Oligosaccharide Synthesis using α-Glucosidases of Different Origin

ŠÁRKA MALÁ, PETRA KARASOVÁ, MICHAELA MARKOVÁ and BLANKA KRÁLOVÁ

Institute of Chemical Technology – Department of Biochemistry and Microbiology, Prague, Czech Republic

Abstract

MALÁ Š., KARASOVÁ P., MARKOVÁ M., KRÁLOVÁ B. (2001): **Oligosaccharide synthesis using α-glucosidases of different origin**. Czech J. Food Sci., **19**: 57–61.

α-Glucosidase from *Aspergillus awamori* and intestinal α-glucosidase (saccharase-isomaltase complex) exhibited high transglycosylation activity and were able to synthesize tri- and tetrasaccharides during maltose hydrolysis. Both tested enzymes were also able to transfer the glucose residue to all tested monosaccharide acceptors (D-mannose, D-xylose, L-sorbose and D-galactose). Their transfer activity towards respective acceptors varied and their acceptor preference also depended on the origin of the enzyme. Out of the acceptors tested, both enzymes exhibited high transfer activity in xylose.

Keywords: α-glucosidase; transglycosylation; synthesis of glucooligosaccharides

Oligosaccharides are relatively new functional food ingredients. They have the potential of improving the quality of numerous foods and provide useful modification to food flavour and physiochemical properties. Many of them also possess properties beneficial to consumers' health (TOMOMATSU 1994).

Oligosaccharides provide several benefits that make their use as food ingredients particularly attractive. They are water soluble and mildly sweet, their higher molecular weight results in increased viscosity. They can also be used to alter the freezing point and to control the browning in heat-processed foods caused by Maillard reactions. They prevent excessive drying. Currently available food-grade oligosaccharides are not utilized by mouth microflora to form acids or polyglucans and humans do not digest many of these. This property makes them suitable for use in sweet, low-calorie diet foods (GIBSON *et al.* 1994). Many oligosaccharides are able to stimulate the growth of beneficial microflora in the colon, but the effective dose varies among different oligosaccharide types (CRITTENDEN & PLAYNE 1996).

Oligosaccharides find their major use in beverages, but they are also added to probiotic yoghurts and yoghurt drinks (galacto- and fructooligosaccharides – HIDAKA *et al.* 1991). Oligosaccharides are also widely used in confectionery. Other current applications include desserts such as jellies and ice creams, bakery products including pastries, biscuits and breads, spreads as jams and marmalades, and infant milk formulas. Besides that, oligosaccharides are used in livestock industry. Some non-food applications also have been proposed, including drug delivery, cosmetics and mouthwashes (PLAYNE & CRITTENDEN 1996).

Besides the chemical synthesis of oligosaccharides and catalysis by glycosyltransferases, there is another frequently used method – the use of glycosidases (NILSSON 1988). Most, if not all, glycosidases can transfer the glycosyl moiety of a substrate to acceptors other than water and hydrolysis merely represents a special case where water serves as the acceptor. Glycosidases occur widely in most organisms and are thus easily available. Another advantage is that they do not need cofactors, use simple, easily available substrates and can use a number of different acceptors. Immobilized enzymes allow reuse in large-scale synthesis (MONSAN & PAUL 1995).

The present paper deals with the possibility of oligosaccharide synthesis by transglycosylation reaction of α-glucosidase from *Aspergillus awamori* and sucrase-isomaltase complex from pig small intestine. Sucrase-isomaltase complex is responsible for α-glucosidase activity in animal

---

This work was supported in part by the internal grant of ICTP and the Research Grant of Ministry of Education, Youth and Sports.

organisms. We studied their transglycosylation activity in maltose solution and the ability of α-glucosidases to transfer glucose to other saccharide acceptors.

