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Crystallization of ClfA and ClfB fragments: the fibrinogen-binding surface proteins of *Staphylococcus aureus*

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Recombinant constructs encoding the fibrinogen-binding domains of ClfA and ClfB from *Staphylococcus aureus* have been crystallized. ClfA was crystallized in the orthorhombic space group $P2_12_12_1$ with unit-cell parameters $a = 39.58$, $b = 81.39$ and $c = 112.65$ Å. A complete data set was recorded to 2.1 Å resolution and had a V_m of 2.3 Å^3 Da^{-1} with 46.5% solvent, suggesting one molecule per asymmetric unit. Co-crystals of ClfA with the 17 amino-acid C-terminal peptide of fibrinogen γ-chain diffracted to 2.1 Å resolution and had unit-cell parameters $a = 39.11$, $b = 81.39$ and $c = 109.51$ Å in the space group $P2_12_12_1$. ClfB was crystallized in the tetragonal space group $P4_12_12$ or $P4_32_12$ with unit-cell parameters $a = 96.31$, $b = 96.31$ and $c = 84.13$ Å and diffracted to 2.45 Å resolution. The estimated V_m of 2.6 Å^3 Da^{-1} with 53% solvent indicated one molecule in the asymmetric unit.

1. Introduction

Staphylococcus aureus expresses several cell-surface adhesins which specifically interact with various mammalian extracellular matrix proteins including collagen, fibronectin, fibrinogen and vitronectin (Patti *et al.*, 1994; Patti & Höök, 1994). These bacterial surface proteins, collectively termed MSCRAMMs (microbial surface components recognizing adhesive matrix molecules), provide a means of adherence to host tissues and are likely to contribute to the pathogenicity (Patti *et al.*, 1994). Two distinct fibrinogen-binding proteins, clumping factor A (ClfA) and clumping factor B (ClfB), have been identified on the surface of *S. aureus* (McDevitt *et al.*, 1995; Ní Edhin *et al.*, 1999). These proteins bind soluble fibrinogen and are responsible for the bacterial clumping that occurs when *S. aureus* is incubated in the presence of fibrinogen. The predicted protein sequence of ClfA consists of 933 amino acids, and is comprised of an N-terminal signal peptide followed by a 520-residue-long A region, a 308-residue-long R region (consisting primarily of serine–aspartate dipeptide repeats) and C-terminal domains responsible for anchoring the protein to the bacterial cell wall (Fig. 1*a*). ClfB is similar to ClfA in that it consists of a similar domain structure (Fig. 1*b*). Furthermore, the fibrinogen-binding activity of both proteins is localized within the A region. However, ClfA is expressed throughout the growth cycle whereas ClfB is expressed primarily on cells in exponential growth (Ní Edhin *et al.*, 1999). Additionally, these proteins appear to recognize different sites in fibrinogen. ClfB binds to

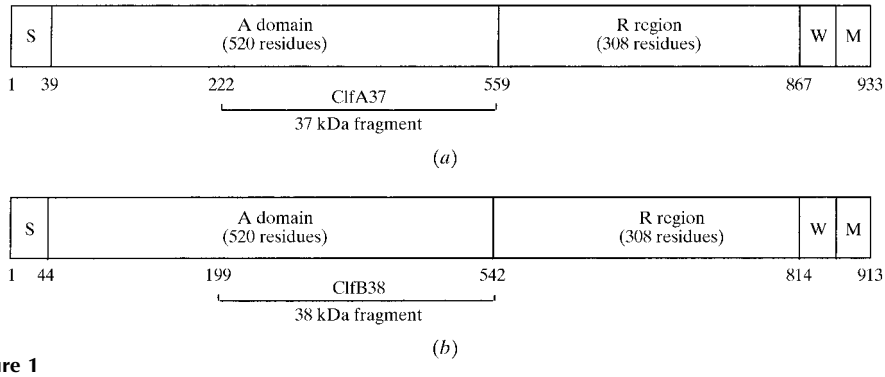

Figure 1
(*a*) ClfA has a signal peptide (S) at the N-terminal end, followed by the A domain which binds to fibrinogen, followed by the aspartate–serine (DS) dipeptide repeat region (R). The C-terminal has a cell-wall domain (W) and a membrane-spanning domain (M). (*b*) ClfB has a similar architecture to ClfA, with the signal peptide (S) at the N-terminal end, followed by the A-domain which binds to fibrinogen, followed by the aspartate–serine (DS) dipeptide repeat region (R). The C-terminal has a cell-wall domain (W) and a membrane-spanning domain (M).

both the α- and β-chains (McDevitt *et al.*, 1997, Ní Edhin *et al.,* 1999) while ClfA binds to two sites in the γ-chain: (i) at the extreme C-terminus which is also the binding site for the platelet integrin αIIbβ3 (O'Connell *et al.*, 1998) and (ii) at an internal site that is recognized by the leukocyte integrin αMβ2 (O'Connell *et al.*, personal communication).

