Crystallization and preliminary X-ray diffraction data of proteinase II from *Crotalus adamanteus* (Eastern Diamond Rattlesnake)

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Proteinase II, a snake venom metalloproteinase from *Crotalus adamanteus*, has been crystallized. The crystals are of the trigonal space group P 3_112 (or P 3_212), diffract well beyond 2 Å and are suitable for X-ray crystal structure analysis.

Protein crystallography *Metalloproteinase* *Snake venom* *Limited proteolysis*

1. INTRODUCTION

A metalloproteinase, designated proteinase II, was isolated in a homogeneous state from *Crotalus adamanteus* venom, its properties and activities described [1]. By SDS gel electrophoresis, its M_r is ~24 000. It is readily inactivated by EDTA, is unaffected by diisopropyl fluorophosphate, and does not require thiol compounds for activation. By its inhibition profile, Zn^{2+} and Ca^{2+} seem to be essential for activity [1]. Proteinase II hydrolyzes general protein substrates such as casein and globin very poorly, and shows no activity on typical synthetic substrates for the serine proteinases trypsin, chymotrypsin, plasmin, thrombin or elastase. Thus proteinase II has all characteristics of a metalloproteinase, but does not digest typical substrates for thermolysin, carboxypeptidase A or B, *Pseudomonas aeruginosa* elastase or *Clostridium histolyticum* collagenase, and shows no activity on native collagen or elastin. Rather, the specificity of proteinase II seems directed towards enzymatic inactivation of native (human) plasma proteinase inhibitors [2,3]. Proteinase II in catalytic amounts cleaves the Ala_{350}–Met_{351} bond [4,5] in human plasma α1 proteinase inhibitor and the Ala_{375}–Ser_{376} bond [6] in human plasma antithrombin III resulting in inactivation of these inhibitors. These bonds are 8 and 9 residues, respectively, N-terminal of the reactive site bonds of those inhibitors [5,7]

2. MATERIALS AND METHODS

Proteinase II from *Crotalus adamanteus* venom (obtained from Miami Serpentarium) was prepared as in [1] except that a final step of gel filtration on S-200 Sephacryl (Pharmacia) in 0.05 M glycine–0.2 M NaCl (pH 9.5) was incorporated to remove a trace amount of higher-M_r contaminant. The purified enzyme was then dialyzed against 0.01 M sodium borate–0.002 M $CaCl_2$ (pH 8.0) concentrated and stored as a suspension in 80% saturated $(NH_4)_2$ SO_4. The enzyme retains full activity under these conditions.

Crystallization was achieved in $(NH_4)_2$ SO_4 at 4°C with a solution containing 20 mg protein/ml, buffered with 0.1 M phosphate and saturated with $CaCl_2$, using the vapour diffusion technique and methods in [8]. The crystal density was determined

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in a Ficoll 400 (Pharmacia) gradient as in [9] but with a density read after 2 min centrifugation at 10 000 × *g*. SDS gel electrophoresis was performed on 7.5% polyacrylamide gels in 0.1 M Tris-phosphate buffer at pH 6.8 in the presence of 0.1% SDS and 8 M urea. The samples were preincubated in the presence of 1% mercaptoethanol and 1% SDS in the same buffer at 90°C for 5 min before application to the gels. Direct M_r determination by sedimentation equilibrium in a Spinco Model E analytical ultracentrifuge (Beckman) was kindly performed by Dr H. Priess (Max-Planck-Institut für Biochemie, Martinsried).

