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Preliminary X-ray crystallographic analysis of a nitric oxide-inducible lactate dehydrogenase from *Staphylococcus aureus*

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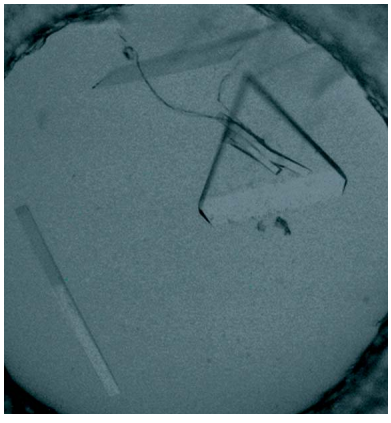

Recent studies have indicated that *Staphylococcus aureus* can survive the nitrosative stress (caused by the radical nitric oxide; NO·) mounted by the immune system of the infected host. It does this by expressing a nitric oxide-inducible L-lactate dehydrogenase (Sa-LDH-1). Therefore, if efficient inhibitors of Sa-LDH-1 can be designed then Sa-LDH-1 could be a potential drug target against the pathogen *S. aureus*. For this purpose, the nitric acid-inducible LDH-1 from *S. aureus* COL strain has been cloned into the expression vector pET-28a(+) and the protein has been expressed, purified and crystallized. The Sa-LDH-1 crystal diffracted to 2.4 Å resolution at a home X-ray source and belonged to space group *C*2, with unit-cell parameters a = 131.4, b = 74.4, c = 103.2 Å, β = 133.4°.

1. Introduction

Lactate dehydrogenase (EC 1.1.1.27), a member of the 2-hydroxyacid oxidoreductase family, is essential for all organisms and has been intensively studied. The enzyme catalyzes the stereospecific conversion of lactate to pyruvate and converts NAD^+ to NADH. This reaction is an important way of regenerating NAD^+, enabling the continuation of glycolysis (Garvie, 1980).

Pathogenic *Staphylococcus aureus* is resistant to the human innate immune system, which produces the antimicrobial radical nitric oxide (NO·); this is the first line of host defence using the inhibition of bacterial respiration by NO· (Fang, 2004). Recent studies have shown that the mechanism by which *S. aureus* survives host nitrosative stress is by the expression of an NO·-inducible L-lactate dehydrogenase (Sa-LDH-1; Richardson *et al.*, 2008). Therefore, if we are able to design and screen for selective inhibitors then Sa-LDH-1 could become a potential drug target for new antibiotics. To gain insight into the structural differences between the LDH isoforms from *S. aureus* and to compare them with human LDH for structure-based drug design and to screen specific inhibitors for drug discovery, the high-resolution structure of Sa-LDH-1 is needed. Pathogenic *S. aureus* contains two structurally undetermined L-lactate dehydrogenases with 53% sequence identity to each other: the NO·-inductive Sa-LDH-1 and the housekeeping constitutive Sa-LDH-2 (Richardson *et al.*, 2008).

Here, we report the cloning, purification, crystallization and preliminary X-ray crystallographic analysis of Sa-LDH-1.

2. Materials and methods

2.1. Cloning and expression

The gene *ldh*-1 was amplified from the genomic DNA of *S. aureus* COL strain by polymerase chain reaction (PCR) using the forward primer 5′-CGCGGATCCATGAACAAATTTAAAGGGAACAA-AG-3′ and the reverse primer 5′-CCCAAGCTTTTATTTAAGTTC-TTCTGCTTCAGCC-3′ containing *Bam*HI and *Hin*dIII digestion site, respectively. After digestion by *Bam*HI and *Hin*dIII at 298 K for 4 h, the PCR product was ligated into the expression vector pET-28a(+) (Novagen, USA) containing an N-terminal His_6 tag at 277 K overnight. The sequence of the recombinant vector was verified by DNA sequencing.

The recombinant vector containing the target gene was transformed into *Escherichia coli* BL21 Rosetta strain (Invitrogen, USA). The transformed cells were grown overnight at 310 K in 20 ml Luria–Bertani (LB) broth medium containing 50 µg ml^{-1} kanamycin. The overnight cultures were then inoculated into 1 l fresh LB medium containing 50 µg ml^{-1} kanamycin and grown at 310 K until the OD_{600} reached 0.6–0.8. The cells were then induced with 1.0 m*M* isopropyl *β*-D-1-thiogalactopyranoside. After further growth at 291 K overnight, the cells were harvested by centrifugation at 6000*g* for 10 min and resuspended in lysis buffer (20 m*M* Tris–HCl, 500 m*M* NaCl pH 7.5).

