Studies on the solution structure of chitinase A1 composed of four domains by small angle X-ray scattering

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Introduction

Chitin, an insoluble linear β-1,4 homopolymer of N-acetylglucosamin, is one of the most abundant polysaccharides in nature. Chitinase hydrolyzes the β-1,4-glycosidic-linkages of chitin.

Chitinase A1 from Bacillus circulans WL-12 is composed of a catalytic domain (CatD), two fibronectin type III like domains (FnIIID), and a chitin binding domain (ChBD), and the 3D-structures of these domains have been determined separately. Removal of the ChBD results in a significantly decreased binding activity to chitin as well as insoluble chitin-hydrolyzing activity [1]. Deletion of the FnIIIDs, on the other hand, does not affect chitin-binding activity but results in a significantly decreased insoluble chitin-hydrolyzing activity [1]. These observations suggest that cooperative action of these domains is crucial in insoluble chitin hydrolysis. To obtain information about entire chitinase A1 molecule, solution structure of chitinase A1 was studied by small angle X-ray scattering.

Materials and Methods

Chitinase A1 was produced in E. coli HB101 cells carrying recombinant plasmid and purified as previously described [2]. For SAXS experiments, chitinase A1 was dissolved in 20 mM Tris-HCl pH8.0.

SAXS experiments were performed using synchrotron radiation with an instrument for SAXS installed at BL-10C of Photon Factory. An X-ray wavelength of 1.488 Å was selected. The scattering vector is defined as \( Q = 4 \sin \theta / \lambda \), where \( \theta \) is the scattering angle and \( \lambda \) is the X-ray wavelength. All measurements were done at 25 °C. A series of measurements at different protein concentrations ranging from 5 to 14 mg/ml were performed for chitinase A1. As a molecular mass standard, SAXS measurements for lysozyme (Sigma L-6876) were carried out at the protein concentration of 7 mg/ml. Scattering data were collected for 300 seconds.

The values of radius of gyration (\( R_g \)) were derived from Guinier approximation using SAXSANA [3]. The distance distribution functions \( P(r) \) were calculated by the Fourier inversion of the scattering intensity using GNOM [4]. The low-resolution structure of chitinase A1 was determined \textit{ab initio} from the scattering curves using DAMMIN [5].

Results

A linear Guinier region was observed at low angles. The \( R_g \) value was 38.9 Å and the effect of protein concentration was not observed. A molecular mass of chitinase A1 in the solution estimated from \( I(0) \) value of chitinase A1, making reference to the value of lysozyme, was 66 kDa, which corresponds approximately to the mass of a monomer of 70 kDa. This result indicates that chitinase A1 exists as a monomer in the solution.

The \( P(r) \) profile displays not a bell-shape which is characteristic of a globular protein but a biphasic pattern, and maximum dimension was 135 Å.

The solution structure of chitinase A1 was determined using DAMMIN and the crystal structures of the isolated domains were fitted into the determined structure (Fig1). The determined structure is in extended form and ChBD is distantly located from CatD.

Fig. 1. Proposed structure of chitinase A1. Gray represents a bead model of chitinase A1 obtained with DAMMIN by three independent calculations. Structures of CatD, FnIIID and ChBD are shown in blue, yellow and green, respectively.

References


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