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Expression, purification and crystallization of *Streptococcus dysgalactiae*-derived mitogen

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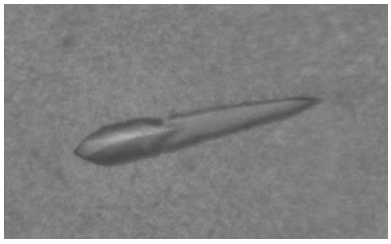

Superantigens are bacterial or viral toxins with potent immunostimulatory properties. *Streptococcus dysgalactiae*-derived mitogen, a 25 kDa protein, is a recently discovered superantigen isolated from *S. dysgalactiae* culture supernatant. Sequence considerations suggest that it belongs to a new superantigen family distinct from other superantigens. The protein was expressed in *Escherichia coli* cells and purified to homogeneity. Crystals were grown at pH 4.2–4.4 in the presence of 18–20%(*w*/*v*) PEG 3350 and 0.4 *M* lithium nitrate. A complete data set to 2.4 Å resolution was collected from a single crystal at liquid-nitrogen temperatures using synchrotron radiation. The crystals belong to space group $P3/P3_1/P3_2$, with unit-cell parameters $a = b = 52.7$, $c = 62.4$ Å, $\gamma = 120^\circ$ and one molecule in the crystallographic asymmetric unit.

1. Introduction

Superantigens are protein toxins of bacterial or viral origin able to cross-link major histocompatibility complex class II (MHCII) molecules with T-cell receptors (TcR). The formation of the trimolecular TcR–superantigen–MHCII complex results in the activation of approximately one in five resting T cells, while conventional antigens normally activate only one in 10^5–10^6 T cells (Fraser *et al.*, 2000; Papageorgiou & Acharya, 2000). Superantigen-induced T-cell activation leads to a massive production of inflammatory cytokines such as tumour necrosis factors α and β and interleukin-2. Consequently, superantigens have been implicated in various pathological situations including food poisoning and toxic shock syndrome. Furthermore, it has been suggested that superantigens play a role in immune-mediated diseases and induce long-term effects on autoimmune diseases and immunodeficiency (Torres *et al.*, 2001).

Bacterial superantigens secreted by *Staphylococcus aureus* and *Streptococcus pyogenes* are the best characterized superantigens to date. These superantigens show a similar fold that consists of two domains at the N- and C-termini of the molecule, respectively, separated by a long central α-helix. Structural homology with the C-terminal domain of superantigens has been detected in the *Staph. aureus* extracellular adherence protein (Geisbrecht *et al.*, 2005). However, the three-dimensional structures of *Yersinia pseudotuberculosis* YPM (Donadini *et al.*, 2004) and *Mycoplasma arthritidis* MAM (Zhao *et al.*, 2004) superantigens revealed folds unrelated to the prototype superantigen fold.

Crystal structures of superantigen complexes with either MHCII or TcR molecules have been determined (Petersson *et al.*, 2004). It is now well established that superantigens have evolved a number of different ways to interact with MHCII and TcR molecules despite their overall similarity in the three-dimensional structure. The presence of a zinc-binding site in several superantigens has significantly contributed to variations observed in the binding modes between superantigens and MHCII molecules.

S. dysgalactiae-derived mitogen (SDM), a 25 kDa protein (212 amino acids), is a novel superantigen recently purified from *S. dysgalactiae* culture supernatant (Miyoshi-Akiyama *et al.*, 2003). It activates human T cells having T-cell receptors with Vβ1+ or Vβ23+ variable regions. SDM shows about 30% homology with other superantigens at the amino-acid sequence level. Phylogenetic tree analysis has shown that SDM belongs to a family distinct from other

Table 1
Data-collection statistics.

Values in parentheses are for data in the highest resolution shell.

Space group	$P3/P3_1/P3_2$
Unit-cell parameters	
a (Å)	52.7
b (Å)	52.7
c (Å)	62.4
γ (°)	120
Resolution range (Å)	20–2.4 (2.44–2.40)
Wavelength (Å)	0.8031
Temperature (K)	100
Total reflections	42040
Unique reflections	7479
Completeness (%)	99.8 (98.3)
R_{merge} (%)	7.6 (45.8)
Average $I/\sigma(I)$	17.2 (2.2)
Mosaic spread (°)	0.48

known bacterial superantigens. In addition, a zinc-binding site has been predicted for SDM. *S. dysgalactiae* belongs to the group C/G of streptococci implicated in sepsis, cellulitis and necrotizing fasciitis (Igwe *et al.*, 2003). The pathogenic role of SDM in infectious diseases caused by *S. dysgalactiae* is currently under investigation. To gain further insights into the role and function of SDM, the protein was expressed, purified and crystallized in a form suitable for structural studies.