MATERIAL AND METHODS

Materials: Saccharides and *p*-nitrophenyl-α-D-glucopyranoside (PNPG) were purchased from Sigma (USA). α-Glucosidase from *Aspergillus awamori* was a kind gift by Dr. E. Ralyse (Fermentas, Latvia) and intestinal α-glucosidase (saccharase-isomaltase complex) was prepared as described by Kolínska (KOLÍNSKÁ & SEMENZA 1967).

Enzyme Assay: The α-glucosidase activity was measured using maltose as a substrate. The enzyme was incubated at 37°C with 4% (w/w) maltose solution in 0.1M sodium phosphate buffer pH 6.8 for intestinal α-glucosidase and in 0.1M acetate buffer in the case of α-glucosidase from *Aspergillus awamori*. The reaction was terminated by adding 10% (w/v) Na_2CO_3 and the liberated glucose was assayed by the glucoseoxidase-peroxidase method using the Oxochrom Test (Lachema,CR). One unit of activity was defined as the amount of the enzyme that produces 1 mmol of glucose per 1 min at pH 6.8 (4.2) and 37°C.

Transglycosylation Reaction: Experiments on the transglycosylation activity of α-glucosidase were carried out under the following conditions: 0.1M acetate buffer pH 4.2, 50°C for α-glucosidase from *Aspergillus awamori* and 0.1M sodium phosphate buffer pH 6.8, 37°C for intestinal α-glucosidase. The enzyme (0.5 U/ml) was incubated with 1 ml of maltose solution of various concentrations (15, 30, and 50% w/w) or with maltose (15% w/w) as glycosyl donor and other monosaccharide (15% w/w) as acceptor. Samples (30 µl) were taken at various time intervals from the reaction mixture and 30 µl of the internal standard was added. The mixture was heated for 3 min at 100°C to denature the enzyme. After filtration, 20 µl aliquots were analysed by HPLC.

High Performance Liquid Chromatography (HPLC): Oligosaccharide synthesis was monitored by HPLC (Waters, USA). All reaction products were identified and quantified on Supelcogel Ca column with elution by deionised water at a flow rate of 0.5 ml/min at 80°C. Elution was monitored by a differential refractometer. Retention times of analysed saccharides: tetrasaccharide 8 min, trisaccharide 9.4 min, maltose 10.5 min, glucose 12.9 min, xylose 23.7 min, sorbose 14.8 min, mannose 14.4 min, galactose 14.2 min. Comparing their retention times with standards identified the saccharides analysed. Product quantification was based on the comparison of peak areas with those of standard sugars and galactose as internal standard using calibration curves.

RESULTS

Transglycosylation Reaction: Transglycosylation activity of α-glucosidase from *Aspergillus awamori* and sucrase-isomaltase complex was studied in 15% (w/w) maltose solution. The results are shown in Fig. 1. Both enzymes were able to synthesize higher oligosaccharides (trisaccharides and tetrasaccharides) during maltose hydrolysis. However, the reaction courses of transglycosylation differed from each other. The enzyme from *Aspergillus awamori* exhibited higher total transfer activity and trisaccharides were rapidly synthesized up to the maximum value of 116 mM (yield about 24% on a molar basis). Tetrasaccharides were still synthesized at that time up to the concentration of 39 mM. Transglycosylation activity of intestinal α-glucosidase was lower and the enzyme was able to form trisaccharides up to the maximum of 72 mM and tetrasaccharides up to 42 mM. In both cases tri- and tetrasaccharides started to disappear from the reaction mixture after reaching their maximum, as they could be subjected to secondary hydrolysis. Next to the above mentioned different transglycosylation activity of both enzymes, difference in the course of tetrasaccharide syn-

