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Crystallization and preliminary X-ray analysis of HCE-1, a hatching enzyme of medaka fish, *Oryzias latipes*

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The hatching enzyme of medaka fish, high choriolytic enzyme (HCE-1; MW = 22.7 kDa), was crystallized by the hanging-drop vapour-diffusion method using PEG 10 000 as the precipitant. The hatching enzyme is a metalloproteinase which is secreted from the embryo at the time of hatching. The crystals diffracted X-rays to beyond 1.34 Å resolution using a synchrotron-radiation source. The crystals belonged to the monoclinic space group *C*2, with unit-cell parameters a = 99.0, b = 30.4, c = 79.6 Å, β = 123.6°. The crystal contains one molecule in an asymmetric unit (V_M = 2.2 Å^3 Da^{-1}) and has a solvent content of 43.7%.

1. Introduction

The hatching enzyme is an embryo-secreted proteinase that digests the hardened egg envelope (chorion) allowing the embryo to emerge at the time of hatching. Thus, the enzyme is a unique proteinase synthesized exclusively in developing embryos. In medaka (*Oryzias latipes*), two hatching enzymes were identified and studied extensively: HCE (high choriolytic enzyme; choriolysin H; EC 3.4.24.67) and LCE (low choriolytic enzyme; choriolysin L; EC 3.4.24.66). Analyses of the cDNAs for HCE and LCE showed that both of them are synthesized as preproenzymes and that the mature forms both consist of 200 amino acids. The amino-acid sequence identity was 53% between HCE and LCE (Yasumasu *et al.*, 1992). Both enzymes belong to the astacin family of metalloendopeptidases (Dumermuth *et al.*, 1991; Stöcker *et al.*, 1993; Zwilling & Stöcker, 1997), which are classified into the metzincin clan (Gomis-Rüth, 2003). HCE and LCE have an additional N-terminal ten-amino-acid sequence including two cysteine residues, which distinguishes them from the other proteinases in the family (Yasumasu *et al.*, 1994).

HCE and LCE act cooperatively to digest the chorion. HCE partially digests the inner layer of the chorion, swelling this major structure of the chorion (Yasumasu *et al.*, 1989*b*). This partial choriolysis by HCE is ascribed to the release of proline-rich polypeptides from the chorion (Lee *et al.*, 1994). LCE cannot efficiently digest the intact chorion, but digests the HCE-swollen chorion to completion (Yasumasu *et al.*, 1989*a*,*b*). Therefore, the proteolytic action of HCE is the first step of the chorion-digesting process. Kinetic studies and binding experiments (Yasumasu, Katow *et al.*, 1989) have shown that HCE tightly binds to the chorion prior to partial digestion and swelling. To understand hatching, it is necessary to characterize the structural features of the chorion that are recognized by HCE and the molecular mechanism of the release of the proline-rich polypeptides.

To elucidate the mechanism of chorion recognition and binding by HCE, we have crystallized and performed preliminary X-ray diffraction analysis of HCE-1, one of the two isoforms of HCE. This is the first report of the crystallization of a hatching enzyme.

2. Methods and results

2.1. Protein purification and crystallization

HCE-1 was purified according to the procedure described previously (Yasumasu *et al.*, 1989*a*), with a minor modification. The hatching liquid, the filtrate of the culture medium of about 50 000 embryos, was applied to an SP Sepharose Fast Flow column (Amersham Biosciences) and eluted with 0.4 *M* NaCl, 50 m*M* Tris–HCl pH 8.5. The fractions having proteolytic activity were concentrated by ammonium sulfate precipitation and further fractionated using a Toyopearl HW50S column equilibrated with 50 m*M* bicarbonate buffer pH 10.0. The HCE fraction was dialyzed against 50 m*M* Tris buffer pH 8.5 and introduced into a HPLC system with a Source 15S column (Pharmacia Biotech). Two peaks (HCE-1 and HCE-2) were obtained by cationic column chromatography. The HCE-1 fraction was used for crystallization. Crystallization trials were performed by the hanging-drop vapour-diffusion method using the crystallization screening kits Crystal Screen and Crystal Screen 2 (Hampton Research). To prevent autolysis of HCE-1 at low pH (Yasu-

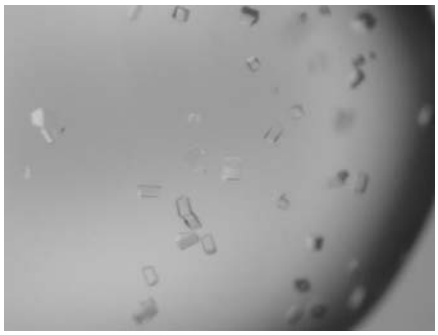

Figure 1
Single crystals of HCE-1 grown at 278 K using PEG 10 000 as precipitant.

