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Crystallization and preliminary X-ray crystallographic studies of native elastase from North Atlantic salmon (*Salmo salar*).

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Abstract

Crystals of elastase from North Atlantic salmon have been grown from 2-methyl-2,4-pentanediol by the hanging-drop vapour-diffusion method at room temperature. They grow to dimensions of 0.7 × 0.4 × 0.3 mm in three weeks. The crystals belong to the tetragonal space group $P4_12_12$ or $P4_32_12$ with cell dimensions $a = b = 68.0$ Å and $c = 84.0$ Å. There are eight molecules in the unit cell. The crystals diffract to at least 1.6 Å resolution and are suitable for a high-resolution crystal structure determination.

Introduction

Elastase is an endopeptidase which has the ability to cleave peptide bonds at carbonyl groups of amino acids carrying small non-polar side chains like alanine, leucine and valine (Thompson & Blout, 1973). The enzyme belongs to the family of serine proteinases, which among others includes trypsin and chymotrypsin. The main function of the pancreatic family of elastases is presumably the digestion of proteins by endopeptidic cleavage carried out in the duodenum and intestines (Shotton, 1970) in conjunction with other proteases. Elastases are also found in granules of polymorphonuclear leukocytes. Leukocyte elastase probably plays an essential role in defence against infection by invading microorganisms and in digestion during phagocytosis (Janoff, 1985).

Poikilothermic organisms, such as fish, have adapted their enzymes, through evolution, to maintain full physiological activities in their cold habitats. Elastases from cold-adapted species have been reported to possess higher catalytic efficiency (k_{cat}/K_m) than their homologous mammalian enzymes (Gildberg & Øverbø, 1990). The observed higher catalytic efficiency of cold-adapted elastases is due to higher turnover (k_{cat}) and not to a higher substrate affinity. In addition to the higher efficiency, elastases from these species have also been shown to be less pH and temperature stable than their mammalian counterparts. These phenomena, which have also been observed for other serine proteinases from cold-adapted species (Àsgeirsson, Fox & Bjarnason, 1989) may be explained by the need for ectothermic species to compensate for lower body temperature.

The three-dimensional structures of both human leukocyte and porcine pancreatic elastase have been determined to high-resolution by X-ray crystallographic analysis and are well characterized (Sawyer, Shotton, Campell, Wendell, Muirhead, Watson, Diamond & Ladner, 1978; Meyer, Cole, Radhakrishnan & Epp, 1988).

Of general interest in protein chemistry is the elucidation of the factors responsible for catalytic efficiency and thermal/pH stability of enzymes. To facilitate comparative studies between the warm-blooded mammalian elastases and the cold-adapted elastase from North Atlantic salmon, we have crystallized salmon elastase with the aim being to solve its three-dimensional structure by X-ray crystallography.

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Experimental

Purification and crystallization

Pancreatic elastase from North Atlantic salmon was purified from a defatted pancreatic extract by hydrophobic interaction, ion-exchange and gel-filtration chromatography. The purified elastase was homogeneous as judged by SDS–PAGE. Chymotrypsin and trypsin activity was not detected in the pure elastase preparation. The purified enzyme used for crystallization was dialysed against distilled water. and concentrated by ultrafiltration to about 10 mg ml^{-1}.

Initial crystallization conditions for salmon pancreatic elastase were screened using the sparse-matrix method (Jancarik & Kim, 1991). Droplets of 6 µl initial volume were prepared on siliconized coverslips by mixing 3 µl elastase in distilled water (9.5 mg ml^{-1}) and 3 µl of the different reservoir solutions. The coverslips were placed over 0.7 ml reservoir solution in each well of a tissue-culture plate and made airtight with grease.

X-ray analysis of the crystals

Crystals were mounted in thin-walled glass capillaries with a small volume of reservoir solution. X-ray data were collected using an Enraf–Nonius FAST area-detector system. Graphite-monochromated Cu $K\alpha$ radiation was provided by an Enraf–Nonius FR-571 rotating-anode generator operated at 40 kV, 70 mA and equipped with a 0.4 mm collimator. Data were collected and processed using the program system *MADNES* (Messerschmidt & Pflugrath, 1987). Profile fitting was carried out by the Kabsch's procedure (1988) and the data were scaled using *AGROVATA/ROTAVATA* programs of the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994).

Results and discussion

Out of the 50 different conditions tested, droplets initially containing crystals of elastase from North Atlantic salmon grew in 20%(*v*/*v*) 2-methyl-2,4-pentanediol (MPD), 0.1 *M* sodium citrate in 50 m*M* HEPES buffer, pH 7.3 and in a combination of 10%(*w*/*v*) polyethylene glycol (PEG) 4000 and 5%(*v*/*v*) 2-propanol in 50 m*M* HEPES buffer, pH 7.15. The MPD crystals grew as well defined prisms to a maximum size of 0.7 × 0.4 × 0.3 mm in three to four weeks (Fig. 1), while the PEG/2-propanol crystals grew to a maximum size of 0.8 × 0.3 × 0.15 mm in about 10 d. The faces and edges of the PEG/2-propanol crystals were not as well formed as the

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MPD crystals. The better formed MPD crystals were used for X-ray analysis.

The diffraction limit for the MPD crystals examined was approximately d_{min} = 1.6 Å resolution. Analysis of the diffraction data shows that the crystals exhibit tetragonal symmetry with unit-cell parameters $a = b = 68.0$ and $c = 84.0$ Å (1 Å = 0.1 nm). This corresponds to a unit-cell content of eight molecules. Assuming a molecular weight of 25 000 Da for salmon elastase, the packing parameter $V_m = 1.95$ Å^3 Da^{-1}, corresponds to a solvent content of 36.8%. This value is within the range found for other protein crystals (Matthews, 1968). Inspection of axial reflections indicate one fourfold and one twofold screw axis, identifying the space group as $P4_12_12$ or its enantiomorph, $P4_32_12$. The native data set from this crystal was scaled and merged to give 21 574 unique reflections (83.6% of possible) between 14.0 and 1.61 Å resolution with an overall R_{merge} = 2.4% [$R_{merge} = (\Sigma_h\Sigma_i|I_{hi} - \langle I_h\rangle|/\Sigma_h\Sigma_i|\langle I_h\rangle|) \times 100\%$, where I_{hi} is the intensity of the ith measurement of an reflection, $\langle I_h\rangle$ the mean intensity for that reflection with summations over all measurements].

The crystals of native pancreatic elastase from Atlantic salmon are suitable for high-resolution X-ray crystallographic analysis, and further studies are now underway to obtain the three-dimensional structure.

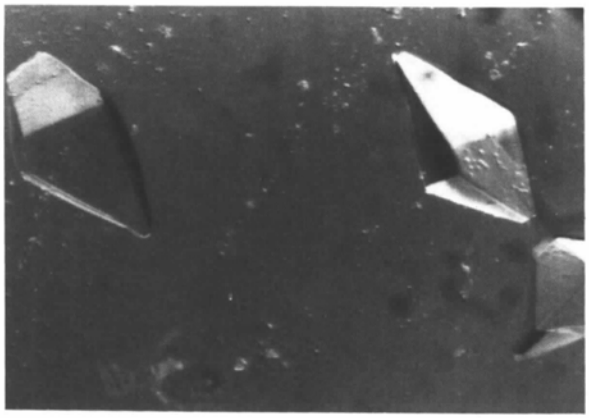

Fig. 1. Crystals of native pancreatic elastase from the North Atlantic salmon. The crystals has approximate dimensions of 0.7 × 0.4 × 0.3 mm. See text for detailed crystallization conditions.

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