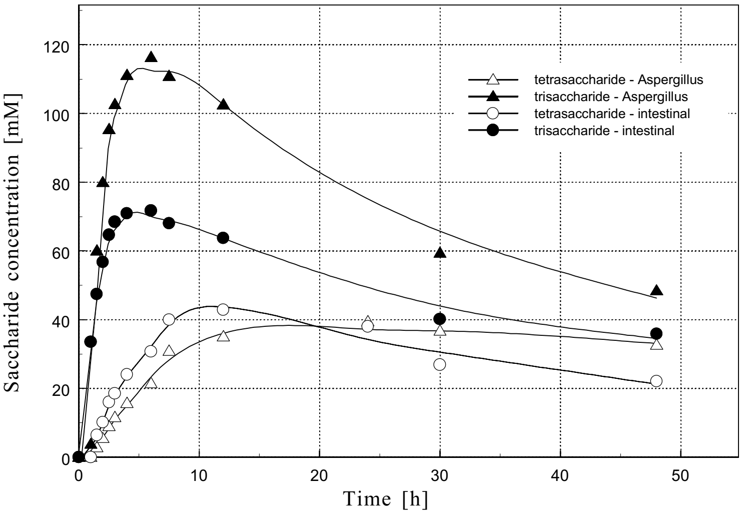

Fig. 1. Time course of trisaccharide and tetrasaccharide synthesis during reaction in 15% maltose catalyzed by α-glucosidase from *Aspergillus awamori* and sucrase-isomaltase complex

thesis can be found between them. In intestinal enzyme their synthesis starts at much lower trisaccharide concentration but their maximum concentration reached is comparable to that formed by the enzyme from *Aspergillus awamori*.

Transglycosylation activity was further examined as a function of maltose concentration. Maltose concentration had the same effect on both enzymes: the increase of maltose concentration in the reaction mixture caused an increase of maximum trisacharide and tetrasaccharide concentrations. The highest tri- and tetrasaccharide concentrations were obtained using the highest maltose concentration tested (50%): trisaccharide, 304 mM and tetrasaccharide, 125 mM by the intestinal enzyme; and trisaccharide, 408 mM and tetrasaccharide, 66 mM by α-glucosidase from *Aspergillus awamori*. Consequently, the time needed for reaching the above-mentioned maximum concentrations increased, too. In these experiments we observed differences in the course of tetrasaccharide synthesis among the enzymes tested, similar to the reaction in 15% maltose.

Transglycosylation Reaction with Different Acceptors: Substrate glycosylation is one of the possible pathways of transglycosylation reaction. Here a glucose residue from the donor is transferred to a substrate molecule (acceptor). D-Mannose, D-xylose, L-sorbose and D-galactose were employed as acceptors and the capability of both enzymes to transfer glucose from maltose to these acceptors was evaluated. As some of transglycosylation products (disaccharides) were not completely separated from maltose, the course of transglycosylation reaction was determined on the basis of the decrease of acceptor saccharide concentration in the reaction mixture by HPLC. The results are shown in Figs. 2 and 3.

These data indicate that both tested enzymes were able to transfer the glucose residue to all tested monosaccharide acceptors and their transfer activity toward respective acceptors varied. From the view of tested acceptors

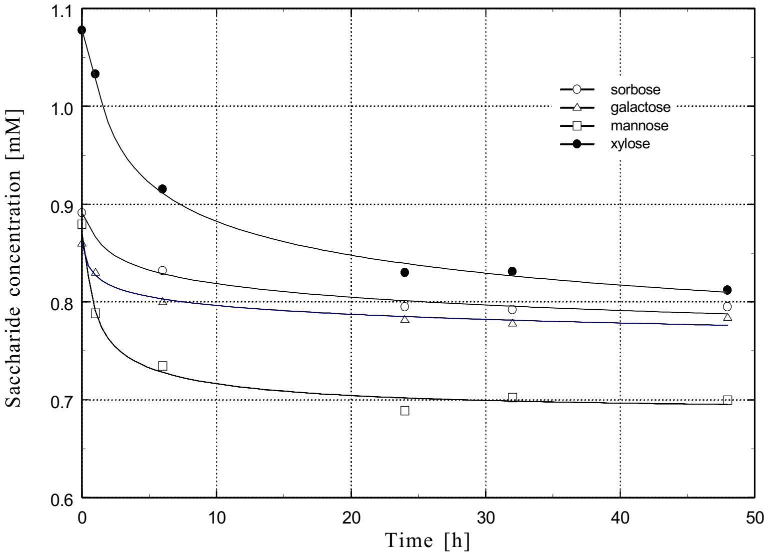

Fig. 2. Time course of consumption of saccharide acceptors from reaction mixture during transgly-cosylation reaction in presence of intestinal α-glucosidase