In order to investigate the mechanism of *S. aureus* binding to fibrinogen, we have initiated the crystal structure analysis of ClfA and ClfB. This paper presents purification, crystallization and preliminary X-ray diffraction studies on the fibrinogen-binding A regions of ClfA and ClfB.

2. Materials and methods

2.1. Purification and crystallization of ClfA

DNA encoding the fibrinogen-binding A region of ClfA (amino-acid residues 40–559) was cloned into the pQE-30 expression vector (Qiagen Inc., Chatsworth, CA) and expressed in *E. coli* as a polyhistidine fusion protein containing six histidine residues at the N terminus (McDevitt *et al.*, 1995). Recombinant protein was initially purified using metal-chelating chromatography. Bacterial lysates were applied to an HR10/10 (Pharmacia Biotech Inc., Piscataway, NJ) column of nickel-charged iminodiacetic acid Sepharose (Sigma Chemical Company, St Louis, MO), and bound protein was eluted with a 160 ml linear gradient of 0–120 m*M* imidazole in 4 m*M* Tris–HCl, 100 m*M* NaCl, pH 7.9. Fractions corresponding to ClfA, as determined by SDS–PAGE, were pooled and further purified using ion-exchange and gel-filtration chromatography. Samples were applied to an HR10/10 column of Q-Sepharose (Pharmacia Biotech Inc., Piscataway, NJ) and eluted with a 160 ml linear gradient of 100–500 m*M* NaCl in 25 m*M* Tris–HCl pH 8.0. Protein was then concentrated and applied to a 2.6 × 60 cm column of Sephacryl S-100 and eluted with 20 m*M* Tris–HCl, 100 m*M* NaCl, 1 m*M* EDTA, pH 8.0 at a flow rate of 1.0 ml min^{-1}. Protein purity was estimated to be 98% following SDS–PAGE and staining with silver nitrate. The predicted molecular mass of the purified protein was 57 kDa. However, during this purification protocol, the ClfA preparation breaks down to a single 37 kDa fragment (as determined by electrospray ionization mass spectroscopy). N-terminal sequencing revealed the first amino acid of the fragment to be alanine 222, indicating that a significant portion of the N terminus was missing. This 37 kDa fragment (ClfA37, residues 222–550) exhibited fibrinogen-binding activity similar to that observed (McDevitt *et al.*, 1997) for the recombinant construct Clf33 (residues 221–550). It was therefore decided to crystallize this smaller fibrinogen-binding fragment ClfA37. The protein was concentrated to 16 mg ml^{-1} (as determined by optical density at 280 nm with $\varepsilon = 36330$ cm^{-1} M^{-1}) using a micro-ultrafiltration Amicon concentrator with a YM10 membrane. Crystallization screening of the ClfA37 fragment was performed using the hanging-drop vapor-diffusion method. Crystals were formed under the following conditions. The reservoir contained 1.0 ml of 1.2 *M* sodium citrate, 50 m*M* HEPES buffer pH 8.0, and was equilibrated against 6 µl drops which were produced by mixing 3 µl of 16 mg ml^{-1} protein solution and 3 µl of reservoir solution. Crystals (Fig. 2) grew to full size (0.4 × 0.3 × 0.1 mm) in two to three weeks at room temperature and diffracted to 2.1 Å resolution. A peptide corresponding to the 17 C-terminal residues of the fibrinogen γ-chain (GEGQQHHLGGAKQAGDV) was synthesized and purified as described by McDevitt *et al.* (1997) and co-crystallized with ClfA37 at a 2:1 molar ratio. The 2:1 molar ratio was chosen based on earlier studies indicating a K_d of 10^{-6} *M* between Clf33 and the γ-chain peptide (McDevitt *et al.*, 1997). Crystallization conditions for the ClfA37–peptide complex were similar to those for the native ClfA37 crystals. The reservoir contained 1.0 ml of 1.2 *M* sodium citrate and 50 m*M* Tris–HCl at pH 9.0. This was equilibrated against 6 µl drops produced by mixing 2 µl of protein, 2 µl of peptide and 2 µl of the well solution. Crystals grew to 0.5 × 0.3 × 0.1 mm in two to three weeks at room temperature. Preliminary diffraction analysis revealed that crystals of the complex (Fig. 3) were non-isomorphous with those of native ClfA37 and diffracted much more strongly to 2.1 Å resolution.

