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Evaluation of the glucoamylase Glm from *Saccharomycopsis fibuligera* IFO 0111 in hydrolysing the corn starch

Viera Horváthová[1]*, Katarína Šlajsová[1] & Ernest Šturdík[1,2]

[1] *Department of Biotechnologies, Faculty of Natural Sciences, University of St. Cyril and Methodius in Trnava, Nám. J. Herdu 2, SK-91701 Trnava, Slovakia; phone: ++ 421 33 5565 384, fax ++421 33 5565 185, e-mail: viera.horvathova@ucm.sk*

[2] *Department of Biochemical Technology, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, SK-81237 Bratislava, Slovakia*

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The starch hydrolysis is an important step in the processes of starch technologies and treatment of starch substrates. High attention has been paid to the industrial hydrolysis of starch substrates by enzymatic preparations. In this work the glucoamylase Glm from *Saccharomycopsis fibuligera* IFO 0111 was used in an attempt to obtain starch hydrolysates. This enzyme very probably possesses the raw starch-binding site within the structure of catalytic domain allowing it to split the native (i.e. raw) starch. The main goal of the present work was to test the *S. fibuligera* IFO 0111 glucoamylase in hydrolysing the raw starches. Two substrates were used: starch milk and corn starch (initial concentration 23% w/w). The effects of enzyme dosage and thermal processing of substrates on kinetics and efficiency of hydrolysis were observed. The results suggested that the glucoamylase was sufficiently effective in hydrolysing tested raw starches and thermal modification of substrates was not necessary.

Key words: enzymatic hydrolysis, starch substrates, glucoamylase, amylolysis, corn starch degradation.

Introduction

Starch is an important material for starch industry and fermentation technologies. From a chemical view of point starch is a composition of the homopolysacharides – linear amylose and branched amylopectin. Starch is degraded mainly by amylolytic enzymes, namely by α-amylase, β-amylase, glucoamylase, pullulanase, and isoamylase (Horváthová et al., 2001; Bertoldo & Antranikian, 2002). These enzymes are produced by a wide variety of organisms (Horváthová et al., 2000; Pandey et al., 2000). More then 150 yeast strains are able to degrade starch (Mc Cann & Barnett, 1986). The strains of *Saccharomycopsis fibuligera* have been known as good producers of various amylolytic enzymes for a long time (Itoh et al., 1987a, 1987b; Hosti-

* Corresponding author

Table 1. Some properties of glucoamylases from *Saccharomycopsis fibuligera* (HOSTINOVÁ, 2002).

Enzyme	M_w [kDa]	pH_{opt}	T_{opt} [°C]	Residual activity[a] [%]	Raw starch digestion
Gla[b]	62	5–6	40–50	55	No
Glm[c]	55	5.5	40	0	Yes

[a]Residual activity after 10 min boiling. [b]Glucoamylase from *Saccharomycopsis fibuligera* KZ. [c]Glucoamylase from *Saccharomycopsis fibuligera* IFO 0111.

NOVÁ et al., 1991, 2003). However, the starch substrates have to be thermally modified before use (GAŠPERÍK et al., 1985, 1988).

The glucoamylases (EC 3.2.1.3) from *S. fibuligera* belong to important amylolytic exoenzymes. They degrade starch completely to β-D-glucose (SAUER et al., 2000). Maltose can also be hydrolyzed (NIGAM & SINGH, 1995). At higher starch concentrations glucoamylases can cause polymerization (reverse reactions) of glucose (OATES & CHRISTOPHER, 1997).

