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Characterization, crystallization and preliminary X-ray analysis of the adhesive domain of SdrE from *Staphylococcus aureus*

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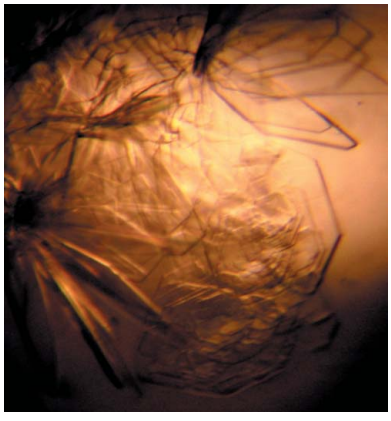

The adhesive domain of SdrE from *Staphylococcus aureus* was recombinantly expressed in *Escherichia coli*. The purified protein was identified by SDS–PAGE and MALDI–TOF MS. The protein was crystallized using the vapour-diffusion method in hanging-drop mode with PEG 8000 as the primary precipitating agent. X-ray diffraction data were collected to 1.8 Å resolution from a single crystal of the protein. Preliminary X-ray analysis indicated that the crystal belonged to space group $P1$, with unit-cell parameters a = 40.714, b = 66.355, c = 80.827 Å, α = 111.19, β = 93.99, γ = 104.39°.

1. Introduction

The natural habitat of *Staphylococcus aureus* in humans is the skin and nasopharynx. Although the organism is part of normal human flora, it can cause a number of infections ranging from minor skin infections to life-threatening endocarditis and osteomyelitis when there is a break in the skin or mucous membrane that grants it access to the surrounding tissues (Cheung *et al.*, 2004; Lindsay & Holden, 2004).

The success of *S. aureus* as a pathogen is in general due to two types of virulence determinants: cell-surface-associated proteins and extracellular protein toxins (Diekema *et al.*, 2001; Lowy, 1998). The molecular pathogenesis of bacteria is initiated by adherence of the bacteria to host tissues.

Adhesins called MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) are specialized surface-located proteins that mediate bacterial adhesion. *S. aureus* expresses a number of adhesins on its cell surface that promote the binding of the organism to components of the host extracellular matrix and play an important role in bacterial virulence (Patti *et al.*, 1994).

A family of covalently attached surface proteins exist in *S. aureus* which are characterized by the presence of a domain containing extensive Ser-Asp dipeptide repeats and a similar structural arrangement (Josefsson, McCrea *et al.*, 1998; Foster & Hook, 1998). ClfA and ClfB are the most studied members of this family from *S. aureus*. The fibrinogen-binding clumping factor protein of *S. aureus* (ClfA) was the first fibrinogen-binding adhesin to be identified in *S. aureus* (Hartford *et al.*, 2001). ClfB binds to cytokeratin 10 and to fibrinogen (O'Brien, Walsh *et al.*, 2002; Perkins *et al.*, 2001). Additional important members of this family are the Sdr proteins, which differ from ClfA and ClfB by having two, three or five (in SdrC, SdrE and SdrD, respectively) B-repeats of 110–113 residues located between the A and R domains. Each B-repeat contains a consensus EF-hand loop for binding Ca^{2+} with high affinity (Josefsson, McCrea *et al.*, 1998; Josefsson, O'Connell *et al.*, 1998).

SdrE, when expressed heterologously in *Lactococcus lactis*, is able to promote platelet aggregation, which most likely to be mediated by binding to a plasma protein that acts as a bridge between the bacterium and a platelet receptor (O'Brien, Kerrigan *et al.*, 2002). Studies have indicated that SdrE is involved in fibrinogen-bridged *S. aureus*–platelet interaction. Both ClfA and SdrCDE are required on the bacterial surface for stable adhesion to platelets in the high-shear regime (George *et al.*, 2006).

To date, the detailed structural characteristics of SdrE have remained unknown. The three-dimensional structure of the adhesive domain of SdrE will help to further elucidate its biological function in infection by *S. aureus* and may expose new targets for the development of novel therapeutics.