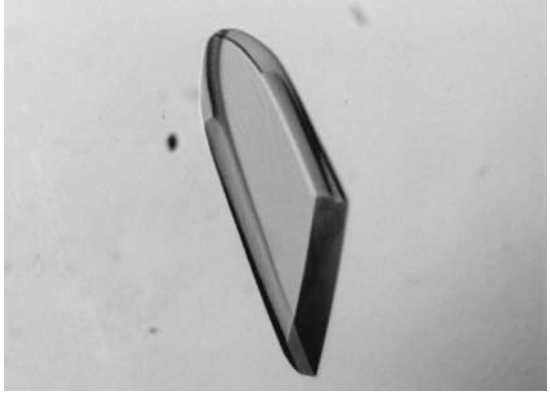

Figure 2
Orthorhombic crystals of ClfA37 grew in the presence of 1.2 *M* sodium citrate and HEPES pH 8.0 to maximum dimensions of 0.4 × 0.3 × 0.1 mm. Crystals diffract to 2.1 Å resolution.

Figure 3
Crystal of ClfA37 plus 17 amino-acid γ-chain peptide. Crystals diffract strongly to 2.1 Å resolution.

2.2. Purification and crystallization of ClfB

DNA encoding the fibrinogen-binding A region of ClfB (amino-acid residues 45–542) was cloned into the pV4 expression vector (Van Dyke *et al.*, 1992) and expressed in *E. coli* as a polyhistidine fusion protein containing six histidine residues at the C-terminus (Ní Edhin *et al.*, 1999). Recombinant protein was then purified in exactly the same manner as ClfA with the following exceptions. A 200 ml linear gradient of 0–200 m*M* imidazole was used to elute ClfB from the nickel-charged iminodiacetic acid Sepharose column, and gel filtration was performed using a 2.6 × 60 cm column of Sephacryl S-200 with 20 m*M* Tris–HCl, 100 m*M* NaCl, 2 m*M* EDTA, pH 7.6 as the mobile phase. As was observed for ClfA, the ClfB construct also degraded to a protein of smaller than expected size. The predicted molecular mass of the ClfB A region is 56 kDa, whereas the purified fragment (ClfB38) had a mass of 38 kDa (as determined by electrospray ionization mass spectroscopy). N-terminal sequencing indicated that this fragment started at residue alanine 199. This fragment still retained fibrinogen-binding activity, as determined by a Western ligand blot assay. Protein purity

Table 1
Crystallographic data-collection details of ClfA37, ClfA37 plus peptide and ClfB38.

Protein	Unit-cell dimensions (Å)			Space group	Resolution (Å)	$I/\sigma(I)$	R_{sym}† (%)	Completeness (%)	Unique reflections (%)	Reflections above 3σ (%)
	a	*b*	*c*							
ClfA	39.58	81.39	112.65	$P2_12_12_1$	2.10	16.5	7.4	99.1	21973	64.3
ClfA plus peptide	39.11	81.39	109.51	$P2_12_12_1$	2.10	27.4	5.0	99.1	21008	84.1
ClfB	96.31	96.31	84.13	$P4_12_12$ or $P4_32_12$	2.45	19.4	6.1	98.9	15061	79.9

† $R_{sym} = \sum_h \sum_i |I_{hi} - \langle I_{hi}\rangle| / \sum_h \sum_i |I_{hi}|$, where h specifies unique reflections and i indicates symmetry-equivalent observations of h.

was estimated to be 96% following SDS–PAGE and staining with silver nitrate. The purified ClfB38 was concentrated to 18 mg ml^{-1} (as determined by OD at 280 nm with ε = 39310 cm^{-1} M^{-1}) using a micro-ultrafiltration Amicon concentrator with a YM10 membrane. Initial crystallization trials were set up using the hanging-drop vapor-diffusion method. Good-quality crystals (Fig. 4) of dimensions 0.5 × 0.1 × 0.1 mm were obtained from a 6 µl droplet containing protein at 9 mg ml^{-1} and 0.7 *M* sodium formate precipitant, when equilibrated against a reservoir solution containing 1 ml of 1.4 *M* sodium formate.