Knowledge concerning the amylolytic enzymes produced by the strains of *S. fibuligera* (GAŠPERÍK et al., 1991; HOSTINOVÁ et al., 1991; GAŠPERÍK & HOSTINOVÁ 1993; REISER & GAŠPERÍK, 1995; SOLOVICOVÁ et al., 1999) has recently been augmented by the studies on glucoamylase Glm from the strain *S. fibuligera* IFO 0111 (HOSTINOVÁ, 2002). This glucoamylase is able to split the native (i.e. raw) starch (HOSTINOVÁ et al., 2003). The other two glucoamylases from this yeast, Glu and Gla, do not exhibit this property (ITOH et al., 1987a; HOSTINOVÁ et al., 1991). The determination of the primary structure of Glm and its comparison with all available glucoamylase sequences confirmed that it belongs to the family GH-15 glycoside hydrolases (HENRISSAT, 1991). Since the size of this enzyme is comparable with the size of the catalytic domain of usual GH-15 glucoamylases, the raw-starch degradation ability has to be organised as a raw starch-binding site within the catalytic domain, i.e. not as a separate starch-binding domain (HOSTINOVÁ et al., 2003). There are two related starch-binding domains present, in addition to glucoamylases, also in α-amylases and β-amylases (SVENSSON et al., 1989; JANEČEK & ŠEVČÍK, 1999). They are positioned at either the C- or the N-terminal end of the protein and have been classified as carbohydrate-binding module families CBM-20 and CBM-21, respectively (COUTINHO & HENRISSAT, 1999). Recently, the homologues of the starch-binding domain have been identified in various non-amylolytic proteins, e.g. laforin (MINASSIAN et al., 2001) and genethonin 1 (JANEČEK, 2002). It is worth mentioning that the starch-binding domain retains its properties despite the fact whether or not it is joined to the catalytic domain (DALMIA et al., 1995; PALDI et al., 2003). The ability to degrade the raw starch without the presence of a separate starch-binding domain is known also in α-amylases, especially in those from barley (SØGAARD et al., 1993; TIBBOT et al., 2002).

Some enzymatic properties of glucoamylases Gla and Glm from the strains of *S. fibuligera* are shown in Table 1. It is obvious that the yeast *Saccharomycopsis fibuligera* (strains KZ and IFO 0111) is a good producer of glucoamylases with industrially interesting properties, such as raw starch degradation by Glm or the capability of Gla to retain the enzyme activity after boiling. The main aim of the present study was to test the hydrolytic activity of glucoamylase Glm from *Saccharomycopsis fibuligera* IFO 0111 on the native starch substrates at different conditions.

Material and methods

Substrates

Two substrates were used: A – starch milk, dry matter 46% (w/w) (AMYLUM SLOVAKIA a. s., Boleráz, Slovakia) and B – corn starch (LABETA, Staré Jesenčany, Czech republic).

Enzymes

Glucoamylase Glm from *Saccharomycopsis fibuligera* IFO 0111 (a gift from the Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava, Slovakia) in the form of a lyophilised crude enzyme preparation, specific activity of lyophilisate: 320 U/mg of protein.

For determination of glucose concentration, glucosidase as commercial set Glukosa GOD 1500 (LACHEMA, Brno, Czech Republic) was used.

Hydrolysis of native starch substrates

The suspension of substrates (A, B) with starch concentration 23% (w/w) was adjusted to pH 5.5. The concentrations of glucoamylase were 33, 50, 75 U/g of starch or 15, 300, 500, 1000 U/g of starch. The entire composition, with occasional mixing, was incubated for 10 to 15 hours at 40 °C. During the incubation the 50 μL samples were taken that were used for determination of glucose concentration.

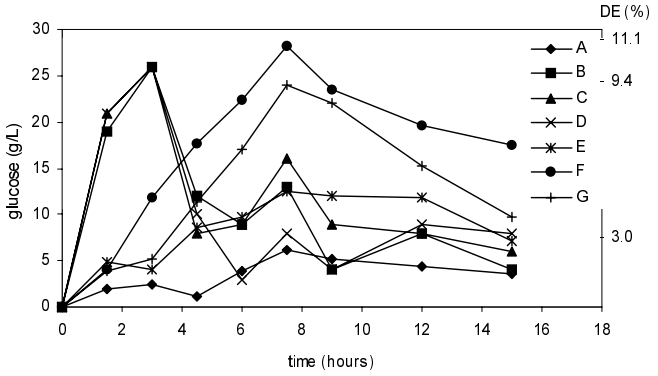

Fig. 1. The course of glucoamylase hydrolysis of native starch milk (concentration 23% w/w) at pH 5.5 and 40 °C and its efficiency. Concentration of glucoamylase (in U/g of starch): A – 15, B – 33, C – 50, D – 75, E – 300, F – 500, and G – 1000.

