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Cloning, purification, crystallization and preliminary crystallographic analysis of *Bacillus subtilis* LuxS

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LuxS of *Bacillus subtilis* is a member of a novel family of proteins with a potential role in quorum sensing, controlling important aspects of cellular physiology in a range of microbial species. *B. subtilis luxS* was cloned, expressed in *Escherichia coli*, purified and crystallized using the hanging-drop method of vapour diffusion with ammonium sulfate as the precipitant. The crystals belong to one of the enantiomorphic space groups $P6_122$ or $P6_522$, with approximate unit-cell parameters $a = b = 63.6$, $c = 151.5$ Å and one subunit in the asymmetric unit, corresponding to a packing density of 2.5 Å^3 Da^{-1}. The crystals diffract X-rays to at least 1.55 Å resolution on a synchrotron-radiation source. Determination of the structure will provide insights into the key determinants of function of this class of proteins, for which no structures are currently available.

1. Introduction

It is only recently that complex social interactions within many bacterial populations have begun to be recognized. The term quorum sensing (Fuqua *et al.*, 1994) has been coined to describe the ability of diverse types of bacteria to respond to changes in population density by monitoring the concentration of extracellular chemical signals produced by themselves (autoinducers). Population-density increase itself is a marker of likely nutrient exhaustion. Thus, quorum sensing allows a concerted, often pleiotropic, response to environmental conditions, bringing about such mechanisms as induction of toxins, endospore production and initiation of genetic competence (Winans & Zhu, 2000).

The characterized chemical signals produced by bacteria are diverse but fall into two main categories. Many Gram-negative bacteria produce related acyl-homoserine lactone derivatives whose synthesis, structure and recognition have been well established, particularly in the marine organisms *Vibrio fischeri* and *V. harveyi* (Winans & Zhu, 2000). Conversely, in several Gram-positive bacteria short-peptide (sometimes modified) pheromones have been identified. In the soil organism *B. subtilis* at least three pheromone peptides are produced: one specific for sporulation, one for competence and the third involved in both phenomena (Perego, 1999; Lazazzera *et al.*, 1999).

Recently, a novel type of chemical signal has been found (first in *V. harveyi*) that has been shown to be produced by a number of Gram-negative species (Surette *et al.*, 1999). A gene involved in its biosynthesis (*luxS*) has been characterized from several species and shown to be functionally homologous. Database searching reveals the presence of likely *luxS* homologues in many species, including important Gram-negative and Gram-positive pathogens. Thus, the LuxS autoinducer system may form an intra- and interspecies communication mechanism with an important role in cellular physiology and pathogenicity. LuxS therefore constitutes a potential novel drug target present in many important pathogens. In order to address this and to further understand the mechanism of LuxS activity it is important to analyze this protein at the molecular level.

B. subtilis has a single LuxS-like protein, YtjB, which shows 38% identity over 145 residues to the prototypical *V. harveyi* protein. This paper describes the cloning, purification, crystallization and preliminary X-ray analysis of the protein.

2. Materials and methods

The *luxS* gene from *B. subtilis* 168 was PCR amplified from genomic DNA using appropriate primers and cloned into pETBlue-1 using an AccepTor vector kit (Novagen), creating pSKD1. Plasmid containing the *luxS* gene (pSKD1) was used to transform competent cells of the overexpression strain *E. coli* Tuner (DE3) pLacI. This strain was grown in LB medium at 310 K with vigorous aeration until the OD_{590} reached 0.9. IPTG was added (2 m*M* final concentration) to induce expression and the cells were grown for a further 4 h to achieve a maximal yield of LuxS.

To purify LuxS, cell paste was suspended in buffer *A* (50 m*M* Tris–HCl pH 8.0) and disrupted by sonication. Debris was removed by centrifugation at 70 000*g* for 10 min. The supernatant fraction was loaded onto a column with DEAE-Sepharose Fast Flow (Pharmacia) and the proteins were eluted by a gradient of NaCl concentration from 0 to 0.5 *M* in buffer *A*. Fractions containing LuxS were combined, concentrated on a Viva Spin concentrator with molecular-weight cutoff 10 kDa (Viva Science) to a volume of 1.5 ml and loaded onto a Hi-Load Superdex-200 column (Pharmacia) equilibrated with 0.1 *M* NaCl in buffer *A*. LuxS elutes from the column with an approximate molecular weight of 35 kDa, consistent with a dimeric quaternary structure. The purity of protein estimated by SDS–PAGE (4–12% bis-tris Nu-PAGE gel, Novex) was 85–90%. Typical yield was about 50 mg [estimated by the method of Bradford (1976) using the Bio-Rad Protein Assay reagent] of protein from 1 l of culture.

