Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and X-ray analysis of the salmon-egg lectin SEL24K

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Received 14 March 2007
Accepted 29 March 2007

The 24 kDa egg lectin of Chinook salmon (*Oncorhynchus tshawytscha*) is released from the egg during the cortical reaction. The lectin functions in blocking polyspermy during the fertilization process. The egg lectin was purified by affinity chromatography from salmon eggs and crystallized by the hanging-drop vapor-diffusion method using 15/4 EO/OH (pentaerythritol ethoxylate) as a precipitant. The crystal diffracted synchrotron-radiation X-rays to 1.63 Å resolution. The crystal belongs to the monoclinic space group $P2_1$, with unit-cell parameters $a = 93.0$, $b = 73.6$, $c = 113.6$ Å, $\alpha = 90$, $\beta = 92.82$, $\gamma = 90^\circ$. The crystal is likely to contain eight molecules in the asymmetric unit (V_M = 2.3 Å^3 Da^{-1}), corresponding to a solvent content of 45.5%. A self-rotation function suggests an arrangement with 222 point symmetry within the asymmetric unit.

1. Introduction

In teleost eggs, the existence of lectin molecules, particularly D-galactose/rhamnose-binding lectins, have often been reported (for a review, see Krajhanzl, 1990). The lectins are sometimes localized in vesicles immediately adjacent to the egg plasma membrane called cortical granules. It was suggested that fish-egg lectins might function as antibacterial agents against pathogenic bacteria, a defense mechanism that protects the embryo during development (Voss *et al.*, 1978; Krajhanzl, 1990; Tateno *et al.*, 2002; Booy *et al.*, 2005). We recently proposed a role for a galactose/rhamnose-specific lectin from the Chinook salmon egg in providing a block to polyspermy during fertilization (Yasumasu *et al.*, 2000; Murata *et al.*, 2000; Murata, 2005). The 24 kDa Chinook salmon-egg lectin (SEL24K) may contribute to the polyspermy block by agglutinating and immobilizing the sperm after being released from the cortical granules during the fertilization process. To elucidate the mechanisms of sperm agglutination and immobilization by the lectin, we purified SEL24K from salmon eggs in order to determine its structural properties. It was subsequently crystallized and X-ray diffraction analysis of SEL24K crystals was performed. This is the first report of the crystallization and diffraction analysis of an egg lectin.

2. Methods and results

2.1. Protein purification and crystallization

Chinook salmon (*Oncorhynchus tshawytscha*) eggs were obtained from mature females at Nimbus State Hatchery, Department of Fish and Game, Sacramento, CA, USA. The eggs were transported to the laboratory at 277 K and were used immediately or frozen for later use.

To isolate the egg lectin, 250 g of eggs in an equivalent amount of ice-cold Tris-buffered saline (TBS; 0.15 *M* NaCl, 50 m*M* Tris–HCl pH 7.4) were homogenized in a Waring blender. After filtration with 1 mm square-mesh cheesecloth to remove the egg envelope, the egg mixture was centrifuged at 10 000*g* for 15 min at 277 K. The orange supernatant solution was subsequently dialyzed against a 20-fold excess of ice-cold 50 m*M* Tris–HCl pH 7.4 overnight at 277 K and then centrifuged at 10 000*g* for 15 min to remove precipitated yolk protein. Solid NaCl was added to the supernatant solution to a final

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

X-ray source	SSRL BL 9-2
Wavelength (Å)	0.97977
Resolution (Å)	1.63 (1.67–1.63)
Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 93.0, b = 73.6, c = 113.6, β = 92.82
Monomers per ASU	8
V_M (Å^3 Da^{-1})	2.3
Solvent content (%)	45.5
No. of reflections	675422
No. of unique reflections	185184
R_{merge}† (%)	7.3 (35.9)
Mean $I/\sigma(I)$	15.1 (2.2)
Completeness (%)	97.2 (96.6)

† $R_{merge} = \sum_h \sum_i |I_h - I_{hi}| / \sum_h \sum_i I_{hi}$, where I_h is the mean of I_{hi} observations of reflection h.

concentration of 0.15 *M* and the solution was applied onto a rhamnose-conjugated affinity column. The affinity-column matrix was prepared using epoxy-activated Sepharose 6B (Amersham Biosciences Corp., NJ, USA) following the manufacturer's instructions. After washing the protein-bound column with ten volumes of TBS, SEL was eluted with TBS containing 0.2 *M* L-rhamnose. The peak fractions containing SEL isoforms were collected, transferred into dialysis tubing and dialyzed against TBS overnight at 277 K.