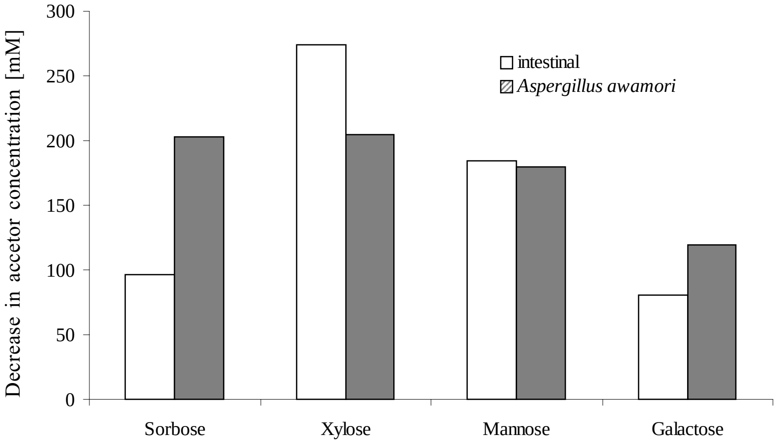

Fig. 3. Decrease of acceptor concentration in reaction mixture observed after 48 h of trans-glycosylation reaction in mixture of maltose and saccharide acceptor catalyzed by intestinal α-glucosidase and α-glucosidase from *Aspergillus awamori*

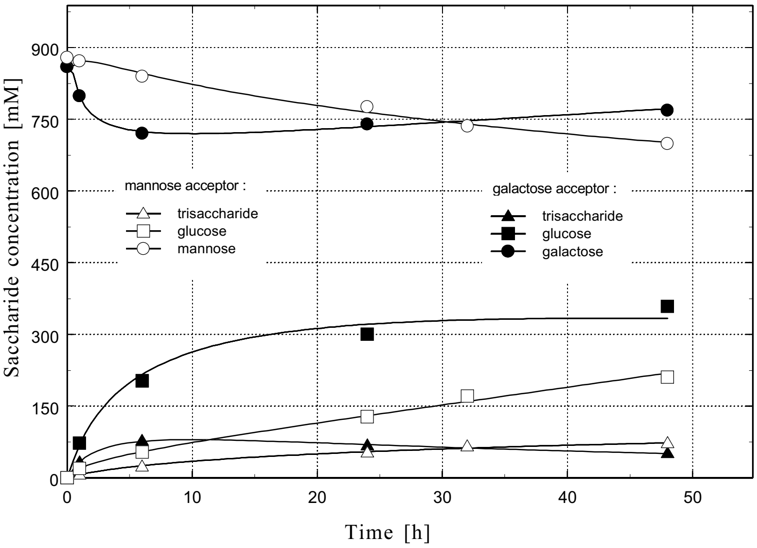

Fig. 4. Effect of presence of two different acceptors (galactose and mannose) on transglycosylation reaction catalyzed by α-glucosidase from *Aspergillus awamori*

both enzymes exhibited high transfer activity for xylose. The reason for this high activity of tested α-glucosidases in xylose could be attributed to the structure of the acceptor in a solution where the cyclic form prevails. Glucose has the same configuration at carbon atoms 1, 2, 3 and 4; unlike in the other acceptors tested no steric interference can therefore be expected during transfer. However, the order of preference of the other acceptors was dependent on enzyme origin. In intestinal α-glucosidase, mannose ranked as the second acceptor next to xylose in concentration decrease (yield approximating 21% on molar basis), followed by sorbose and galactose (yield near 11% and 9% respectively). In α-glucosidase from *Aspergillus awamori* the preferences of the tested acceptors are much less pronounced than in the intestinal enzyme.