3. RESULTS AND DISCUSSION

In 1.8 M $(NH_4)_2$ SO_4 at pH 5, triangular prism-shaped crystals (fig. 1) could be grown to a maximal length of 1 mm and up to 0.3 mm diam. Precession photographs show the Laue group $3\,\overline{m}$, systematic extinctions along 001 for $l = 3n + 1$ and $l = 3n + 2$ ($n = 0,1,2\ldots$) and *mm* symmetry of the h01 zones. Consequently these crystals belong to the trigonal space group $P3_112$ or to its enantiomorphic space group $P3_212$. The unit cell constants as measured from zero layer precession photographs are $a = b = 73.3$ Å, $c = 95.8$ Å, $\alpha = \beta = 90°$, $\gamma = 120°$. Due to SDS gel electrophoresis, the crystalline material is identical with the original proteinase II material and migrates like a normally behaving protein of M_r 24 000 (the sedimentation equilibrium runs indicate M_r 22 400 ± 2000 under the assumption of a partial specific volume of 0.74 ml/g for this proteinase). Because of a crystal density of 1.20 ± 0.01 g/ml determined in the aqueous Ficoll gradient for 4 different crystals, the protein content of the asymmetric unit should have M_r 34 500 ± 2000. Thus, the asymmetric unit may either contain one or two molecules. If this proteinase, however, behaves normally with respect to the M_r determination methods applied, only one molecule/asymmetric unit is consistent with the crystal volumes/protein M_r unit observed [10]. The reason for this uncertainty is unclear. These trigonal crystals are stable in 3 M $(NH_4)_2$ SO_4 up to pH 9 (i.e., in the optimal pH-range of this enzyme) and they tolerate either EDTA or Zn^{2+}, properties which could be helpful for the preparation of heavy metal derivatives. They diffract well to Bragg spacings beyond 2.0 Å and prove to be quite suitable for X-ray structure analysis. The collection of intensity data from these trigonal crystals is now under way in our laboratory.

Besides these well-diffracting trigonal crystals, which could only be grown at pH 5, another crystal modification growing in thin stacked plates (fig. 2) of approximate dimensions 0.5 × 0.3 × 0.03 mm was frequently observed in 1.8–2.0 M $(NH_4)_2$ SO_4 between pH 5–8. These crystals show reflections to ~3 Å resolution, but are considerably disordered and not suitable for X-ray diffraction studies.

At present the metalloproteinases for which 3-dimensional structures are known are thermolysin [11], carboxypeptidases A [12] and B [13], and a D-alanyl-D-alanine-cleaving carboxypeptidase from *Streptomyces albus* [14]. With the ex-

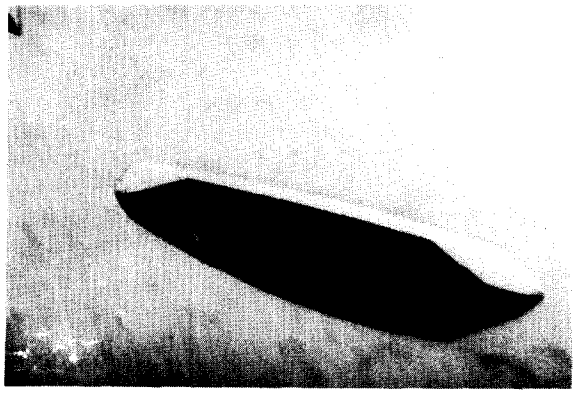

Fig. 1. Trigonal proteinase II crystal of approximate size 0.8 × 0.2 × 0.1 mm^3.

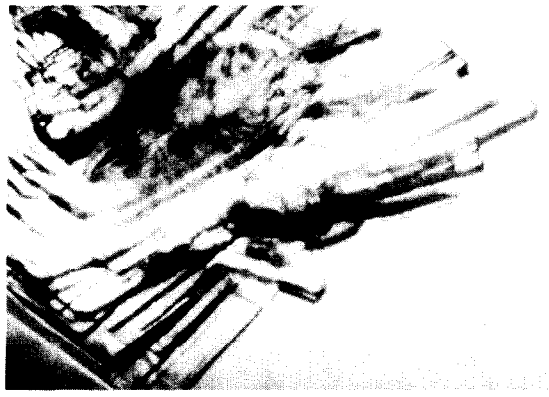

Fig. 2. Stacked plates of the second (presumably monoclinic) crystal form of proteinase II.

ception of the close homology between both bovine carboxypeptidases, these metalloenzymes including proteinase II seem to be unrelated with respect to molecular size and chain fold. Nevertheless, the molecular parts involved in the catalytic event seem to have several features in common. Thus these proteinase II crystals suggest not only a new proteinase fold, but an alternative investigation of metalloproteinase reaction mechanism.

The unique specificity of this proteinase II must reside in the specific contouring of the substrate-binding surface. Since the structure of a split human α1 proteinase inhibitor is now being determined [15], knowledge of the crystal structure of proteinase II could help us to understand the specific and selective cleavage on a structural basis. Finally, no previous crystal structure determinations have been reported for any reptilian or snake venom proteinase. Comparison of proteinase II with metalloproteinases from other sources will be important in determining the extent of structural homologies which have evolved in this category of proteinases.

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