3. Results

3.1. Data collection: ClfA37

Attempts to flash-freeze crystals were not successful, as this procedure increased the crystal mosaicity. Diffraction data were recorded for native and complex crystals using an R-AXIS IV image-plate detector mounted on a Rigaku rotating-anode generator operating at 50 mA and 100 kV power at room temperature. Initial exposures showed good diffraction, and 180° of data were collected to 2.1 Å using a 15 min exposure, 1° oscillation and a crystal-to-detector distance of 160 mm. Data were integrated and reduced using *DENZO* and scaled with *SCALEPACK* (Otwinowski, 1993). The crystallographic details of the data are presented in Table 1. The Matthews constant V_m (Matthews, 1968) of 2.3 Å^3 Da^{-1} results in an estimated solvent content of 46.5%, suggesting one molecule in the asymmetric unit. The co-crystals of ClfA–peptide were exposed to X-rays and data were collected under similar experimental conditions (details are presented in Table 1).

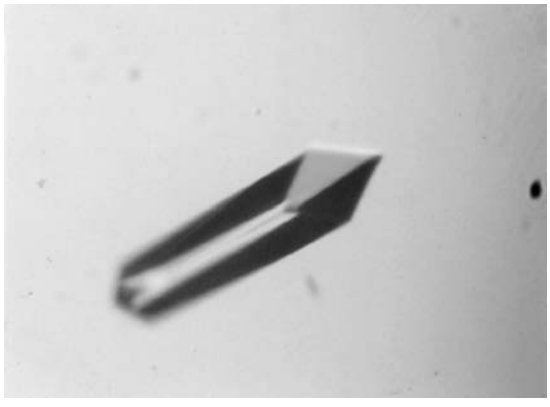

Figure 4
Rod-shaped orthorhombic crystals of ClfB38 grown in the presence of 1.4 *M* sodium formate. Crystals diffract to 2.45 Å resolution.

3.2. Data collection: ClfB38

Individual crystals were picked up on a nylon cryo-loop (Hampton Research Inc, Laguna Hills, CA), dropped into a cryo-protectant solution containing 15% ethylene glycol in 3.8 *M* sodium formate for less than 30 s and were then flash-frozen at 100 K using an X-stream cryogenic crystal cooler. Crystals diffracted to 2.45 Å resolution on an R-AXIS IV image-plate detector mounted on a Rigaku rotating-anode generator operating at 50 mA and 100 kV. A total of 120° of data were collected to 2.45 Å using a 20 min exposure, 1° oscillation and a crystal-to-detector distance of 200 mm. Diffraction data were processed using *DENZO* and scaled with *SCALEPACK* (Otwinowski, 1993). The crystallographic details are presented in Table 1. The Matthews constant V_m (Matthews, 1968) of 2.6 Å^3 Da^{-1} results in an estimated solvent content of 53%, suggesting one molecule per asymmetric unit. Screening for heavy-atom derivatives is in progress.

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References

McDevitt, D., Francois, P., Vaudaux, P. & Foster, T. (1995). *Mol. Microbiol.* **16**, 895–907.
McDevitt, D., Nanavathy, T., Pompeo, K. H., Bell, E., Turner, N., McIntire, L., Foster, T. & Höök, M. (1997). *Eur. J. Biochem.* **247**, 416–424.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Ní Edhin, D., Perkins, S., Francois, P., Vaudaux, P., Höök, M. & Foster, T. J. (1999). In preparation.
O'Connell, D. P., Nanavathy, T., McDevitt, D., Gurusiddappa, S., Höök, M. & Foster, T. J. (1998). *J. Biol. Chem.* **273**, 6821–6829.
Otwinowski, Z. (1993). *DENZO. A Film Processing Program for Macromolecular Crystallography*. Yale University, New Haven, CT, USA.
Patti, J. M., Allen, B. L., McGavin, M. J. & Höök, M. (1994). *Annu. Rev. Microbiol.* **48**, 585–617.
Patti, J. M. & Höök, M. (1994). *Curr. Opin. Cell. Biol.* **6**, 752–758.
Van Dyke, M. W., Sirito, M. & Sawadogo, M. (1992). *Gene*, **111**, 99–104.