2.2. Protein purification and crystallization

The resuspended cells were disrupted by sonication on ice and centrifuged at 34 700*g* for 20 min twice. The supernatant was loaded onto a 5 ml Ni^{2+}-chelating affinity column (HiTrap; GE Healthcare, USA) previously equilibrated with lysis buffer. The column was eluted with a linear gradient of imidazole from 0.0 to 0.5 *M* in lysis buffer. The fractions containing the target protein were further purified by gel filtration using a Superdex 75 XK 16/60 gel-filtration column (GE Healthcare, USA) with elution buffer (20 m*M* Tris–HCl, 200 m*M* NaCl pH 7.5). The purity of the target protein was verified by SDS–PAGE as shown in Fig. 1.

The purified Sa-LDH-1 protein was concentrated to 15 mg ml^{-1} in the final elution buffer using a Millipore centrifugal filter device (Ultra-15, 10 kDa cutoff; Millipore, USA). Crystallization experiments were carried out at 289 K using the sitting-drop vapour-diffusion method in a 48-well plate (XtalQuest Inc., Beijing, People's Republic of China). Crystallization screening kits such as Crystal Screen, Crystal Screen 2 and Index (Hampton Research, California, USA) were used as initial screening conditions. 1 µl protein solution was mixed with an equal volume of reservoir solution and equilibrated against 100 µl reservoir solution. Thin crystal plates of equilateral triangular shape appeared in Crystal Screen condition No. 14 [0.2 *M* calcium chloride dihydrate, 0.1 *M* Na HEPES pH 7.5, 28%(*v*/*v*) PEG 400] after 3 d. Single crystals (Fig. 2) of diffraction quality were obtained when the protein concentration was reduced to 5 mg ml^{-1}.

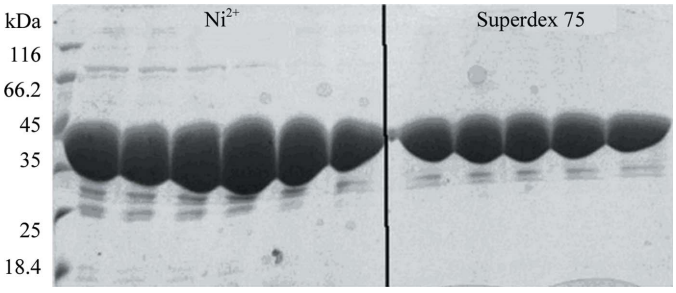

Figure 1
SDS–PAGE of Sa-LDH-1 during purification. The left part shows fractions containing the target protein eluted from the Ni^{2+}-chelating affinity column. The right part shows the protein further purified using a Superdex 75 gel-filtration column.

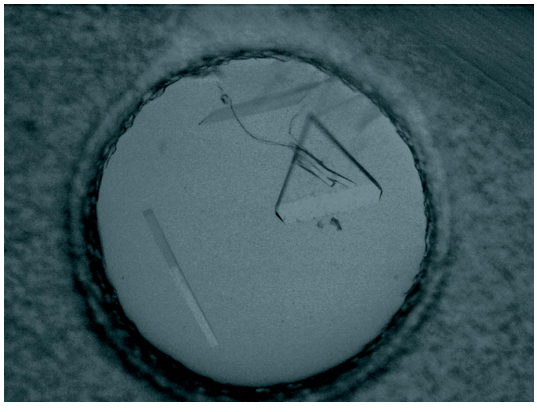

Figure 2
Crystals of Sa-LDH-1 from *S. aureus* have equilateral triangular shapes with typical dimensions of 0.5–0.8 mm on the edges and 0.1–0.2 mm in thickness.