Each SEL isoform was further purified using C4 reverse-phase HPLC. The affinity-purified SEL was dialyzed against 200 m*M* ammonium acetate and applied onto a C4 reverse-phase column (Phenomenex) equilibrated with 0.1% TFA. The SEL isoforms were eluted with an increasing acetonitrile concentration gradient in 0.1% TFA (0–25% in 5 min followed by 25–45% in 65 min). The first peak eluted contained the SEL24K isoform and was used for crystallization. Crystallization trials were performed by the hanging-drop vapor-diffusion method using the Index HT crystallization screening kit (Hampton Research, CA, USA). Crystals appeared in the presence of 15/4 EO/OH (pentaerythritol ethoxylate) as a precipitant. Crystals suitable for X-ray analysis were obtained in approximately one week after mixing 2 µl protein solution (3.18 mg ml^{-1} in 5 m*M* Tris–HCl pH 7.4, 0.15 *M* NaCl) and 2 µl of a reservoir solution containing 30%(*v*/*v*) 15/4 EO/OH, 0.05 *M* bis-Tris pH 6.5 and 0.05 *M* ammonium sulfate.

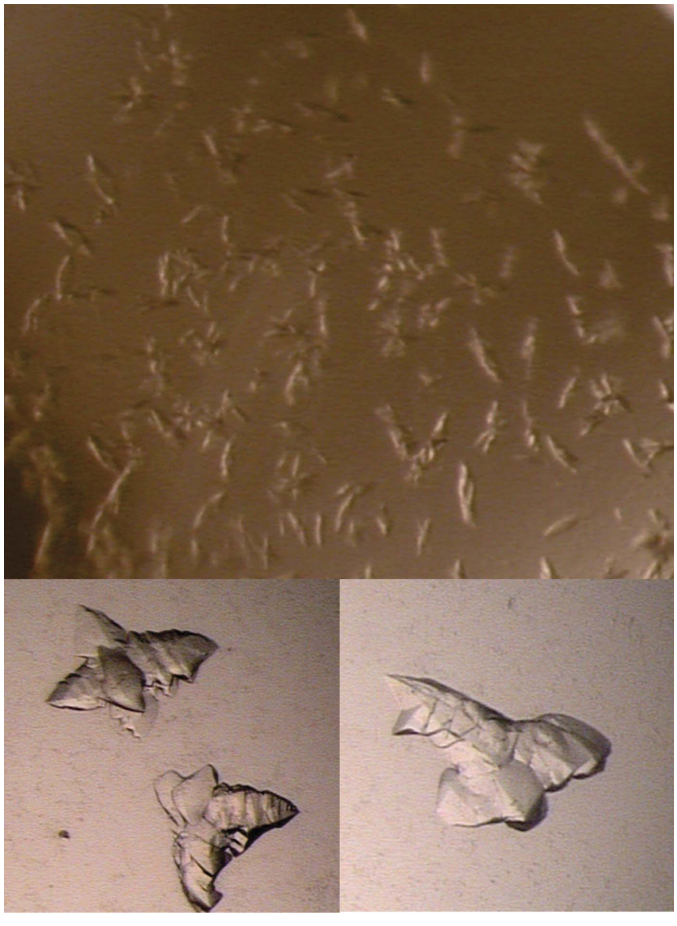

Figure 1
SEL24K crystals.

2.2. X-ray data collection and processing

The crystals of SEL24K had an 'arrowhead' morphology and appeared to be highly mosaic (Fig. 1). A crystal was mounted for data collection by dragging it through Paratone-N oil to remove surface mother liquor prior to freezing it in a stream of nitrogen at 100 K. A single diffraction lattice was observed, which indexed in the monoclinic space group $P2_1$ with unit-cell parameters a = 93.0, b = 73.6, c = 113.6 Å, β = 92.82° (Fig. 2). A complete data set was collected on beamline 9-2 at Stanford Synchrotron Radiation Laboratory (SSRL). The data were processed and scaled to 1.63 Å resolution with an R_{merge} of 7.3% using the programs *MOSFLM* and *SCALA* (Collaborative Computational Project, Number 4, 1994; Leslie, 1992). Data-collection statistics are given in Table 1. No sequence homologues of known three-dimensional structure were found in the PDB database that could assist in structure solution. Since the protein was purified directly from salmon eggs, which precludes SeMet incorporation, structure solution will be determined by a combination of multiple isomorphous replacement combined with multiwavelength anomalous scattering (MIRAS) by screening heavy-atom soaks.