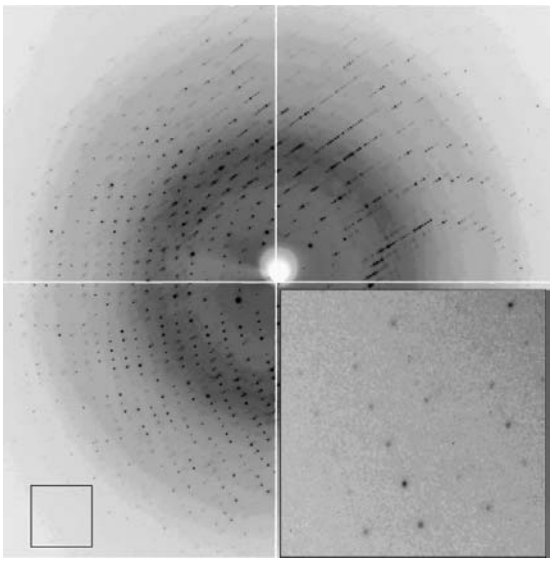

Figure 1
A representative 0.5° oscillation image of data collected from a LuxS crystal on a Quantum CCD Q4 detector on station 9.6 at the SRS Daresbury Laboratory. The bottom left corner of the selected rectangle corresponds to a resolution of 1.52 Å.

The protein was concentrated to 12 mg ml^{-1} using a Viva Spin concentrator and dialyzed against buffer *B* (10 m*M* Tris–HCl pH 8.0). Crystals were grown using the standard hanging-drop vapour-diffusion technique by mixing 2 µl of the protein solution with an equal volume of precipitant (1.8–2.4 *M* ammonium sulfate and 0.1 *M* Tris–HCl, pH range 7.5–8.5) and equilibrating against the same precipitant at 290 K. Hexagonal bipyramidal crystals up to 0.2 mm in size appeared within several days.

To perform data collection at cryogenic temperatures, the crystals were soaked in a solution containing 2.2 *M* ammonium sulfate, 0.1 *M* Tris–HCl and 25% glycerol and flash-frozen in a stream of nitrogen gas at 100 K using an Oxford Cryosystems Cryostream device. X-ray diffraction data was collected by the rotation method with 0.5° rotation per frame at an X-ray wavelength of 0.879 Å on station 9.6 at the CLRC Synchrotron Source, Daresbury using a CCD Q4 detector. The data were processed using the *DENZO*/*SCALEPACK* package (Otwinowski & Minor, 1997).

3. Results and discussion

Analysis of a preliminary data set collected on a LuxS crystal with the autoindexing routine in *DENZO* (Otwinowski & Minor, 1997) is consistent with a primitive crystal system, class 622, with unit-cell parameters $a = b = 63.6$, $c = 151.5$ Å. Analysis of systematic absences along the c^* axis show that the crystals belong to one of the enantiomorphic space groups $P6_122$ or $P6_522$. Taking the subunit molecular weight to be 17.7 kDa, this would suggest that the crystals contain a monomer in the asymmetric unit with a V_M of 2.5 Å^3 Da^{-1}, which is within the range given by Matthews (1977).

Significant reflections were observed beyond 1.55 Å resolution (Fig. 1) and a data set to this resolution was collected, with the average $I/\sigma(I)$ value being 40 for all reflections and 6.7 in the highest resolution shell (1.58–1.55 Å). A total of 185 641 measurements were made of 25 510 independent reflections. Data processing gave an $R_{\rm merge}$ of 0.046 for intensities (0.126 in the resolution shell 1.58–1.55 Å) and this data set is 93.6% complete (60.1% completeness in the highest resolution shell).

Our efforts are currently being directed towards a search for heavy-atom derivatives and the solution of the structure using multiple isomorphous replacement.

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