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Carbohydrate Research 326 (2000) 264–276

CARBOHYDRATE RESEARCH

Hydrolysis of low-molecular-weight oligosaccharides and oligosaccharide alditols by pig intestinal sucrase/isomaltase and glucosidase/maltase

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Received 21 November 1998; received in revised form 27 October 1999; accepted 20 December 1999

Abstract

The ability of purified pig intestinal sucrase/isomaltase (SI; EC 3.2.1.10/48) and glucosidase/maltase (GM; EC 3.2.1.20) to hydrolyze di- and oligosaccharides consisting of D-glucose and D-fructose residues and the corresponding alditols was studied. The products, after incubation, reflect different binding patterns at both catalytic sites of SI. The active site of the sucrase subunit cleaves α,β-(1→2) glycosidic bonds, and only two monomer units of the substrates bind with favorable affinity. Oligosaccharides and reduced oligosaccharides containing α-(1→6) and α-(1→1) glycosidic bonds are hydrolyzed by isomaltase, and for the active site of this subunit more than two subsites were postulated. Moreover, different binding sites for various aglycons seem to exist for isomaltase. Oligosaccharide alcohols are cleaved at lower rates if the reduced sugar residue occupies the aglycon binding site. GM also hydrolyzes α-(1→1) linkages, but at a lower rate. The enzyme has the ability to bind compounds containing residues other than D-glucose. There are indications for similarities between GM and the isomaltase subunit of SI in the binding mode of oligosaccharides.

Keywords: Pig intestinal sucrase; Isomaltase; Glucosidase; Maltase; Affinity to monomeric units of substrates; Characteristic of active sites; Enzyme specificity

1. Introduction

Sucrase/isomaltase (SI; EC 3.2.1.10/48) and glucosidase/maltase (GM; EC 3.2.1.20) belong to the intestinal brush border glycoside hydrolases and are responsible for the final steps of carbohydrate digestion, leading to absorbable monosaccharides. Both enzyme complexes consist of two noncovalently linked subunits, each harboring one active site. SI is an exohydrolase that acts at the nonreducing end of oligosaccharides specifically cleaving α,β-(1→2), α-(1→4) and α-(1→6) bonds, and GM exclusively cleaves α-(1→4) glycosidic bonds.

Corresponding to the substrate specificity of their catalytic sites, the subunits of SI are called sucrase and isomaltase. In humans [1,2], rats [3] and rabbits [4], both subunits also show maltase activity. In monkeys, this activity is restricted to the isomaltase subunit [5], and in pigs to the sucrase subunit [6,7]. At both subunits, the binding site for the glycon moiety only accepts pyranoside structures,

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0008-6215/00/$ - see front matter
PII: S0008-6215(00)00008-2

whereas the aglycon component can be a furanosyl as well as a pyranosyl ring. Different kinds of aglycons, however, occupy individual binding sites [7].

In contrast to SI, the two active sites in GM cannot be distinguished with respect to substrate specificity [8]: both cleave α-(1→4) glycosidic bonds and hydrolyze maltooligosaccharides up to maltoheptaose. As described for glucoamylases of different origins [9–12], the active sites of GM are divided into a series of binding sites [2,5,13], called subsites, which interact with the monomer units of the substrates. According to Heymann [7], the subsites of the active sites of GM have no affinity for furanoside structures.

The purpose of this work was to gain new information about the specificity of the active sites of SI and GM for certain glycosidic bonds and the affinity for the different monomeric parts of the substrates. This subject is of importance as there are not many definitive reports on the specificity of these enzymes on oligosaccharides and their alditols. Therefore, the hydrolysis of selected oligosaccharides, consisting of D-glucose and D-fructose residues linked via α-(1→1), α-(1→6) or α,β-(1→2) glycosidic bonds (Fig. 1(A)) and of their corresponding sugar alco-

