X-RAY CRYSTALLOGRAPHIC STUDIES OF LACTONOHYDROLASE

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The enzymes catalyzing the reversible or irreversible hydrolysis of carboxylic esters to the respective carboxylic acids and alcohols (EC 3.1.1.) play an important role in the biosynthesis and biodegradation of various compounds. Lactonohydrolases catalyze the hydrolysis of carboxylic esters, but act on the intramolecular ester bonds of lactone compounds. They are involved in the synthesis and degradation of various lactone compounds *in vivo*. Lactonohydrolases acting on aldonate lactones are involved in the oxidative degradation of aldoses through the ring-opening of aldonate lactones, which are formed by enzymatic oxidation of aldoses. There has been no report on the three-dimensional structure of any lactonohydrolase. It was found that *Fusarium oxysporum* produced a novel lactonohydrolase catalyzing the hydrolysis of aldonate lactones and aromatic lactones [1]. The three-dimensional structure of lactonohydrolase of *F. oxysporum* will give a significant inshight into the mechanisms underlying the stereoselectivity and substrate specificity of the enzyme.

Crystals of lactonohydrolase from *Fusarium oxysporum* suitable for X-ray diffraction studies were obtained by the following protocol [2]. A 4μl protein droplet, with a protein concentration of 7 mg ml^{-1} in 100 mM sodium citrate buffer (pH 6.2) containing 3.5 mM KCl and 11.0% (*w*/*v*) PEG 4000, was vapor-equilibrated against 0.2 ml of the same buffer solution containing 7mM KCl and 21.5% (*w*/*v*) PEG4000 at 4°C. The crystals diffracted X-rays from synchrotron radiation beyond 3.0 Å resolution. The crystals belong to the monoclinic space group $P2_1$. The unit-cell dimensions were determined as a = 156 Å, b = 100 Å, c = 94.1 Å, and β = 91.7°. Assuming that there are two lactonohydrolase dimer molecules in the asymmetric unit, the crystal volume per unit molecular mass, V_M, was calculated to be 2.94 Å^{3}Da^{-1}.

The best data set of the native crystal was collected at BL6A using a crystal with the approximate size of 0.5×0.4×0.1 mm. A total of 61,465 unique reflections was obtained, which corresponds to 79 % of the possible reflections of a crystal at 2.7 Å resolution. The merging R factor was 0.075 for 91,627 measurements. Search for heavy atom derivatives is underway.

References

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