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Crystal Structure Determination of New Subtilisin Family Protease

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Introduction

Subtilisins and related "high-alkaline" serine proteases are important materials to improve the efficiency of laundry detergents. The activities of these proteases are reduced by oxidizing agents such as hydrogen peroxide, a component of bleach. These reagents oxidize a conserved methionine residue adjacent to an active serine residue. No mutagenesis experiments improve the resistance to oxidization without reducing their activity. Recently, an oxidatively stable alkaline serine protease, KP-43 has been isolated from *Bacillus* sp. strain KSM-KP43 [1]. This protease also possesses the conserved methionine residue. In order to understand structural features for oxidative stability, we have determined the crystal structures of native and oxidized KP-43 protease.

Materials and Methods

Crystals were obtained using 1.7 M $(NH_4)_2SO_4$ as a precipitant [2]. The KP-43 protease structure was solved at room temperature by the multiple isomorphous replacement method with anomalous scattering (MIRAS) method using samarium and platinum derivatives. The datasets for the native and platinum derivative crystals were collected at the BL6A beamline of the Photon Factory, whereas that for the samarium derivative was collected at SPring-8.

Results

Crystals belong to the space group $C222_1$ with cell dimensions of a = 43.5 Å, b = 110.4 Å and c = 168.9 Å. The processed native dataset has shown an overall R_{merge} of 6.1 % and a completeness of 94.1 % at 1.50 Å resolution. The initial model was constructed using the program *O* on the electron density map at 2.5 Å resolution. All 434 residues were assigned successfully and the model was refined to the crystallographic *R*-factor of 0.126 at 1.50 Å resolution using the program *CNS* and *SHELX-97*.

KP-43 protease consists of two domains, an N-terminal subtilisin-like α/β domain and a C-terminal jelly roll β-barrel domain. Three Ca^{2+} ion binding sites exist in a KP-43 molecule and these are different from the sites conserved in other subtilisins. Two of them are located in the C-terminal domain, and the other is in the N-terminal domain. Further structure refinements and investigation into the oxidative stability by comparison between the native and the oxidized structures are in progress.

References

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