In this study, we report the expression, crystallization and preliminary crystallographic analysis of the adhesive domain of SdrE from *S. aureus*.

2. Materials and methods

2.1. Materials

The enzymes for recombinant DNA technology such as *Taq* polymerase, T4 DNA ligase, *Bam*HI and *Xho*I were purchased from New England Biolabs. PCR amplification kits (including PCR buffer and dNTP Mix) were also obtained from New England Biolabs.

2.2. PCR amplification

The primers spanning amino acids 269–597 of SdrE were designed according to the published nucleotide sequence of *S. aureus* subsp. *aureus* TCH60 (GenBank Accession No. GG668725). In order to facilitate the subsequent cloning, two restriction-endonuclease sites, *Bam*HI and *Xho*I, were attached to the 5′-termini of the upstream and the downstream primer, respectively. The forward primer was 5′-ATCGTCGGATCCAATAATGTAAATGACTTAATTACA-3′ and the reverse primer was 5′-AATGCTCTCGAGCTAAGGTTTAACAGTACCGTCGCCACC-3′. The polymerase chain reaction was carried out using genomic DNA of *S. aureus* ATCC 25923 as a template. The PCR product was separated on an agarose gel containing 1% agarose and was purified using a quick DNA purification/recovery kit (Omega).

2.3. Cloning, expression and purification

The purified PCR product was digested with *Bam*HI and *Xho*I overnight and cloned into pGEX-6p-1 vector (Merck Biosciences) treated with the same enzymes. The recombinant plasmid was transformed into *Escherichia coli* strain BL21.

Protein expression was induced by the addition of IPTG (0.5 m*M* final concentration) when the OD_{600} of the culture reached ∼0.8. The cultures were allowed to grow for another 12 h at 291 K. The cells were then harvested by centrifugation at 5000 rev min^{-1} for 10 min at 277 K. The bacterial cells were resuspended in lysis buffer (1× PBS, 1 m*M* DTT and 1 m*M* PMSF) and homogenized by sonication. The cell lysate was centrifuged at 20 000*g* for 45 min at 277 K to remove cell debris completely.

All of the following purification steps were performed at 289 K. The clear supernatant was applied onto a self-packaged GST-affinity column (2 ml glutathione Sepharose 4B; GE Healthcare) and contaminant proteins were removed with wash buffer (lysis buffer plus 200 m*M* NaCl). The fusion protein was then digested with PreScission protease at 277 K overnight. The protein with an additional five-amino-acid tag (GPLGS) at the N-terminus was eluted with lysis buffer. The eluant was concentrated using an Ultrafree 5000 molecular-weight cutoff filter unit (Millipore) and further purified using a Superdex-75 (Pharmacia) column (10 m*M* Tris–HCl pH 8.0, 200 m*M* NaCl, 1 m*M* DTT buffer). The purified protein was analyzed by SDS–PAGE and MALDI–TOF MS. The fractions containing the target protein were pooled and concentrated to 30 mg ml^{-1}.

2.4. Crystallization

The protein sample was centrifuged at 20 000*g* for 45 min to clarify the solution before initiating crystallization trials. Initial screening was performed at 291 K in 24-well plates by the hanging-drop vapour-diffusion method using sparse-matrix screening kits from Hampton Research (Crystal Screens I and II), followed by refinement of the conditions by variation of the precipitants, pH, protein concentration and additives. Typically, droplets consisting of 1 µl protein solution and an equivalent volume of reservoir solution were equilibrated against 200 µl reservoir solution.

2.5. X-ray crystallographic studies

X-ray diffraction data sets were collected from the crystals using a MAR CCD 245 image-plate detector on Shanghai Synchrotron Radiation Facility (SSRF) beamline BL17U with a wavelength of 0.9794 Å.