The presence of saccharide acceptors in the reaction mixture also had other effects on the transglycosylation reaction. The comparison of the reaction time course with the acceptor present or absent reveals that the quantity of synthesized oligosaccharides and released glucose are mainly affected. In the presence of any tested acceptor lower amounts of synthesized trisaccharides and released glucose were determined and no synthesis of tetrasaccharides was observed. However, the quantity of synthesised oligosaccharides and released glucose also depended on acceptor structure. The effect of the presence of two acceptors (galactose and mannose) on the transglycosylation reaction catalyzed by α-glucosidase from *Aspergillus awamori* is shown in Fig. 4.

As can be seen from Fig. 4, the presence of mannose instead of galactose resulted in slower release of glucose during the reaction, along with a slower decrease in acceptor concentration. Due to this slow reaction rate a higher yield could be obtained if the reaction time with mannose acceptor were prolonged.

DISCUSSION

α-Glucosidases from various microorganisms can perform not only hydrolysis but also transglucosylation to D-glucosyldonor substrates and acceptor substrates. The transglycosylation activity in maltose solution was tested in α-glucosidase from *Aspergillus awamori* and sucrase-isomaltase complex. These enzymes belong to α-glucosidases that exhibit high activity toward the easily available substrate, maltose (RODRIGUEZ *et al.* 1984) and would therefore be suitable for glucooligosaccharide synthesis. Our experiments demonstrated the ability of α-glucosidase from *Aspergillus awamori* and sucrase-isomaltase complex to catalyse the synthesis of tri- and tetrasaccharides. Relatively high transglycosylation activity of both enzymes is probably derived from their hydrolytic substrate specificity. Our previous experiments (MALÁ *et al.* 1999) indicated that an α-glucosidase having high ability to hydrolyze tri- and tetrasaccharides most probably also has high ability to synthesize these, as the binding conditions for enzyme-substrate complex will be identical in both ways. This conclusion is also in good accordance with results obtained for α-glucosidase from *Aspergillus awamori* and sucrase-isomaltase complex and low transglycosylation activity found in yeast α-glucosidases (MALÁ *et al.* 1999; KELLY & FOGARTY 1983). However, there are still differences between enzymes with higher transglycosylation activity. For example, α-glucosidase from *Aspergillus carbonarius* (DUAN *et al.* 1994), which can hydrolyse higher oligosaccharides, was only able to synthesise trisaccharides during maltose hydrolysis.

The ability of α-glucosidase from *Aspergillus awamori* and sucrase-isomaltase complex to synthesise heterooligosaccharide was also examined. Both enzymes were able to transfer the glucose residue to all tested monosaccharide acceptors (D-mannose, D-xylose, L-sorbose and D-galactose). The reason could consist in their broad substrate specificity towards different linkage position of the enzymes tested, as was observed in previous experiments (MALÁ *et al.* 1999). *Bacillus stearothermophilus* α-glucosidase, which is highly specific towards α(1→4)-linkage, did not transfer the glucosyl residue to the same tested acceptors with the exception of structurally similar xylose, unlike α-glucosidase from brewer's yeast.

CONCLUSIONS

α-Glucosidase from *Aspergillus awamori* and the sucrase-isomaltase complex were able to synthesize higher oligosaccharides (trisaccharides and tetrasaccharides) during maltose hydrolysis. However, the reaction courses of transglycosylation were different. In 15% maltose solution transglycosylation activity of intestinal α-glucosidase was lower and the enzyme was able to form trisaccharides up to the maximum of 72 mM and tetrasaccharides up to 42 mM. The enzyme from *Aspergillus awamori* exhibited higher total transfer activity and trisaccharides were rapidly synthesized up to the maximum value of 116 mM and tetrasaccharides up to 39 mM.

