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Crystallization and preliminary crystallographic studies of antibacterial polypeptide LCI expressed in *Escherichia coli*

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LCI is a type of novel antibacterial polypeptide secreted by a *Bacillus subtilis* strain. It consists of 47 residues with a molecular weight of 5468 Da. Using bioengineering, LCI was expressed in *Escherichia coli* DH5α with recombinant plasmid pBVAB16. It was crystallized using PEG 4000 as a precipitant. The crystal belongs to space group $P6_222$ or $P6_422$, with unit-cell parameters $a = b = 29.30$, $c = 187.09$ Å, and diffracts to 2.44 Å. A set of diffraction data to 2.8 Å was collected.

1. Introduction

Antibacterial proteins are a class of very important proteins that exist widely in the bodies or secretions of plants (Roberts & Selitrennikoff, 1986), microorganisms (Von Tersch & Carlton, 1983), insects (Okada & Natori, 1985) and animals (Scheit *et al.*, 1985). Liu *et al.* (1990) screened and isolated a *B. subtilis* strain (named A014) whose secretions possess very strong antibacterial activities, especially against the pathogen (*Xanthomonas campestris* pv. *oryzea*) of rice leaf-blight disease, which is a serious threat to rice production and causes great losses in yields in most rice fields annually. Until now, there has been no efficient method to control this disease. It was found that the antibacterial functions are carried out by several types of protein in the secretions (Liu *et al.*, 1991, 1992, 1993). The proteins are named LCI (Liu *et al.*, 1990), LCII (Liu *et al.*, 1992) and LCIII (Liu *et al.*, 1993), respectively, according to the order of their elution peaks from a CM-52 chromatographic column. The sequence of LCI was determined by protein sequencing. LCI consists of 47 residues, with a molecular weight of 5468 and a pI of 10.25. No homologous protein was found in the protein information resources the Protein Sequence Database (PIR-PSD) and the Protein Data Bank (PDB).

After incubation for 20 min at 333, 353 or 373 K, LCI retains 100, 85.3 or 12.5% of its antibacterial activity, respectively (Liu *et al.*, 1990), indicating that LCI is thermostable and that its three-dimensional structure may be highly stable, although there is no cysteine in its sequence and hence no disulfide bonds in the structure. Owing to the low yield of LCI secreted by wild *B. subtilis* A014, it is difficult to carry out crystallographic studies. Recently, LCI was expressed in large quantities with an *E. coli* expression system using DH5α with the recombinant plasmid pBVAB16 in our laboratory. Its molecular weight is 5464 Da as measured by mass spectrometry and it shows comparable thermal stability and antibacterial activities to the native protein (to be published elsewhere). The present work reports the crystallization and preliminary crystallographic studies of the antibacterial polypeptide LCI expressed by engineering *E. coli.* with DH5α. Study of its three-dimensional structure will be helpful in revealing the mechanisms of its antibacterial activities and the thermal stability for the protein.

2. Methods and results

Crystallization was carried out using the hanging-drop vapour-diffusion method at 293 K. A 11 µl droplet containing 3.64 mg ml^{-1} LCI, 3.09%(*w*/*v*) PEG 4000, 0.55%(*v*/*v*) dioxane and 0.01 *M* NaOAc–HOAc buffer pH 5.7 was equilibrated against 0.4 ml reservoir solution containing 34%(*w*/*v*) PEG 4000 in double-distilled water. Crystals were obtained with maximum dimensions of 0.3 × 0.1 × 0.1 mm after 7–10 d (Fig. 1). Only twin crystals were obtained in the absence of dioxane in the droplet. A crystal was mounted in a glass capillary and X-ray diffraction data were collected on a MAR 345 image-plate system using Cu $K\alpha$ ($\lambda = 1.5418$ Å) radiation from a sealed-tube generator operated at 50 kV and 40 mA with a crystal-to-detector distance of 200 mm. The data were collected at 293 K. The scan range was 90° with an oscillation of 1°. The data were processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The crystal diffracts to 2.44 Å and the effective resolution is 2.8 Å. A total of 27 210 observations of 1506 unique reflections within the resolution range 30.0–2.80 Å were collected with an $R_{\rm merge}$ of 10.9% (35.9% in the

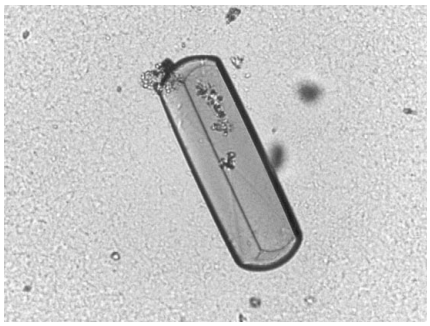

Figure 1
The crystal of antibacterial polypeptide LC1 expressed in *E. coli*. The longest dimension is approximately 0.3 mm.

highest resolution shell 2.87–2.80 Å) and a multiplicity of 7.65 (7.47 in the highest resolution shell 2.87–2.80 Å). The completeness of the overall data set is 99.8% (100.0% in the highest resolution shell 2.87–2.80 Å). The percentage of the overall data set with $I > 3\sigma(I)$ is 85.2% and with $I > 2\sigma(I)$ is 89.1%. The crystal belongs to one of the enantiomorphous space groups $P6_222$ or $P6_422$. The unit-cell parameters are $a = b = 29.30$, $c = 187.09$ Å, with a χ^2 of 0.751. Assuming one LCI molecule per asymmetric unit cell, the calculated V_M is 2.12 Å^3 Da^{-1}, corresponding to a solvent content of 39.7% (Matthews, 1968). The values lie within the normal range for protein crystals. As there is no homologous protein available whose three-dimensional structure has been determined, the preparation of heavy-atom derivatives and further crystallographic studies are under way.

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