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Crystal structure of *Arthrobacter globiformis* I42 glucodextranase

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Introduction

Enzymes classified into glycosyl hydrolase (GH) family 15, whose major members are glucoamylases (EC 3.2.1.3), are commercially important in the production of glucose, and has been widely used in starch-processing industries. The enzymes are exohydrolases that release β-D-glucose from the non-reducing ends of starch or related oligo- and polysaccharides. Although there are numerous reports on the fungal enzymes, relatively little is known about the prokaryotic GH family 15 enzymes. We have reported that the properties of some prokaryotic enzymes are markedly different from those of the fungal enzymes in spite of the similarities of their primary structures. A glucoamylase from *Thermoactinomyces vulgaris* R-47 efficiently hydrolyzes small maltooligosacchrides. Also, a glucodextranase (EC 3.2.1.70) from *Arthrobater globiformis* I42 (iGDase) efficiently releases β-D-glucose from the non-reducing end of dextran. Unlike the typical fungal enzymes, both enzymes hydrolyze starch less efficiently. In this report, we describe the crystal structure of iGDase [1].

Materials and Methods

Crystals of iGDase were grown at 20 °C using the hanging drop vapor-diffusion method, with a well solution of 3.0 % (w/v) PEG 8000, 80 mM potassium dihydrogen phosphate in 50 mM sodium acetate buffer (pH 5.1) and a protein solution of 8 mg/ml. The structure was solved by molecular replacement. Although the whole structure of *Thermoanaerobacterium thermosaccharolyticum* glucoamylase (PDB No. 1LF6) was initially used as the search model, the reasonable phases were not obtained. Therefore, the structure of *T. thermosaccharolyticum* glucoamylase was divided into two models, namely, the part of domains N and A, respectively, and independently used as the starting models. After clear 2Fo-Fc electron density of domains N and A was observed, the density modification protocol was applied, and the adequate phases to all domains were clearly obtained. We also determined a complex structure of iGDase with an inhibitor, acarbose, which is a pseudotetrasaccharide inhibitor that possesses the acarviosine unit at the non-reducing end.

Results and Discussion

The structure of iGDase is composed of four domains, N, A, B and C (Fig. 1). Domain N, uniquely found in the bacterial and archaeal glucoamylases and glucodextranases, is composed of 17 antiparallel β-strands, which are divided into two β-sheets. Domain A is an (α/α)6-barrel structure that is common among the GH15 family enzymes, and between domain N and A, two α-helices connect those domains and form a linker region. Domain B consists of antiparallel eight-strand β-strands. Domain C is composed of 17 antiparallel β-strands forming three typical β-sheets.

The most distinguishing features of iGDase structure are domains B and C. A short linker, consists of nine amino acid residues, AGTPLSSPE, connects an α-helix in domain A and a β-strand in domain B. It is likely that this linker functions as a hinge in the solution. The primary structure of domain C is homologous to those of surface layer homology (SLH) domains, thus it is a reasonable assumption that these domains serve as cell wall anchors. Our report is the first crystal structure of SLH domains.

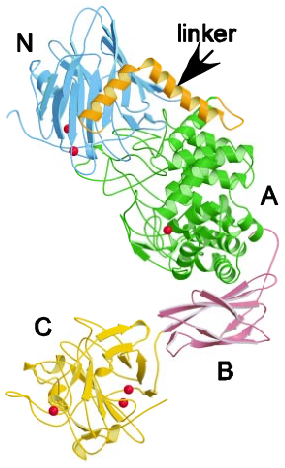

Fig. 1. Structure of iGDase

References

[1] Mizuno et al., J. Biol. Chem., 279, 10575-10583 (2004).

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