The transfer activity of α-glucosidase from *Aspergillus awamori* and sucrase-isomaltase complex varied with respective acceptors tested. The order of preference of the other acceptors was dependent on enzyme origin.

References

CRITTENDEN R.G., PLAYNE M.J. (1996): Production, properties and applications of food-grade oligosaccharides. Trends Food Sci.Technol., **7**: 353–361.

DUAN K.J., SHEU D.CH., LIN M.T., HSUEH H.CH. (1994): Reaction mechanism of isomaltooligosaccharides synthesis by α-glucosidase from *Aspergillus carbonarius*. Biotech. Let., **16**: 1151–1156.

GIBSON G.R., WILLIS C.L., VAN LOO J. (1994): Non-digestible oligosaccharides and bifidobacteria – implications for health. Int. Sugar J., **96**: 381–387.

HIDAKA H., HIRAYAMA M., YAMADA K. (1991): Fructooligosaccharides: Enzymatic preparation and biofunctions. J. Carbohydr. Chem., **10**: 509–522.

KELLY C.T., FOGARTY W.M. (1983): Microbial α-glucosidases. Process Biochem., **6**: 6–12.

KOLÍNSKÁ J., SEMENZA G. (1967): Studies on intestinal sucrase and on intestinal sugar transport. V. Isolation and properties of sucrase-isomaltase from rabbit small intestine. Biochim. Biophys. Acta, **146**: 181–195.

MALÁ Š., DVOŘÁKOVÁ H., HRABAL R., KRÁLOVÁ B. (1999): Towards regioselective synthesis of oligosaccharides by use of α-glucosidases with different substrate specificity. Carbohyd. Res., **322**: 209–218.

MONSAN P., PAUL F. (1995): Enzymatic synthesis of oligosaccharides. FEMS Microbiol. Rev., **16**: 187–192.

NILSSON K.G.I. (1988): Enzymatic synthesis of oligosaccharides. Trends Biotechnol., **6**: 256–264.

PLAYNE M. J., CRITTENDEN R. (1996): Commercially available oligosaccharides. Bull. Int. Dairy Fed., **313**: 10–22.

RODRIGUEZ I.R., TARAVEL F., WHELAN W.J. (1984): Characterization and function of pig intestinal sucrase-isomaltase and its separate subunits. Eur. J. Biochem., **143**: 575–582.

TOMOMATSU H. (1994): Health effects of oligosaccharides. Food Technol., **48**: 61–65.

Received for publication November 21, 2000
Accepted for publication February 20, 2001

Souhrn

MALÁ Š.,KARASOVÁ P., MARKOVÁ M., KRÁLOVÁ B. (2001): **Syntéza oligosacharidů použitím α-glukosidas různého původu**. Czech J. Food Sci., **19**: 57–61.

α-Glukosidasy z *Aspergillus awamori* a prasečích střev (sacharaso-isomaltasový komplex) vykazovaly vysokou transglykosylační aktivitu a byly schopné syntetizovat tri- a tetrasacharidy v průběhu hydrolýzy maltosy. Oba testované enzymy byly schopny přenášet glukosylový zbytek na všechny testované monosacharidové akceptory (D-mannosu, D-xylosu, L-sorbosu a D-galaktosu). Jejich transferasová aktivita k jednotlivým akceptorům se lišila a pořadí jejich preference záviselo i na původu enzymu. Z použitých akceptorů obě α-glukosidasy vykazovaly nejvyšší transferasovou aktivitu vůči xylose.

Klíčová slova: α-glukosidasa; transglykosylace; syntéza glukooligosacharidů

Corresponding autor:

Ing. ŠÁRKA MALÁ, Vysoká škola chemicko-technologická, Ústav biochemie a mikrokrobiologie, Technická 5, 166 28 Praha 6, Česká republika, tel.: + 420 2 24 35 30 28, fax: + 420 2 24 35 51 67, e-mail: sarka.mala@vscht.cz