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Expression, purification, crystallization and preliminary crystallographic analysis of laminin-binding protein (Lmb) from *Streptococcus agalactiae*

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Laminin-binding protein (Lmb), a surface-exposed lipoprotein from *Streptococcus agalactiae* (group B streptococcus), mediates attachment to human laminin and plays a crucial role in the adhesion/invasion of eukaryotic host cells. However, the structural basis of laminin binding still remains unclear. In the context of detailed structural analysis, the *lmb* gene has been cloned, expressed in *Escherichia coli*, purified and crystallized. The crystals diffracted to a resolution of 2.5 Å and belonged to the monoclinic space group $P2_1$, with unit-cell parameters a = 56.63, b = 70.60, c = 75.37 Å, β = 96.77°.

1. Introduction

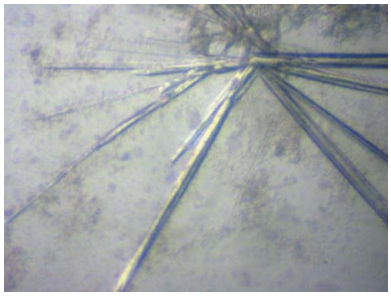
Streptococcus agalactiae, also named group B streptococcus (GBS), is one of the most important neonatal pathogens, causing septicaemia and meningitis. It is commonly found in the gastrointestinal and genitourinary tract and is also the predominant cause of invasive bacterial disease in neonates (Spellerberg *et al.*, 1999).

Adherence and colonization of human tissues is the primary step in any bacterial infection. Bacterial adhesion and aggregation are mediated by several cell-surface structures, mostly proteins and lipoproteins termed adhesins, which bind to components of the extracellular matrix molecules (ECM). The ECM include glycoproteins such as collagen, fibrinogen, fibronectin and laminin that form the underlying basement membrane of the epithelial and endothelial cells (Hay, 1991).

Laminin-binding protein (Lmb), a surface-exposed lipoprotein from *S. agalactiae* (Uniprot Q9ZHG8), mediates the attachment of *S. agalactiae* to human laminin. Studies revealed that *lmb*$^-$ mutants show decreased adherence to immobilized human placental laminin when compared with the wild type, indicating that Lmb is directly involved in the attachment of GBS to laminin. Lmb is expressed in most if not all human GBS strains, making it an attractive target for a GBS vaccine (Spellerberg *et al.*, 1999).

Reports indicate that Lmb shows significant homology (20–61%) to the LraI family of proteins (Spellerberg *et al.*, 1999). The LraI family of lipoproteins is located in the ABC transporter-type operons and plays a dual role in adhesion and transport. This family of surface-associated lipoproteins is involved in the co-aggregation of *S. gordonii* with *Actinomyces naeslundii*, the adherence of *S. sanguis* to the salivary pellicle, the binding of *S. parasanguis* to a platelet fibrin matrix (Viscount *et al.*, 1997; Jenkinson, 1994) and the adherence of *S. pneumoniae* to type II pneumocytes (Berry & Paton, 1996). It has been postulated that together with other proteins LraI proteins constitute a large family of metal transporters (Dintilhac *et al.*, 1997).

Laminin, a 900 kDa glycoprotein, is a major component of the basement membrane. Invasion of *S. agalactiae* into the bloodstream as well as its entry into cerebrospinal fluid, as observed in the case of meningitis, requires the passage of bacteria through the basement membrane. The interaction of bacterial surface proteins such as Lmb with laminin could be a crucial mechanism in this context (Spellerberg *et al.*, 1999). As a part of our initiative to study the structure of Lmb and its complex with laminin, we have expressed, purified and crystallized it, the details of which are described in this paper.



2. Materials and methods

2.1. Expression and purification of rLmb

The *lmb* gene spanning residues 19–305 (devoid of the signal peptide) was cloned into the pET21a expression vector (Novagen, Madison, Wisconsin, USA) containing a C-terminal 6×His tag and transformed into *Escherichia coli* BL21 (DE3) (Novagen) as reported previously (Spellerberg *et al.*, 1999). A single transformed colony was picked and used to inoculate a 5 ml starter culture. This was transferred to 1 l LB medium supplemented with ampicillin (100 µg ml^{-1}) and grown for 4 h at 310 K with vigorous shaking. The culture was then induced with 1 m*M* isopropyl *β*-D-1-thiogalactopyranoside and growth was continued at 310 K for another 3 h. The cells were harvested by centrifugation at 4000 rev min^{-1} for 20 min at 277 K. The pellet was resuspended gently in 10 ml 20 m*M* Tris pH 7.0, 300 m*M* NaCl, 1 m*M* PMSF, 0.1% Triton X-100, 5% glycerol, 5 m*M* *β*-mercaptoethanol and lysed by sonication until a clear lysate was obtained. The lysate was spun at 10 000 rev min^{-1} for 30 min and the pellet and supernatant were analyzed by 12.5% SDS–PAGE to check the solubility of the protein. The protein was found to be soluble and the clear lysate was used for subsequent purification. rLmb was purified by IMAC (immobilized metal-affinity chromatography) using Ni–NTA matrix (Bio-Rad Inc.). The column was loaded with the culture supernatant at 277 K, followed by elution of the protein with a linear gradient of imidazole from 20 to 500 m*M*. rLmb started to elute at 250 m*M* imidazole. Fractions containing more than 80% homogenous protein, as observed on SDS–PAGE, were pooled, concentrated and buffer exchange was carried out using an Amicon concentrator (Centriprep) to remove the imidazole. The protein was further purified by gel filtration using a Superdex S-75 column (GE Healthcare Life Sciences) and eluted with buffer containing 20 m*M* Tris pH 7.0, 300 m*M* NaCl, 0.1% Triton X-100, 5% glycerol, 5 m*M* *β*-mercaptoethanol. The peaks obtained were checked on a 12.5% SDS–PAGE gel and the fractions corresponding to rLmb were pooled and concentrated. The concentration of the protein was measured using a UV spectrophotometer (A_{280}) and assuming a calculated absorption coefficient of 0.853 (*PROTPARAM*; Gasteiger *et al.*, 2005); the yield of the protein was found to be 35 mg per litre of culture.