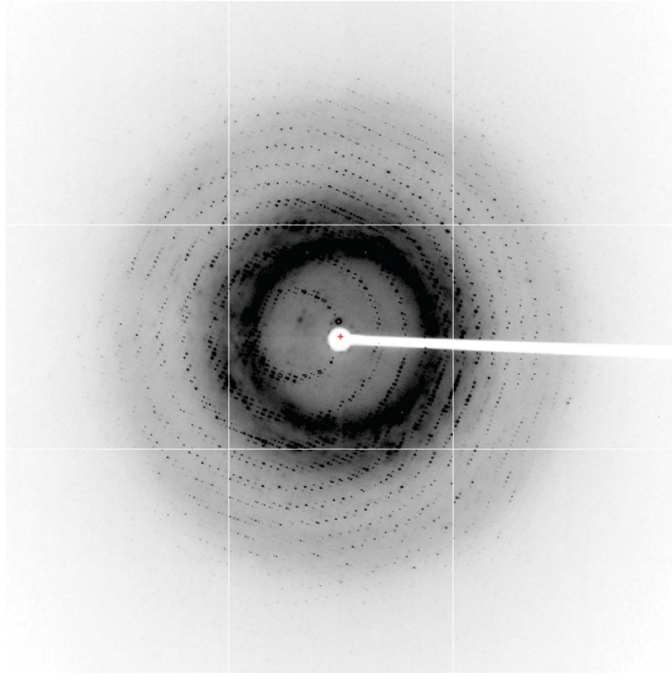

Figure 2
Diffraction of an SEL24K crystal on beamline 9-2 at SSRL. Diffraction extends to 1.8 Å resolution.

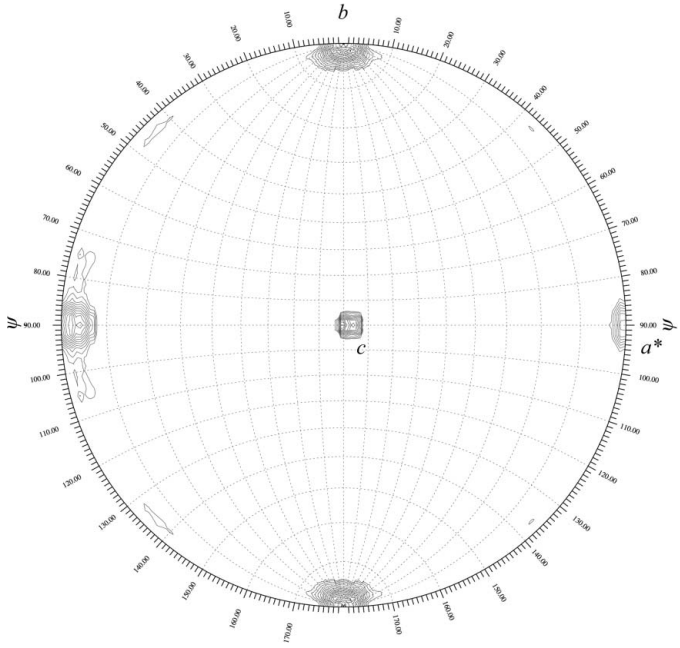

Figure 3
Self-rotation functions of the SEL24K data were calculated using data between 10.0 and 5.0 Å resolution and a radius of integration of 20 Å. Shown here is the self-rotation function using a κ angle of 180°. The plot was contoured starting at four standard deviations (sd) with steps of 0.5 sd. The plot suggests D_2 point symmetry with one twofold parallel to the crystal 2_1 screw axis (along *b*). Rotation searches using κ angles of 60, 90 and 120° did not reveal any significant peaks.

2.3. Rotation function

The crystals of SEL24K were likely to have crystallized with eight monomers in the asymmetric unit. 5–9 monomers per asymmetric unit gave reasonable V_M values of 3.6–2.0 Å^3 Da^{-1}, but considering that the crystals diffract with high resolution it is likely that the solvent content is low. Eight monomers in an asymmetric unit yielded a solvent content of ∼45%. Preliminary observations from other experiments suggested that SEL24K may be present as a dimer in aqueous solutions. The rotation-function program *GLRF* (Tong & Rossmann, 1990) was used to calculate a general self-rotation function to find noncrystallographic symmetry within the asymmetric unit. The rotation function was calculated with a number of κ angles (κ = 60, 90, 120, 180°) to test for the presence of noncrystallographic sixfold, fourfold, threefold and twofold axes, respectively. The only rotation function with significant peaks was calculated using κ = 180°. The crystallographic 2_1 screw axis appeared as the expected strong peak along the *b* axis, but other strong peaks also appeared about 5° away from the *a** and *c* axes but perpendicular to the *b* axis (Fig. 3). These data suggested a possible tetrameric assembly with D_2 symmetry. A possibility is that one of the noncrystallographic twofolds is parallel to the 2_1 screw axis. This model is consistent with eight monomers or two tetramers in the crystallographic asymmetric unit (the two tetramers are related by the 2_1 screw axis). Alternatively, there may be an arrangement of dimers with twofolds that lie almost parallel to the *a** and *c* axes. A third possibility is a single dimer that lies close to either the *a** axis or *c* axis and is perpendicular to the crystal 2_1 screw axis along *b*, which would produce another strong peak perpendicular to both the two twofolds.

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