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Structural determination of glucooligosaccharide oxidase from *A. strictum*

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Only a few oxidases exhibit high specificity toward oligosaccharides. The glucoologisaccharide oxidase (GOOD) from *Acremonium strictum* has been screened from sugar oxidase-producing microorganisms in soil for industrial application. GOOD contains a 25-residue leader peptide and a 474-residue mature protein with a covalently linked FAD and glycosylation. This novel oxidase oxidizes the oligosaccharides with glucose unit on the reducing end and each sugar unit is jointed by α or β-1,4 linkage. Kinetic measurements show that cellobiose has the lowest *Km*, followed by lactose, maltose and glucose, and lactose has the highest *kcat*, followed by cellobiose, maltose and glucose. Interestingly, maltose is the preferred substrate and this oxidase can react with maltoheptaose, which contains up to seven monosaccharide units and is the largest commercially available maltooligosaccharides.

Recombinant GOOD has been expressed successfully in *Pichia*, but not in *E. coli*. Recombinant proteins possess similar enzymatic activity to the native fungal enzyme. The crystal has been grown in 25% polyethylene glycol MM 550, and belongs to the $P2_12_12_1$ space group with unit cell dimensions of a = 53.66 Å, b = 91.40 Å, c = 112.78 Å. The native data at 1.4 Å resolution and S-SAD data at wavelengths of 1.7, 1.8 and 2.2 Å from the GOOD crystals have been collected. Only the S-SAD data at wavelengths of 2.2 Å showed some significant peaks in the difference Patterson maps. However, these peaks were not strong enough to solve the phase problem. Expression of the selenomethionyl protein in *Pichia* was not successful. Alternatively, a conventional heavy-atom screening is carried out. The in-house data showed that $(NH_4)_2OsBr_6$ might be a good candidate, and hence the Os-MAD data at 2.2 Å resolution were collected as listed in Table I.

TABLE I

Statistics of data collection

	Wavelength	Reflection	Completeness	Rmerge
Os peak	1.1404 Å	29281	99.8 %	5.7 %
Os edge	1.1407 Å	29058	99.9 %	3.4 %
Os remote	1.1198 Å	28968	99.8 %	3.4 %

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Structure of a new crystal form of XpsE N-terminal domain

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In pathogenic Gram-negative bacterium *Xanthomas campestris*, secretion of extracellular proteins across the bacterial outer membrane is achieved by a gigantic complex constituted of the protein products of twelve genes. Among them, XpsE protein appears to be cytoplasmic for its lack of membrane spanning sequence. And with a conserved nucleotide binding Walker A motif, it has been suggested to possess either ATPase or kinase activity and hypothesized to serve as an energy generating component in the secretion complex. To understand the structural basis of type II secretion pathway, we have previously determined the crystal structure of the 21 kDa N-terminal domain of XpsE protein ($XpsE_N$) in space group $P4_32_12$ by collecting diffraction data at three different wavelengths at Taiwan beamline SP12B2 (SPring8, Japan). The structure of $XpsE_N$ is bilobal: the N-subdomain of $XpsE_N$ resembles a distorted four-helix bundle, while the C-subdomain forms an α/β sandwich. Packing analysis revealed the presence of a crystallographic $XpsE_N$ dimer. As the dimerization of $XpsE_N$ has been implicated by results obtained from chemical cross-linking and gel-filtration analysis, the crystallographic $XpsE_N$ dimer may be of functional importance. In consistent with this prediction, multiple sequence alignment also shows that this interface is composed of highly conserved residues, and disruption of this interface by point mutation alters the activity of $XpsE_N$.

With the Se-MAD data collected in SP12B2, we have recently determined the crystal structure of $XpsE_N$ in space group $I4_122$. A crystallographic $XpsE_N$ dimer was observed with identical dimer interface as that of space group $P4_32_12$, further supports the significance of this interface. Surprisingly, when compared with the structure determined in space group $P4_32_12$, a large tertiary structural change in the first 36 residues of the N-subdomain was detected. This observation suggests the existence of at least two conformational states of $XpsE_N$. The functional relevance of our observation is currently being addressed.