2.2. Crystallization

Homemade screens such as ammonium sulfate *versus* pH and polyethylene glycol *versus* pH were employed to screen the protein. Crystallization of rLmb was carried out by the hanging-drop vapour-diffusion method at 293 K and crystals were obtained by mixing equal volumes (1 µl) of protein solution and reservoir solution containing PEG 2000 monomethyl ether as a precipitant. The hanging drop was equilibrated against 1 ml reservoir solution and thin plate-shaped crystals grew within 3 d. The quality of the crystals was improved after a series of crystallization trials with various additives such as glycerol, ethylene glycol and divalent metal ions. After refinement of the initial conditions, crystals of suitable size (Fig. 1) for crystallographic studies were obtained with 30–35%(*w*/*v*) PEG 2000 monomethyl ether, 0.1 m*M* sodium citrate pH 5.0, 5 m*M* $NiCl_2$ and 10% ethylene glycol.

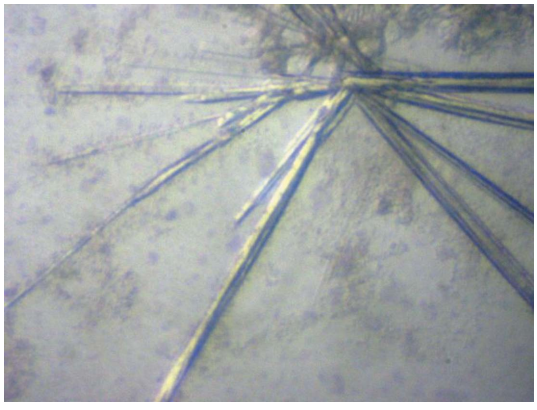


Figure 1
Needle-like crystals of rLmb from *S. agalactiae*.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell (2.59–2.50 Å).

Wavelength (Å)	1.5418
Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 56.63, b = 70.60, c = 75.37, β = 96.77
Resolution range (Å)	30–2.5
Total No. of reflections	75211
No. of unique reflections	19066
Completeness (%)	98.7 (99.8)
Mean $I/\sigma(I)$	5.6 (1.4)
$R_{\rm merge}$	5.4 (27.4)

2.3. Data collection and processing

For X-ray data collection, rLmb crystals were directly taken from the crystallization drop in a loop and flash-cooled in a nitrogen-gas stream at 100 K. Diffraction data were collected at our in-house data-collection facility using a MAR345 image-plate detector and a Bruker Microstar copper rotating-anode generator operating at 60 mA and 45 kV. A total of 100 frames were collected with an oscillation step of 1.5°, an exposure of 300 s per frame and a crystal-to-detector distance of 150 mm. Diffraction images were indexed, integrated, merged and scaled using the *AUTOMAR* software package (Bartels & Klein, 2003). The data-collection statistics are summarized in Table 1.

3. Results and discussion

The rLmb crystals belonged to a monoclinic system with unit-cell parameters a = 56.63, b = 70.60, c = 75.37 Å, β = 96.77°. Systematic absences of reflections indicated that the space group was $P2_1$. Based on the assumption of the presence of two molecules of Lmb in the asymmetric unit, the values for the crystal volume per unit protein mass and solvent content were calculated to be 2.2 Å^3 Da^{-1} and 44%, respectively. Processing of 75 211 reflections led to 19 066 unique reflections with an $R_{\rm merge}$ of 5.4%.

Self-rotation functions were calculated in the resolution range 15–4 Å using the program *POLARRFN* (Collaborative Computational Project, Number 4, 1994). Analysis of self-rotation peaks revealed the presence of noncrystallographic twofold symmetry in the κ = 180° section. The presence of a peak at ω = 95.4°, φ = 180° of height 61.8% of the origin peak most likely suggests that two crystallographically independent molecules of Lmb are present in the asymmetric unit. This is in agreement with the dimer per asymmetric unit which was inferred from the calculations of the Matthews coefficient.

Sequence analysis of Lmb reveals several close homologues, including the metal receptors AdcIIA from *S. pneumonia* with 64% identity (PDB code 3cx3; Loisel *et al.*, 2008), PsaA from *S. pneumonia* with 28% identity (PDB code 1psz; Lawrence *et al.*, 1998), ZnuA from *E. coli* with 22% identity (PDB code 2ogw; Banerjee *et al.*, 2003) and TroA from *Treponema palladium* with 20% identity (PDB

code 1toa; Lee *et al.*, 1999), hinting that the structure could be solved by molecular replacement.

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