth (Life Technologies, Inc., 25 g $l^{-1}$) under an ampicillin pressure of 100 mg $l^{-1}$ with constant agitation] was diluted 1/1000 in the same broth, and growth was allowed to continue under these conditions until the $OD_{600}$ approximated 0.6–0.8 (~6 h). The cells were induced by addition of isopropylthio-β-D-galactoside (IPTG) to a final concentration of 0.2 m*M* and grown for an additional 3 h. Cells were collected by centrifugation and re-suspended in a minimal volume of 5 m*M* imidazole, 100 m*M* NaCl, 4 m*M* Tris–HCl, pH 7.9 and frozen at 203 K.

Cell pellets were thawed, passed through a French press (138 MPa) three times to lyse the cells and centrifuged at 40000 rev $min^{-1}$ for 20 min to remove insoluble proteins. The supernatant was filtered through a 0.45 µm membrane to remove suspended particulates. Recombinant proteins were purified to homogeneity using an immobilized metal-chelate affinity chromatography column attached to a Pharmacia FPLC system as described by Patti *et al.* (1993), run with a stepwise gradient of 4 m*M* Tris–HCl, 100 m*M* NaCl, 200 m*M* imidazole, pH 7.9. In all the purification procedures, the eluate was monitored by absorbance at 280 nm and collected fractions were examined for purity by SDS–PAGE. Fractions containing only the desired product were pooled, concentrated using an Amicon ultrafiltration system, dialyzed exhaustively against 10 m*M* $Na_2HPO_4$, 100 m*M* NaCl, pH 7.0, fast-frozen in a dry ice/ethanol bath and kept at 203 K until use.

### 2.2. Final purification and crystallization of $B_1$ fragment.

The $B_1$ recombinant protein (22.6 kDa) was further purified using gel-permeation chromatography on a Bio-sil SEC250 HPLC column and eluted in 60 m*M* $Na_2PO_4$, 150 m*M* NaCl, pH 6.9. SDS–PAGE confirmed the molecular mass and the purity of the protein. Isoelectric focusing using the PHAST system indicated the p*I* of the protein to be 5.3. The protein was concentrated to approximately 17–18 mg $ml^{-1}$ using an Amicon micro-ultrafiltration system. Crystallization screening was performed using the hanging-drop vapor-diffusion method at room temperature, with the reservoir containing 1 ml of 4 *M* $(NH_4)_2SO_4$, 50 m*M* succinic acid, pH 4.6. This was equilibrated against 4 µl drops, which were made by mixing 2 µl of protein, 1.6 µl of well solution and 0.4 µl of 10%(*w*/*v*) octyl-β-D-glucopyranoside. Crystals (Fig. 2) grew in 4–5 d to a size of 0.5 × 0.5 × 0.2 mm.

### 2.3. Final purification and crystallization of $B_1B_2$ fragment

The $B_1B_2$ recombinant protein (43.8 kDa) was further purified using gel-permeation chromatography on a Bio-sil SEC250 HPLC column and eluted in 60 m*M* $Na_2PO_4$, 150 m*M* NaCl, pH 7.0. The protein was then concentrated to approximately 36 mg $ml^{-1}$ using an Amicon micro-ultrafiltration system. Trials to crystallize the $B_1B_2$ recombinant protein under the conditions described for $B_1$ did not yield crystals. Hanging drops were made by mixing 1 µl of protein, 1 µl of 5 m*M* $CaCl_2$ and 7 µ1 of reservoir solution. The reservoir contained 1 ml of 16%(*w*/*v*) PEG 6000, 100 m*M* sodium cacodylate, pH 7.5. Diffraction quality crystals (Fig. 3) grew in 5–6 d at room temperature.

## 3. Results

### 3.1. Single B-domain repeat unit $B_1$

Crystals of a single B-domain repeat unit, $B_1$, were exposed to X-rays at room temperature on an R-AXIS IIc image-plate detector mounted on a Rigaku RU-200 rotating-anode generator (Cu *Kα* radiation) operating at 100 mA and 40 kV. Complete data sets to a resolution of 2.5 Å were collected over 60 frames (oscillation of 1.5°, exposure time of 40 min and crystal-to-plate distance of 130 mm). The frames were indexed and scaled using *DENZO* and *SCALEPACK* (Otwinowski, 1993). Unit-cell dimensions for crystals of the $B_1$ recombinant protein were $a$ = 96.97, $b$ = 101.32, $c$ = 120.79 Å. The $V_m$ values of 3.2 and 2.2 $Å^3$ $Da^{-1}$ (Matthews, 1968) indicate the possibility of a dimer or a trimer in the asymmetric unit with solvent contents of 62 and 43%, respectively. The absence of reflections with $h + k + l = 2n + 1$ confirmed the body-centered orthorhombic lattice leaving an ambiguity in the space group, which is either $I222$ or $I2_12_12_1$. A total of 97712 reflections were merged to obtain 20953 unique reflections ($R_{merge}$ = 7.0%), 99% of the theoretically possible reflections being recorded.