2.3. Data collection and procession

One crystal was picked up in a nylon loop, flash-cooled immediately without further cryoprotection in liquid nitrogen, mounted and maintained at 100 K in a cold nitrogen-gas stream during data collection. X-ray diffraction data were collected from a single crystal on a Bruker SMART 6000 CCD detector using in-house Cu *Kα* radiation from a Bruker Microstar-H rotating-anode generator operated at 45 kV and 40 mA. The crystal-to-detector distance was set to 6.00 cm. 1500 frames were collected with 0.3° *φ* oscillation and an exposure time of 120 s per frame. Diffraction data were processed using the online Bruker *PROTEUM* software suite (Bruker AXS; http://www.bruker-axs.de/software4.html).

3. Results and discussion

Essentially, only one thick band was visible on SDS–PAGE stained by Coomassie Blue after size-exclusion chromatography purification of Sa-LDH-1 with overloaded samples as shown in Fig. 1, indicating a high degree of protein purity. The purified protein had an estimated molecular mass of about 39 kDa, which is in agreement with the predicted molecular mass of 34.6 kDa plus an additional 4 kDa N-terminal His_6-tag fusion peptide.

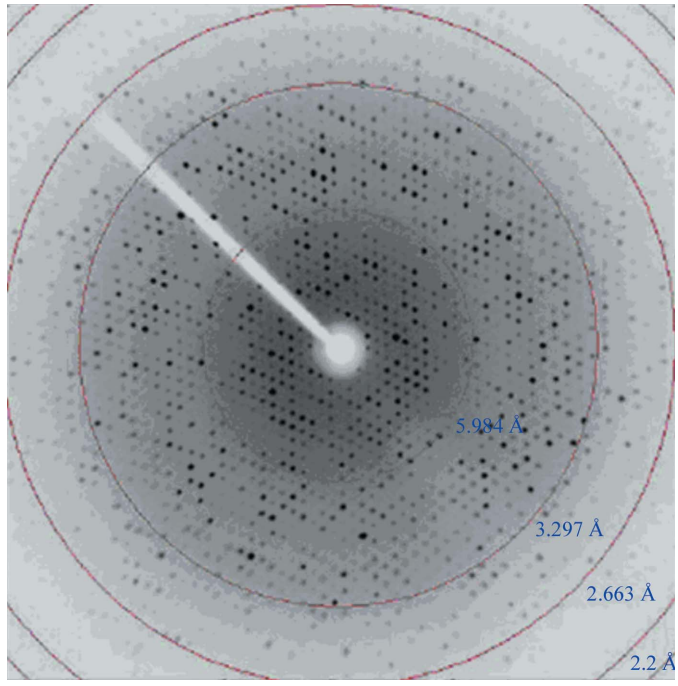

Figure 3
Diffraction pattern of Sa-LDH-1 collected using a home X-ray source (Bruker Microstar-H system) with resolution rings.

Table 1
Diffraction data statistics for the Sa-LDH-1 crystal.

Values in parentheses are for the outer shell.

Wavelength (Å)	1.5418
Resolution (Å)	50.0–2.43 (2.68–2.43)
Completeness (%)	83.24 (55.5)
$R_{\rm merge}$† (%)	5.15 (9.44)
Mean $I/\sigma(I)$	11.05 (5.88)
Redundancy	5.88
Space group	*C*2
Unit-cell parameters (Å, °)	a = 131.4, b = 74.4, c = 103.2, β = 133.4
No. of observed reflections	121795 (11080)
No. of unique reflections	22683 (3240)
No. of molecules in ASU	2
$V_{\rm M}$ (Å^3 Da^{-1})	2.54
Solvent content (%)	51.7

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

The crystals obtained from 0.2 *M* calcium chloride dihydrate, 0.1 *M* Na HEPES pH 7.5, 28%(*v*/*v*) PEG 400 were equilateral triangular plates of about 0.6 mm on the edges and 0.1 mm in thickness (Fig. 2). The crystals diffracted to beyond 2.2 Å resolution (a typical diffraction pattern is shown in Fig. 3) and belonged to space group *C*2. A data set was collected to 2.4 Å resolution with unit-cell parameters a = 131.4, b = 74.4, c = 103.2 Å, β = 133.4°. Data statistics are summarized in Table 1. Assuming the presence of two molecules per asymmetric unit, the Matthews coefficient is 2.54 Å^3 Da^{-1} (Matthews, 1968) and the solvent content is 51.7%. Structure determination is under way by molecular replacement. LDH from *Bacillus stearothermophilus* (PDB code 1ldn; Wigley *et al.*, 1992) will be used as the search model in molecular replacement.

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