The crystals were immersed in a cryoprotectant solution (reservoir solution supplemented with 12% glycerol) for 5–10 s, picked up in a loop and then flash-cooled in a nitrogen-gas stream at 100 K. A data set consisting of 720 frames was collected. The exposure time per frame was 0.8 s, the crystal-to-detector distance was 150 mm and the oscillation range per frame was 0.5°. All intensity data were indexed,

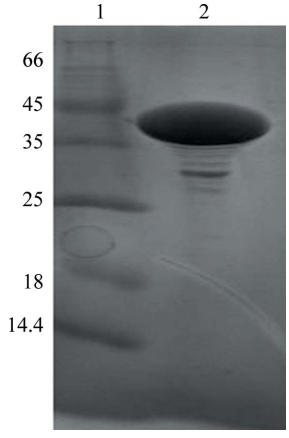

Figure 1
SDS–PAGE of the purified adhesive domain of SdrE. Lane 1, molecular-weight markers (kDa); lane 2, the purified adhesive domain of SdrE used for crystalization.

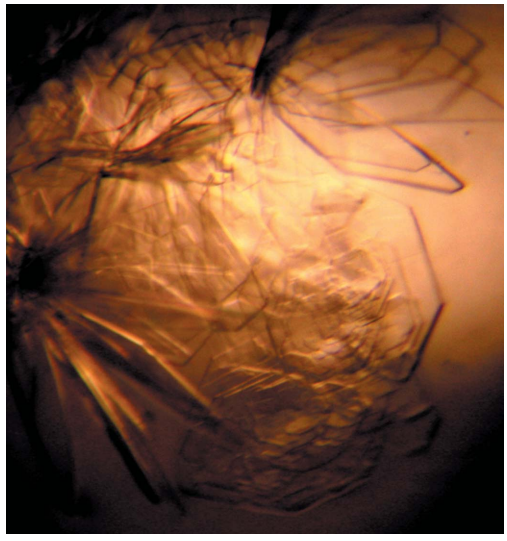

Figure 2
Typical crystal of the adhesive domain of SdrE.

Table 1
Data-collection and processing statistics.

Values in parentheses are for the last shell.

Space group	*P*1
Unit-cell parameters (Å, °)	a = 40.714, b = 66.355, c = 80.827, α = 111.19, β = 93.99, γ = 104.39
Wavelength (Å)	0.9794
Resolution (Å)	50–1.8 (1.83–1.8)
No. of observed reflections	192659
No. of unique reflections	67113
Completeness (%)	96.6 (91)
$\langle I/\sigma(I)\rangle$	27.8 (3.1)
$R_{\rm merge}$ (%)	12.4 (44.1)

integrated and scaled using the *HKL*-2000 program suite (Otwinowski & Minor, 1997).

3. Results and discussion

A 987 bp DNA fragment was obtained by PCR amplification and cloned into pGEX-6P-1. The bacteria containing the recombinant plasmid were identified by PCR and plasmid digestion and confirmed by DNA sequencing.

The adhesive domain of SdrE fused with GST was solubly expressed in *E. coli* in high yield. After purification, the protein was >95% pure on SDS–PAGE stained with Coomassie Brilliant Blue (Fig. 1). MALDI–TOF MS of trypsin-digested purified protein provided convincing evidence that the protein was the SdrE protein from *S. aureus*.

Small SdrE crystals appeared after about 2 d from one condition of Crystal Screen (Hampton Research) containing PEG 8000 as a precipitant. The conditions were further optimized by varying the precipitant, buffer pH and protein concentration, and larger crystals (Fig. 2) were obtained at 291 K using the vapour-diffusion method in hanging-drop mode by mixing 1 µl protein solution with 1 µl reservoir solution (0.2 *M* calcium acetate hydrate, 0.1 *M* sodium cacodylate trihydrate pH 6.5, 18% PEG 8000) and equilibrating against 200 µl reservoir solution. The larger crystals were reproducible and suitable for X-ray diffraction.

An X-ray diffraction data set was collected from a single crystal to 1.8 Å resolution (Fig. 3). The crystal belonged to space group *P*1, with unit-cell parameters a = 40.714, b = 66.355, c = 80.827 Å, α = 111.19, β = 93.99, γ = 104.39° (see Table 1). It was estimated that there were two molecules per asymmetric unit, giving a Matthews coefficient $V_{\rm M}$ of 2.77 Å^3 Da^{-1} and a solvent content of 55.7%. A self-rotation function showed a peak in the κ = 180° section, confirming that there were two molecules per asymmetric unit.