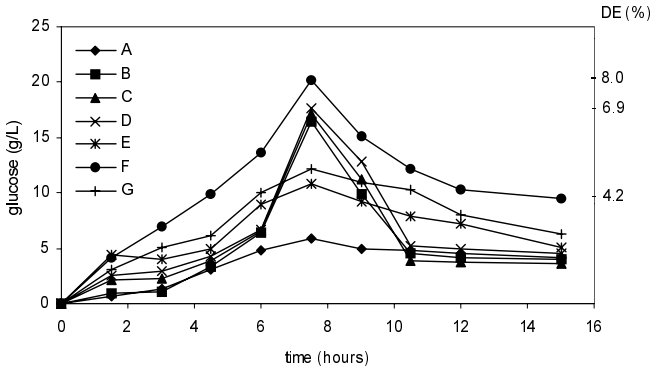

Fig. 2. The course of glucoamylase hydrolysis of native corn starch (concentration 23% w/w) at pH 5.5 and 40 °C and its efficiency. Concentration of glucoamylase (in U/g of starch): A – 15, B – 33, C – 50, D – 75, E – 300, F – 500, and G – 1000.

Hydrolysis of partially thermally processed starch substrates

The suspension of substrates (A, B) with starch concentration 23% (w/w) was preheated for 2 hours at 40 °C in a water bath. After pH adjustment (pH 5.5), properly diluted solution of the enzyme (concentrations 33, 50, 75 U/g of starch or 15, 300, 500, 1000 U/g of starch) was added. The entire composition, with occasional mixing, was incubated for 10 to 12 hours at 40 °C. During the incubation the 50 μL samples were taken that were used for determination of glucose concentration.

Dextrose equivalent computation

Dextrose equivalent (DE) is the expression (in percentages) of the degree of enzyme hydrolysis as a ratio of immediate glucose concentration and the maximum, theoretically obtainable, glucose concentration.

Analytical methods

Glucoamylase activity was determined using the modified Somogy and Nelson micro-method (Somogy, 1952). One unit of the enzyme activity is defined as the amount of enzyme releasing 1 μmol of glucose/1 min from starch under the experimental conditions.

Starch content in the starch milk was determined polarimetrically after its transformation into soluble form using hydrochloric acid (Davídek, 1981). Glucose was determined by the spectrophotometrical commercial set Glukosa GOD 1500 (LACHEMA, Brno, Czech republic).

Results and discussion

The effect of glucoamylase concentrations on hydrolysis of native starch

The starch concentration in both substrates, starch milk (substrate A) and corn starch (substrate B) was 23% (w/w). The courses of hydrolysis for starch milk and corn starch are shown in Figure 1 and Figure 2, respectively. The hydrolysis was monitored by glucose increase and the efficiency was expressed as DE.

In the first series of experiments we choose dosing of glucoamylase 15, 300, 500 and 1000 U/g of starch. At twenty-fold increase of enzyme (e.g., from 15 to 300 U/g of starch) the amount of glucose increased only twice using both substrates: from 6.1 to 12.5 g/L for A and from 5.9 to 10.8 g/L for B. We found out that neither the rate nor the efficiency of the hydrolysis of starch was increased

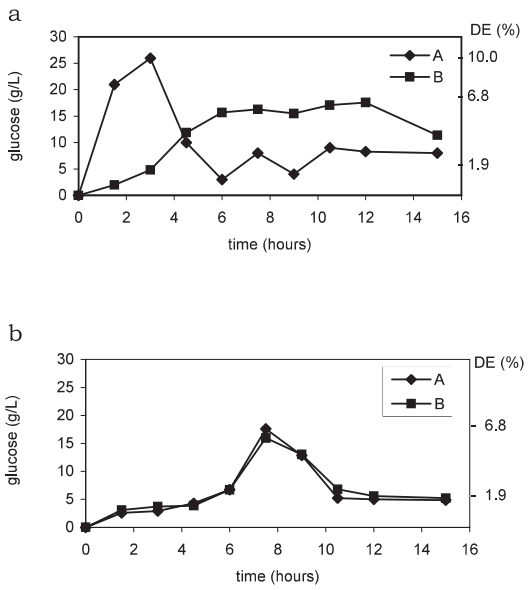

Fig. 3. The course of glucoamylase hydrolysis and its efficiency of the starch milk (**a**) and corn starch (**b**). A – native and B – thermally modified (2 hours at 40 °C) substrate. Concentration of glucoamylase: 75 U/g of starch, concentration of starch: 23% w/w (pH 5.5, 40 °C).

with high dosage of the enzyme. In addition, high dosage of the enzyme can cause reverse reaction of glucose (HOSTINOVÁ et al., 1991). Since no direct proportional relationship was observed concerning the amounts of used enzyme and produced glucose (Figs 1,2), we therefore decreased the dosage of the enzyme in further experiments (up to the level 100 U/g of starch with rising coefficient 1.5). Hydrolysis was more efficient using the substrate A (native starch milk), the maximum increase of glucose being reached already in the 3rd hour of hydrolysis (Fig. 1). Hydrolysis of native corn starch exhibits different behaviour. Maximum DE was lower and maximum increase of glucose was observed in the 7th hour of the process (Fig. 2). The courses of hydrolysis with dosage of glucoamylase 33, 50 and 75 U/g of starch were similar at both substrates.