The self-rotation function calculated using 15–4 Å resolution diffraction data revealed one strong peak (80% of the origin peak) at $\psi = 0°$ and $\varphi = 45°$ in the $\kappa = 180°$ section, indicating the presence of a non-crystallographic twofold axis in the *ac* plane, perpendicular to the *b* axis and 45° to the *a* axis (Collaborative Computational Project, Number 4, 1994). The $\kappa = 120°$ section did not show any significant peaks, thus suggesting the presence of a dimer in the asymmetric unit. Heavy-atom screening is in

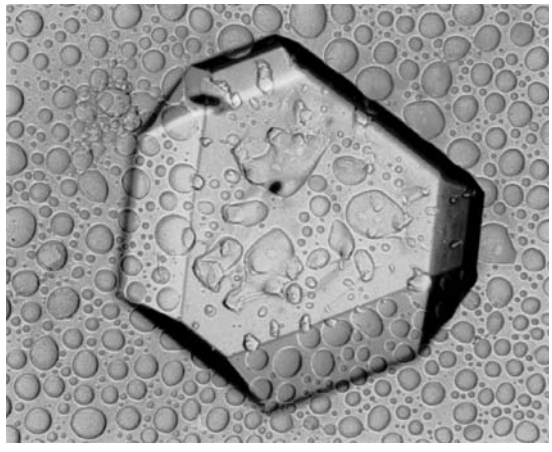

**Figure 2**
Crystal of $B_1$ grown in the presence of $(NH_4)_2SO_4$, octyl-β-D-glucopyranoside and succinic acid at pH 4.6, having maximum dimensions of 0.4 × 0.4 × 0.15 mm.

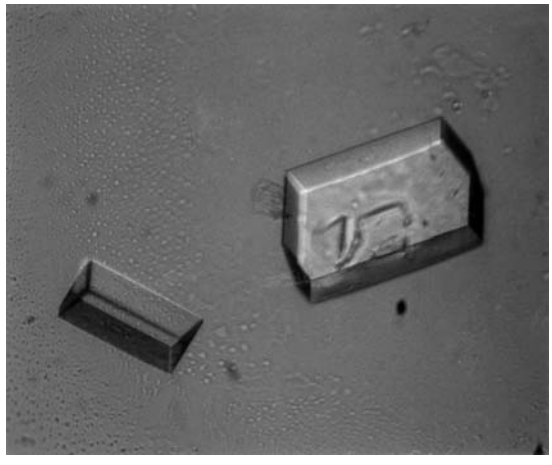

**Figure 3**
Crystals of $B_1B_2$ grown in the presence of PEG 6000, $CaCl_2$ and cacodylic acid at pH 7.5, having maximum dimensions 0.5 × 0.3 × 0.1 mm.

progress for the crystals of the $B_1$ recombinant protein.

### 3.2. Two B-domain repeat unit $B_1B_2$

Diffraction data were collected on the R-AXIS IV image-plate detector (oscillation 1.25°, crystal-to-plate distance 175 mm and exposure time of 20 min) mounted on a Rigaku generator operating at 100 mA and 50 kV. The frames were integrated, reduced and scaled using *DENZO* and *SCALEPACK* (Otwinowski, 1993). Cell dimensions were found to be $a$ = 42.4, $b$ = 79.4, $c$ = 130.4 Å and the space group was $P2_12_12_1$. A total number of 122160 reflections were merged to obtain 19750 unique reflections with an $R_{sym}$ of 6.9%. The data set has an $\langle I/\sigma(I)\rangle$ of 22.3 with 61.5% of reflections above the $3\sigma$ level in the last shell, and has 97% completeness to a resolution of 2.3 Å. The $V_m$ value of 2.4 Å$^3$ Da$^{-1}$ suggests the presence of a monomer in the asymmetric unit (Matthews, 1968).

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