Although we have collected several full sets of diffraction data from the crystals, we have unfortunately so far not been able to determine the structure of the adhesive domain of SdrE using the molecular-replacement method. Only two related structures (SdrG from *S. epidermidis* and ClfA from *S. aureus*) were found in a *BLAST* search of the PDB. The adhesive domain of SdrE shares 54% and 29% sequence identity with SdrG and ClfA, respectively. We attempted to solve the structure of SdrE using SdrG (PDB code 1r17; Ponnuraj *et al.*, 2003) and ClfA (PDB code 1n67; Deivanayagam *et al.*, 2002) as models in the molecular-replacement method but could not obtain a satisfactory result, either because the flexible linker between the N2 and N3 domains causes differing orientations of these two domains or because the sequence identities are too low. The multiple-wavelength anomalous dispersion (MAD) or single-wavelength anomalous dispersion (SAD) methods will be used to solve the structure; the preparation and crystallization of selenomethionine-derivative protein is currently in progress.

Knowledge of the three-dimensional structure of the adhesive domain of SdrE at high resolution will facilitate an understanding of its specific functions and may also be useful in the rational design of drugs.

The diffraction data sets were collected on BL17U at Shanghai Synchrotron Radiation Facility (SSRF). This research was supported by the National Key Project of Scientific and Technical Supporting Programs funded by the Ministry of Science and Technology of China

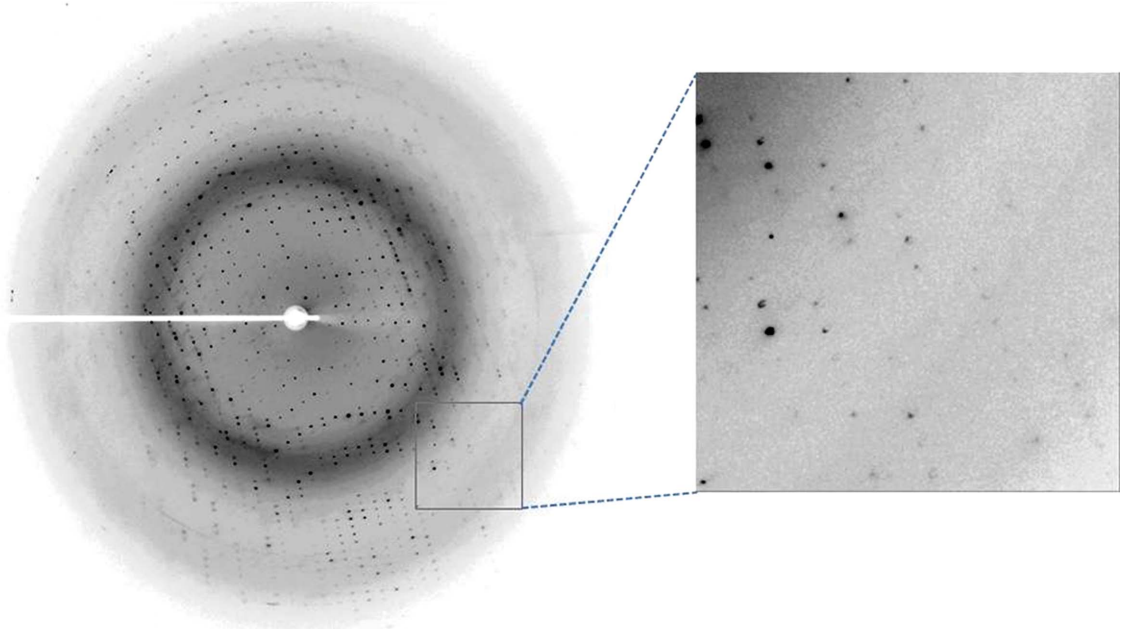

Figure 3
X-ray diffraction pattern from a crystal of the adhesive domain of SdrE. The crystal diffracted to 1.8 Å resolution.

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