Genetic and enzymatic Characterisation of Glucoamylases from Yeast

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Starch belongs to the most abundant rapidly renewable polymeric carbohydrates found in nature It is a heterogeneous polysaccharide composed of amylose and amylopectin, two high-molecular weight components that may be present in different ratios Study of enzymes which degrade this substrate has got theoretical and practical significance Two important enzymes participate in starch degradation endo-acting alpha-amylase, and exo-acting glucoamylase Glucoamylase catalyses the removal of D-glucosyl units from the non reducing ends of starch and maltooligosaccharides Glucoamylases are widely distributed in microorganisms namely in *Aspergillus sp* and *Rhizopus sp* Ability to produce glucoamylases is also distributed among yeast genera In the realm of yeast the best known producers of glucoamylases are *Saccharomycopsis fibuligera* and *Schwanniomyces occidentalis* Glucoamylases are secreted into culture media as glycoproteins often in multiple forms

Glucoamylase has got a wide application in the food and fermentation industries It has got 14% distribution on the world-wide distribution and sales of industrial enzymes Each application of glucoamylase requires an enzyme of a certain technological properties optimal for a given process Structure/function relationship of glucoamylases from various sources should be known to prepare a palette of industrially required enzymes by means of protein engineering

Yeast glucoamylases from *S fibuligera* and *Sch occidentalis* which are the subject of interest of our laboratory, are presented in Table 1

Table 1

Species	M W (kDa)	Optimum pH	Optimum temperature(°C)	Specific activity (U/mg protein)	Gene
Saccharomycopsis fibuligera HUT 7212	57*	5 6	40 50	106	GLU1
Saccharomycopsis fibuligera KZ	62	5 6	40 – 50	55	GLA1
Saccharomycopsis fibuligera IFO 0111	55	5 5	40	88	?
Schwanniomyces occidentalis ATCC26076	135	5 0	50	22	*GAM*1

* recombinant protein

Two glucoamylase genes GLA1 (Hostinová *et al* 1991) and GLU1 (Itoh *et al* 1987) were isolated from two different genomic libraries of *S fibuligera* prepared by means of "shot gun" technique in a shuttle yeast-*Escherichia coli* vector Positive clones were selected after expression cloning in *Saccharomyces cerevisiae* Alignment of the nucleotide sequences of the GLA1 and GLU1 genes showed a high homology Alignment of their amino acid sequences revealed seven amino acid alterations in 492 aa long mature polypeptides which led to differences in specific activity and thermal stability between the Gla1 and Glu1 enzymes Glucoamylase Glu1 has 2-times higher specific activity when compared with the Gla1 The hybrid glucoamylase encoded by the chimerical gene GLA1/GLU1 proved that one of the amino acid alterations in the C-terminus of the enzymes contributed to the changed catalytic activity of the glucoamylases From physico-chemical properties of the Gla1 and Glu1 glucoamylases their ability of renaturation after a thermal denaturation is worth of mentioning Enzymes exposed to boiling for 10 minutes are able to restore about 50% of their catalytic activity within 100 minutes (Gašperik and Hostinova 1993)

Alignment of the primary structures of *S fibuligera* glucoamylases to 16 known primary structures of glucoamylases showed that they also have five highly homologous segments along their polypeptide chains (Coutinho and Reilly 1994) Although it seemed originally that segment five was not important for catalytic activity because it was not present in yeast glucoamylases from *Saccharomyces diastaticus* and *cerevisiae*, structural studies on fungal glucoamylases from *A awamori* and *S fibuligera* revealed that a part of a catalytic site containing a catalytic base was located in this segment

Until recently the characterization of glucoamylase on the basis of its tertiary structure has been done only for a partly deglycosylated proteolytic fragment representing a catalytic domain of a fungal glucoamylase from *Aspergillus awamori* var *X100* (Aleshin *et al* 1992) 3D structure of *S fibuligera* glucoamylase Glu1 is the second known structure among glucoamylases as well as in the family 15 of glycan hydrolases (Ševčík *et al* in press) Our laboratory has chosen a molecular-genetic approach to obtain enzymes for crystallographic studies The genes GLA1 and GLU1 were expressed in *S cerevisiae* The recombinant glucoamylases, however, due to the glycosylation heterogeneity, were not suitable for crystallisation Thus, non-glycosylated enzymes were prepared by the expression of the GLA1 (Solovicova *et al* 1996) and GLU1 genes in *E coli*

Glucoamylase produced by *S fibuligera* IFO 0111 differs from other known yeast glucoamylases in capability to digest raw starch and to hydrolyse, effectively, not only 1,4 but also 1,6-α- glucosidic linkages The sequence of the first 20 amino acid residues from the N-terminus of the mature protein is totally different from the sequence of the N-terminus of the glucoamylases Gla1 and Glu1 Our hypothesis is that this glucoamylase has a raw starch binding domain (SBD) located in the N-terminal region of the protein and that this domain is an integral part of the enzyme It seems that glucoamylase from *S fibuligera IFO 0111* is similar to one of the most active glucoamylases, isolated from *Rhizopus sp* which has a raw starch binding domain located in the N-terminal part of the polypeptide chain On the contrary raw starch digesting glucoamylases from *Aspergillus sp* have the raw starch binding domains located in the C-terminal regions of the enzymes Glucoamylases lacking SBDs have unchanged hydrolytic rates against soluble substrates, but have dramatically slower rates against granular starch The mechanisms by which the substrate specifically interacts with the binding domain and functional relationship between carbohydrate binding and catalysis is not understood yet Glucoamylase from *S fibuligera* IFO 0111 is therefore a potential candidate for the three-dimensional structural studies Our current interest is oriented to crystallisation of a full length raw starch

digesting *S fibuligera* glucoamylase isolated from an original producer

The *Schwanniomyces occidentalis* glucoamylase-encoding gene *GAM1* was isolated from a λ Charon4A genomic library using synthetic oligo probes *GAM1* gene encodes a polypeptide of 958 amino acids which has no homology to all other glucoamylases which are sequenced thus far and have highly conserved regions in common (Dohmen *et al* 1990) On the other hand GAM glucoamylase shows a high sequence similarity to *S fibuligera* α-glucosidase According to the classification of glycosyl hydrolases based on amino acid sequence similarities Gam glucoamylase from *Sch occidentalis* belongs to family 31 The tertiary structure of hydrolases belonging to this group has not been determined, yet *Sch occidentalis* glucoamylase and *S fibuligera* α-glucosidase would be an excellent model for investigation of differences in the mode of action of α-glucosidases and glucoamylases on substrates having different lengths

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Molecular Dynamics Simulations of Nucleic Acids: A) Molecular Dynamics simulations of the Oligonucleotide with the Modified Phosphate/phosphonate Internucleotide LINKAGE

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Impact of the internucleotide linkage modification by inserting a methylene group to the P-O bond, on the modified oligonucleotide binding ability to the natural DNA strand was studied by molecular dynamics simulations (Barvík Jr *et al* 1998)