Influence of thermal modification of substrates on starch hydrolysis

In these experiments we wanted to verify the influence of thermal pre-processing of the substrates on the rate of the hydrolysis and glucose yield. Time of the thermal processing was 30 min, 1 hour and 2 hours (temperature 40 °C). Higher temperatures were not considered due to creating compact gels of starch at these concentrations.

The results are presented in Figure 3. It is obvious that thermal modification of tested substrates was not necessary, because glucoamylase was sufficiently effective to hydrolyse the native substrates. Using the substrate A, the glucoamylase was even more effective in hydrolysing the native starch milk than thermally modified starch milk (Fig. 3A). Concerning the substrate B, the courses of hydrolysis are similar whether or not the corn starch was used as native or thermally adapted (Fig. 3B).

Conclusions

The glucoamylase Glm from *S. fibuligera* IFO 0111 used in the present study represents an exceptional glucoamylase able to degrade the raw starch although it does not contain a separate starch-binding domain (HOSTINOVÁ et al., 2003). The obtained results clearly demonstrate that the glucoamylase was effective in hydrolyzing the native starch substrates (starch milk and corn starch). It was found that the course of hydrolysis depends on type of the substrate and on a dosage of the enzyme. In the hydrolysis of corn starch, the maximum increase of glucose was observed later than in the hydrolysis of the starch milk. Optimum concentration of glucoamylase was determined to be 33–75 U/g of starch at given experimental conditions. The results suggested that thermal modification of the starch substrates (2 hours at 40 °C), when using this glucoamylase, is not necessary.

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References

BERTOLDO, C. & ANTRANIKIAN, G. 2002. Starch-hydrolyzing enzymes from thermophilic archaea and bacteria. Curr. Opin. Chem. Biol. **6:** 151–160.

COUTINHO, P. M. & HENRISSAT, B. 1999. The modular structure of cellulases and other carbohydrate-active enzymes: an integrated database approach, pp. 15–23. In: OHMIYA, K., HAYASHI, K., SAKKA, K., KOBAYASHI, Y., KARITA, S. & KIMURA, T. (eds) Genetics, Biochemistry and Ecology of Cellulose Degradation, Uni Publishers Co., Tokyo.

Dalmia, B. K., Schütte, K. & Nikolov, Z. L. 1995. Domain E of *Bacillus macerans* cyclodextrin glucanotransferase: an independent starch-binding domain. Biotechnol. Bioeng. **47:** 575–584.

Davídek, J. 1981. Laboratorní příručka analýzy potravin. SNTL, Praha, 720 pp.

Gašperík, J. & Hostinová, E. 1993. Glucoamylases encoded by variant *Saccharomycopsis fibuligera* genes – structure and properties. Curr. Microbiol. **27:** 11–14.

Gašperík, J., Hostinová, E. & Zelinka, J. 1985. Production of extracellular amylase by *Endomycopsis fibuligera* on complex starch substrates. Biologia, Bratislava **40:** 1167–1174.

Gašperík, J., Hostinová, E., Mináriková, O., Soldánová, I. & Zelinka, J. 1988. Amylolytic enzymes produced by *Saccharomycopsis fibuligera*. Biologia, Bratislava **43:** 673–679.

Gašperík, J., Kováč, Ľ., Mináriková, O. 1991. Purification and characterization of the amylolytic enzymes of *Saccharomycopsis fibuligera*. Int. J. Biochem. **23:** 21–25.

Henrissat, B. 1991. A classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem. J. **280:** 309–316.

Horváthová, V., Janeček, Š. & Šturdík, E. 2000. Amylolytic enzymes: their specificities, origins and properties. Biologia, Bratislava **55:** 603–613.

Horváthová, V., Janeček, Š. & Šturdík, E. 2001. Amylolytic enzymes: Molecular aspects of their properties. Gen. Physiol. Biophys. **20:** 7–32.

