Structure of the complex of a yeast glucoamylase with acarbose reveals the presence of a raw starch binding site on the catalytic domain

Jozef Ševčík, Eva Hostinová, Adriana Solovicová, Juraj Gašperík, Zbigniew Dauter and Keith S. Wilson

Institute of Molecular Biology, Slovak Academy of Sciences, Dúbravská cesta 21, 84251 Bratislava, Slovakia

Synchrotron Radiation Research Section, Macromolecular Crystallography Laboratory, NCI, Argonne National Laboratory, Building 202, Room Q142, Argonne, IL 60430, USA

York Structural Biology Laboratory, Department of Chemistry, University of York, York YO10 5YW, UK

Glucoamylases (α-1,4-D-glucan glucohydrolase, EC 3.2.1.3) are inverting exo-acting hydrolases that catalyze the removal of α-D-glucose from the non-reducing ends of starch and other related poly- and oligosaccharides. Most glucoamylases have structures consisting of both a catalytic and a starch binding domain. However the structure of a glucoamylase from Saccharomycopsis fibuligera HUT 7212 (Glu) at 1.8 Å resolution, determined a few years ago, consists of a single catalytic domain containing 492 amino acids [1]. Using the classification of glycoside hydrolases, glucoamylase belongs to family 15 [2; http://afmb.cnrs-mrs.fr/CAZY/].

Different strains of the dimorphous yeast S. fibuligera produce a set of closely related glucoamylases. Two of them, Glu (strain HUT7212) and Glm (strain IFO 0111) from the GLU [3] and GLM [4] genes, consist of 492 and 489 amino acid residues, respectively, with an identity of 60 %. The two enzymes differ in biochemical properties, in particular in the ability to digest raw starch. While Glu adsorbs to, but does not digest raw starch, Glm adsorbs well to starch granules and is capable of raw starch digestion.

In this contribution two structures are mentioned: that of the glucoamylase Glu with the resolution extended to 1.1 Å and that of its complex with acarbose at 1.6 Å resolution. The structure at atomic resolution besides higher accuracy shows clearly the influence of cryo-cooling which is manifested in shrinkage of the molecule and lowering the volume of the unit cell. In the structure of the complex, two acarbose molecules are bound, one at the active site at the N-terminal end of the (α/α)₆ barrel and the second at a site remote from the active site, on the surface of the enzyme curved around Tyr464 which resembles the inhibitor molecule in the 'sugar tongs' in the structure of barley α–amylase isozyme 1 complexed with a thiomaltooligosaccharide [5]. We propose the role of the remote site is to fix the enzyme onto the surface of a starch granule while the active site degrades the polysaccharide. Based on the close similarity in sequence of glucoamylase Glu, which does not degrade but adsorbs to raw starch, and that of glucoamylase Glm, a raw starch degrading enzyme for which the 3D structure has been modelled [4], it is reasonable to expect the presence of the remote starch binding site at structurally equivalent positions in both enzymes. Five residues (Arg15, His447, Asp450, Thr459 and Tyr464) which are important in the remote starch binding site in Glu are conserved in Glm (Arg15, His444, Asp447, Thr459 and Phe461). However a key residue which is central for the remote acarbose binding is different in the two enzymes: Tyr464 in Glu versus Phe461 in Glm. Mutation of the above amino acids and test for their ability to adsorb to and digest raw starch confirmed that the remote binding site is essential for raw starch binding.
Figure 1: Stereopicture of the surface acarbose curved around Tyr464 and the interacting partners Arg15, His447, Asp450 and Thr462.

References