Short Communication

DIVERSITY OF MOLECULAR STRUCTURES IN THE YEAST EXTRACELLULAR GLUCOAMYLASES

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Glucoamylase (1,4- α -D-glucan glucohydrolase, EC 3.2.1.3) catalyzes the release of glucose from the non-reducing end of starchy materials. Many authors reported purification of glucoamylases secreted from fungi and yeasts. It has been found that glucoamylases of the following organisms exist in multiple forms varying in size: *Aspergillus niger* (1–4), *Aspergillus oryzae* (5–7), *Rhizopus* sp. (8, 9), *Mucor rouxianus* (10), *Penicillium oxalicum* (11), *Schwanniomyces castellii* (12), *Saccharomycopsis fibuligera* (13–15), and *Saccharomyces diastaticus* (16, 17). Multiple forms of glucoamylase from *Rhizopus* sp. differ in the NH₂-terminal region of the polypeptide chain (8). In *A. niger* glucoamylases, three different biochemical events contribute to the generation of multiple enzyme forms: (i) differential splicing of mRNA transcribed from a single glucoamylase gene (18, 19), (ii) proteolysis of the larger form (1–3), and (iii) different addition of *Trichoderma*-mannosidase-sensitive and *Turbo*-glycosidase-sensitive carbohydrates to multiple proteins (1, 2). However, little is known about the multiplicity of yeast glucoamylases. We have indentified and analyzed regulatory genes for glucoamylase production in the yeast *S. diastaticus* (20–25). Recently, we have cloned an *STA1* DNA which codes for a structural gene of the *S. diastaticus* glucoamylase (26, 27).

In this research, we purified multiple forms of glucoamylase secreted from two widely diverse yeasts, *S. diastaticus* and a transformant of *Schizosaccharomyces pombe*, carrying the glucoamylase gene (*STA1*): *S. diastaticus* strains, 5106-9A and YIY2-12D, each carrying *STA1* on the chromosome, and *S. pombe* HM123 transformed with a plasmid (pSTA1) carrying the cloned *STA1* DNA (25). *S. pombe* cells without the plasmid secrete no amylases (25).

Purified glucoamylases were analyzed with the two criteria, subunit structure and asparagine-linked (N-linked) glycosylation. Results are shown in Table 1.

Table 1. Multiple forms of glucoamylases secreted from *S. diastaticus* and *S. pombe*.^a

Organism	Strain	Genotype	Specific activity ^b (units/ μ g protein)	Molecular weight ^c		Molecular weight of N-linked glycosides ^d
				-Endo H	+Endo H	
<i>S. diastaticus</i>	5106-9A	<i>STA1</i>	7.5	H, 53,000	H, 41,000	H, 12,000
<i>S. diastaticus</i>	YIY2-12D	<i>STA1</i>	9.0			
<i>S. pombe</i>	HM123	[pSTA1]	10.8			

^a Cells of two *S. diastaticus* strains were cultured at 28°C in YEPD medium (1% yeast extract, 2% polypeptone, and 2% glucose). Cells of the *S. pombe* transformant with a plasmid (pSTA1) carrying *STA1* DNA were cultured at 28°C in YPS medium (1% yeast extract, 2% polypeptone, and 3% soluble starch). Glucoamylases were purified from the culture fluids essentially as described previously (24): successive precipitation with ammonium sulfate and acetone, ion exchange column chromatography on DEAE-Sephadex A-50 and CM-Sephadex C-50, and gel filtration through Sephacryl S-200. The data for glucoamylase of the *S. diastaticus* strain 5106-9A are cited from Ref. (24).

^b Specific activity was determined as described previously (24).

^c Tyrosyl residues of the purified proteins were radio-labeled with ¹²⁵I as described previously (24). The ¹²⁵I-labeled protein was treated with Endo H (Seikagaku Kogyo Co.) to remove N-linked glycosyl chains. The reaction mixture contained 0.02 μ g of the ¹²⁵I-labeled protein, 2.5×10^{-3} units of Endo H, and 1 mM diisopropyl fluorophosphate in 10 mM sodium acetate buffer (pH 5.6). The treatment was carried out at 37°C for up to 24 hr. Native and deglycosylated proteins were analyzed in 12.5% acrylamide gel containing sodium dodecyl sulfate (SDS) (28), except for the small subunit in 10% acrylamide gel containing both SDS and urea (29).

^d The molecular weight of the N-linked glycosides was calculated by subtracting the molecular weight of an Endo H-treated sample from that of the untreated one.

The glucoamylase, previously purified from *S. diastaticus* strain 5106-9A carrying *STA1*, was composed of two nonidentical subunits (H and Y), and most, probably all, glycosyl moieties of these subunits were N-linked glycosides which were released with endo- β -N-acetylglucosaminidase (Endo H) (24, 26). Glucoamylases from the other *S. diastaticus* strain YIY2-12D and the *S. pombe* transformant with the plasmid pSTA1 were monomer proteins, the molecular weights of which were much larger than that of the glucoamylase from the *S. diastaticus* strain 5106-9A. This indicates that, in the *S. diastaticus* strain YIY2-12D or the *S. pombe* strain HM123, there are none of the proteolytic activities that were found to generate two subunits in the *S. diastaticus* strain 5106-9A (26). These polymorphic glucoamylases were derived from the *STA1* gene, as they were immunoprecipitated with anti-*STA1*-glucoamylase monoclonal antibodies (27) (data not shown). The glucoamylase of the *S. diastaticus* strain YIY2-12D contained about twice as much N-linked glycosides as that of the *S. diastaticus* strain 5106-9A. It was of particular

interest that glucoamylase secreted from the *S. pombe* transformant contained no detectable N-linked glycosides, although the putative precursor protein of 778 amino acids, deduced from the nucleotide sequence of *STA1*, carries 13 potential N-glycosylation sites (26). These multiple glucoamylases from *S. diastaticus* and *S. pombe* showed similar specific activities (units/ μ g protein). The extra components of glucoamylases besides the N-linked glycosides have not yet been determined.

These results indicate that the diversity of molecular structures in the yeast glucoamylases encoded by *STA1* is partly due to different proteolysis and N-linked glycosylation.

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