Hostinová, E. 2002. Amylolytic enzymes produced by the yeast *Saccharomycopsis fibuligera*. Biologia, Bratislava **57/Suppl.11:** 247–251.

Hostinová, E., Balánová, J. & Gašperík, J. 1991. The nucleotide sequence of the glucoamylase gene GLA1 from Saccharomycopsis fibuligera KZ. FEMS Microbiol. Lett. **83:** 103–108.

Hostinová, E., Solovicová, A., Dvorský, R., Gašperík, J. 2003. Molecular cloning and 3D structure prediction of the first raw-starch-degrading glucoamylase without a separate starch binding domain. Arch. Biochem. Biophys. **411:** 189–195.

Itoh, T., Ohtsuki, I., Yamashita, I. & Fukui, S. 1987a. Nucleotide sequence of the glucoamylase gene GLU1 in the yeast *Saccharomycopsis fibuligera*. J. Bacteriol. **169:** 4171–4176.

Itoh, T., Yamashita, I. & Fukui, S. 1987b. Nucleotide sequence of the α-amylase gene (ALP1) in the yeast *Saccharomycopsis fibuligera*. FEBS Lett. **219:** 339–342.

Janeček, Š. 2002. A motif of a microbial starch-binding domain found in human genethonin. Bioinformatics **18:** 1534–1537.

Janeček, Š. & Ševčík, J. 1999. The evolution of starch-binding domain. FEBS Lett. **456:** 119–125.

McCann, A. K. & Barnett. J. A. 1986**.** The utilization of starch by yeasts. Yeast **2:** 109–115.

Minassian, B. A., Ianzano, L., Meloche, M., Andermann, E., Rouleau, G. A., Delgado-Escueta, A. V. & Scherer, S. W. 2000. Mutation spectrum and predicted function of laforin in Lafora's progressive myoclonus epilepsy. Neurology **55:** 341–346.

Nigam, P. & Singh, D. 1995. Enzyme and microbial systems involved in starch processing. Enzyme Microb. Technol. **17:** 770–778.

Oates, Z. & Christopher, G. 1997. Towards an understading of starch granule structure and hydrolysis. Trends Food Sci. Technol. **8:** 375–382.

Paldi, T., Levy, I. & Shoseyov, O. 2003. Glucoamylase starch-binding domain of Aspergillus niger B1: molecular cloning and functional characterization. Biochem. J. **372:** 905–910.

Pandey, A., Nigam, P., Soccol, C. R., Soccol, V. T., Singh, D. & Mohan, R. 2000. Advances in microbial amylases. Biotechnol. Appl. Biochem. **31:** 135–152.

Reiser, V., Gašperík, J. 1995. Purification and characterization of the cell-wall-associated and extracellular α-glucosidases from*Saccharomycopsis fibuligera.* Biochem. J.**308:** 753–760.

Sauer, J., Sigurskjold, B. W., Christensen, U., Frandsen, T. P., Mirgorodskaya, E., Harrison, M., Roepstorff, P. & Svensson, B. 2000. Glucoamylase: structure/function relationships, and protein engineering. Biochim. Biophys. Acta **1543:** 275–293.

Solovicová, A., Christensen, T., Hostinová, E., Gašperík, J., Ševčík, J., Svensson, B. 1999. Structure-funcition relationships in glucoamylases encoded by variant *Saccharomycopsis fibuligera* genes. Eur. J. Biochem. **264:** 756–764.

Somogy, M. 1952. Notes on sugar determination. J. Biol. Chem. **195:** 19–22.

Søgaard, M., Kadziola, A., Haser, R. & Svensson, B. 1993. Site-directed mutagenesis of histidine 93, aspartic acid 180, glutamic acid 205, histidine 290, and aspartic acid 291 at the active site and tryptophan 279 at the raw starch binding site in barley α-amylase 1. J. Biol. Chem. **268:** 22480–22484.

Svensson, B., Jespersen, H., Sierks, M. R. & MacGregor, E. A. 1989. Sequence homology between putative raw-starch binding domains from different starch-degrading enzymes. Biochem. J. **264:** 309–311.

Tibbot, B. K., Wong, D. W. S. & Robertson, G. H. 2002. Studies on the C-terminal region of barley α-amylase 1 with emphasis on raw starch-binding. Biologia, Bratislava **57/Suppl. 11:** 229–238.

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