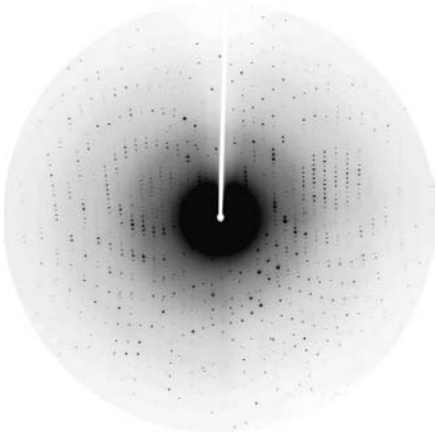

Figure 2
A diffraction image (1° oscillation) of an HCE-1 crystal. The edge of the detector corresponds to a resolution of 1.33 Å.

masu *et al.*, 1989*a*), only buffer conditions above pH 7 were selected.

Monoclinic crystals appeared in the presence of PEG (polyethylene glycol) as a precipitant. After refinement of the crystallization conditions, crystals suitable for X-ray analysis were obtained in a week by mixing 3 µl protein solution (3 mg ml^{-1} in 10 m*M* Tris–HCl pH 7.5, 0.3 *M* NaCl) and 1.5 µl of a reservoir solution consisting of 20%(*w*/*v*) PEG 10 000 and 100 m*M* HEPES buffer pH 7.0. The drop was equilibrated against 500 µl reservoir solution at 278 K.

Table 1
Crystal parameters of HCE-1.

Values in parentheses are for the highest resolution shell.

X-ray source	SPring-8 BL41XU
Wavelength (Å)	0.900
Unit-cell parameters (Å, °)	a = 99.0, b = 30.4, c = 79.6, β = 123.6
Resolution range (Å)	30.0–1.34 (1.39–1.34)
Observed reflections	180573
Unique reflections	44714
Data completeness (%)	99.3 (94.0)
Redundancy	4.1 (3.1)
$R_{\rm merge}$†	0.063 (0.185)
$\langle I\rangle/\langle\sigma(I)\rangle$	12.2 (3.2)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith intensity measurement of reflection hkl, including symmetry-related reflections, and $\langle I(hkl)\rangle$ is its average.

Fig. 1 shows typical crystals (0.05 × 0.05 × 0.05 mm).

2.2. X-ray data collection and processing

The crystal of HCE-1 was picked up in a nylon loop (Hampton Research), transferred to a cryoprotectant solution containing 15%(*v*/*v*) MPD, 24%(*w*/*v*) PEG 10 000 and 100 m*M* HEPES pH 7.5 and then mounted for flash-cooling at 100 K using a Rigaku cryostat. Diffraction data were collected using a MAR CCD detector system at beamline BL41XU at SPring-8. The wavelength was set to 0.90 Å and the distance between the crystal and the detector was 100 mm. The crystals diffracted to 1.34 Å resolution (Fig. 2). The diffraction data were indexed with *HKL*2000 and scaled with *SCALEPACK* (Otwinowski & Minor, 1997). The crystals belong to space group *C*2, with unit-cell parameters a = 99.0, b = 30.4, c = 79.6 Å, β = 123.6°. They contain one molecule per asymmetric unit, according to the Matthews coefficient ($V_{\rm M}$ = 2.2 Å^3 Da^{-1}; Matthews, 1968). The data statistics are given in Table 1. Structure determination by molecular replacement using the coordinates of astacin (PDB code 1ast; Bode *et al.*, 1992) as a search model is currently under way (Collaborative Computational Project, Number 4, 1994).

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