2. Experimental methods

2.1. Expression and purification

Initial crystallization trials were carried out using protein purified according to a previously published protocol (Miyoshi-Akiyama *et al.*, 2003). Very thin rod-like crystals were obtained, but proved to be irreproducible and no structure determination could be pursued. As the protein was expressed with a non-cleavable His tag at its N-terminus, expression of SDM bearing a removable His tag was undertaken. The open reading frame of *sdm* was PCR amplified by AccTaq (Sigma) and the primers 5′-GGGAGGGCATGCGAAA-GATGCTGTGTTGGTTAATAGC-3′ (containing a *Sph*I site and a codon to introduce a lysine residue at the N-terminus of the native SDM protein as a stop point for DAPase; Qiagen) and 5′-GTG-GCGTCGACAGTCTACTAAAACCGCCTAAT-3′ (containing a *Sal*I site). The PCR products were digested with *Sph*I and *Sal*I and cloned into the corresponding sites of TAGzyme pQE2 (Qiagen). The plasmids were used to transform *Escherichia coli* TOP10 cells (Invitrogen) and the DNA sequences were confirmed. *E. coli* cells were induced by incubation with 1 m*M* isopropyl β-D-thiogalactopyranoside (ITPG) for 4 h at 310 K in LB medium. Recombinant SDM was purified by Chelating Sepharose 4B (Amersham) pre-loaded with Ni^{2+} according to the manufacturer's instructions. The purified SDM protein was treated with DAPase (Qiagen) and Chelating Sepharose 4B (Amersham) pre-loaded with Ni^{2+} (Qiagen) to remove the His tag, followed by gel filtration with a Sephadex G-25 column. After gel filtration, the protein was concentrated by ultrafiltration using Amicon YM-10 to 10 mg ml^{-1} in 5 m*M* sodium acetate buffer pH 6.0. The purity of the protein is shown in Fig. 1. The molecular weight as deduced from the SDS–PAGE is higher than that expected, possibly owing to electrical charge or hydrophilicity of the molecule. Accurate measurement with mass spectrometry gave a molecular weight of 25.2 kDa. The yield is approximately 2 mg from 1 l of bacterial culture.

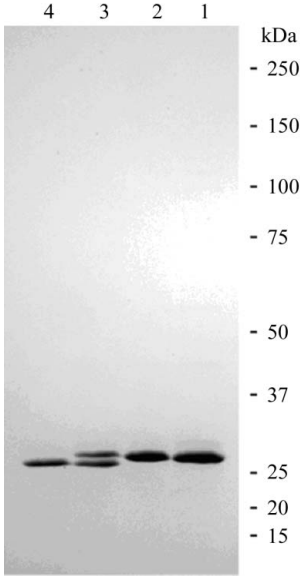

Figure 1
Purity of SDM shown on a 4–20% gradient SDS–PAGE gel. Lane 1, after purification by a Ni^{2+}-column; lane 2, before cleavage; lane 3, cleaved; lane 4, final preparation for crystallization.

2.2. Crystallization and data collection

Initial crystallization trials were carried out using the hanging-drop vapour-diffusion method at 289 K and Hampton Crystal Screens I and II. The drops contained 1.5 µl protein solution and an equal volume of precipitant solution. Inspection of the drops after 1 d revealed precipitation in most of the conditions containing PEG. The PEG/Ion screen from Hampton Research was then employed to find salts that could possibly induce the formation of crystals in the presence of PEG. Small crystals were found after 5 d in conditions containing either 0.2 *M* ammonium nitrate or lithium nitrate as salt additives. Optimization of the conditions was carried out by varying the pH, the PEG 3350 (Fluka) concentration and the type of salt. The best crystals were found to grow at pH 4.2–4.4 in the presence of 0.4 *M* lithium nitrate and 18–20%(*w*/*v*) PEG 3350 (Fig. 2). Use of ammonium nitrate gave crystals that were somewhat smaller than those grown in the presence of lithium nitrate. Data to 2.4 Å were collected on the X11 beamline at EMBL Hamburg (c/o DESY) from a single crystal that had been immersed in a crystallization solution containing 20% glycerol as cryoprotectant prior to data collection at 100 K. A total of 100 images were collected with 1° rotation per image at a wavelength of 0.8031 Å. Data were processed with the

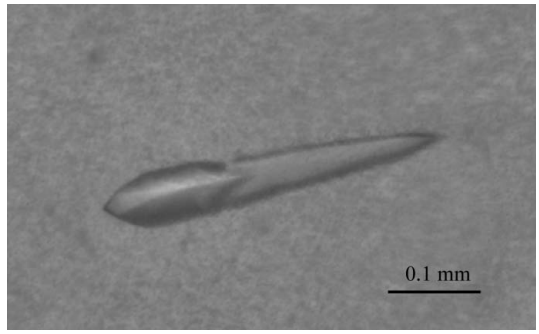

Figure 2
A typical crystal of SDM.

HKL package (Otwinowski & Minor, 1997). The final statistics of data collection are summarized in Table 1.

3. Results and discussion

Crystals of recombinant SDM belong to space group $P3/P3_1/P3_2$, with unit-cell parameters $a = b = 52.7$, $c = 62.4$ Å, $\gamma = 120^\circ$. Assuming the presence of one molecule in the crystallographic asymmetric unit, the Matthews coefficient V_M (Matthews, 1968) is 2.1 Å^3 Da^{-1}, corresponding to a solvent content of ∼40%. Crystal structure determination will be pursued using molecular replacement. SDM has a relatively low sequence identity with other superantigens (30% with SPEC and 28% with SMEZ). Suitable models will be constructed to maximize the success in molecular-replacement efforts. A correct molecular replacement solution will also help in resolving the space-group ambiguity. Structure determination of SDM will allow comparisons with other superantigens and help in understanding evolutionary aspects of superantigen function.

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