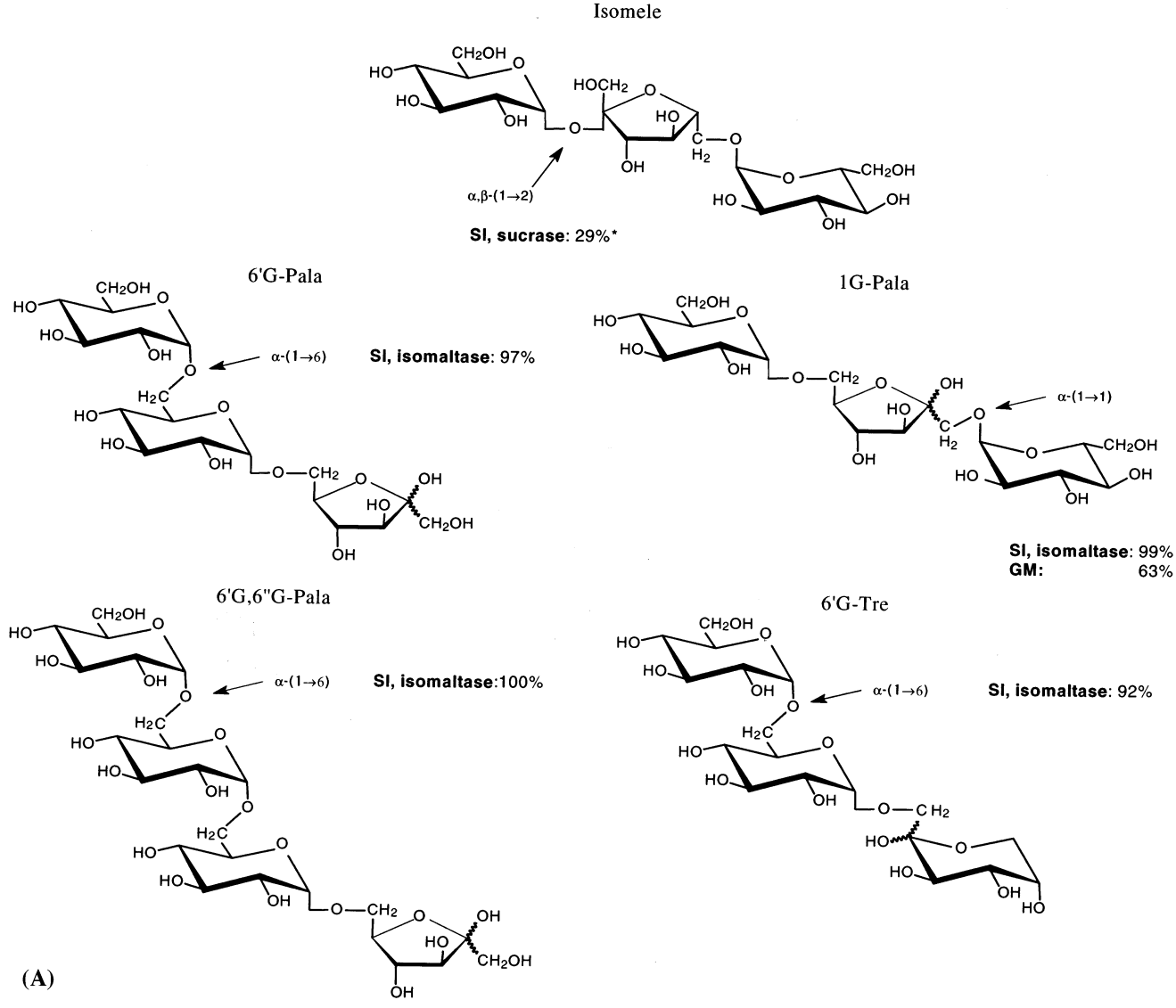


Fig. 1. Structures of (A) oligosaccharides and (B) reduced di- and oligosaccharides (for abbreviations see Table 1), positions of hydrolysis (indicated by arrows), and rate of cleavage by SI and GM, respectively, after 24 h of incubation (for the hydrolysis of the oligosaccharide alcohols by SI, the cleavage rate expresses the sum hydrolysis of Glc-ol and Man components).

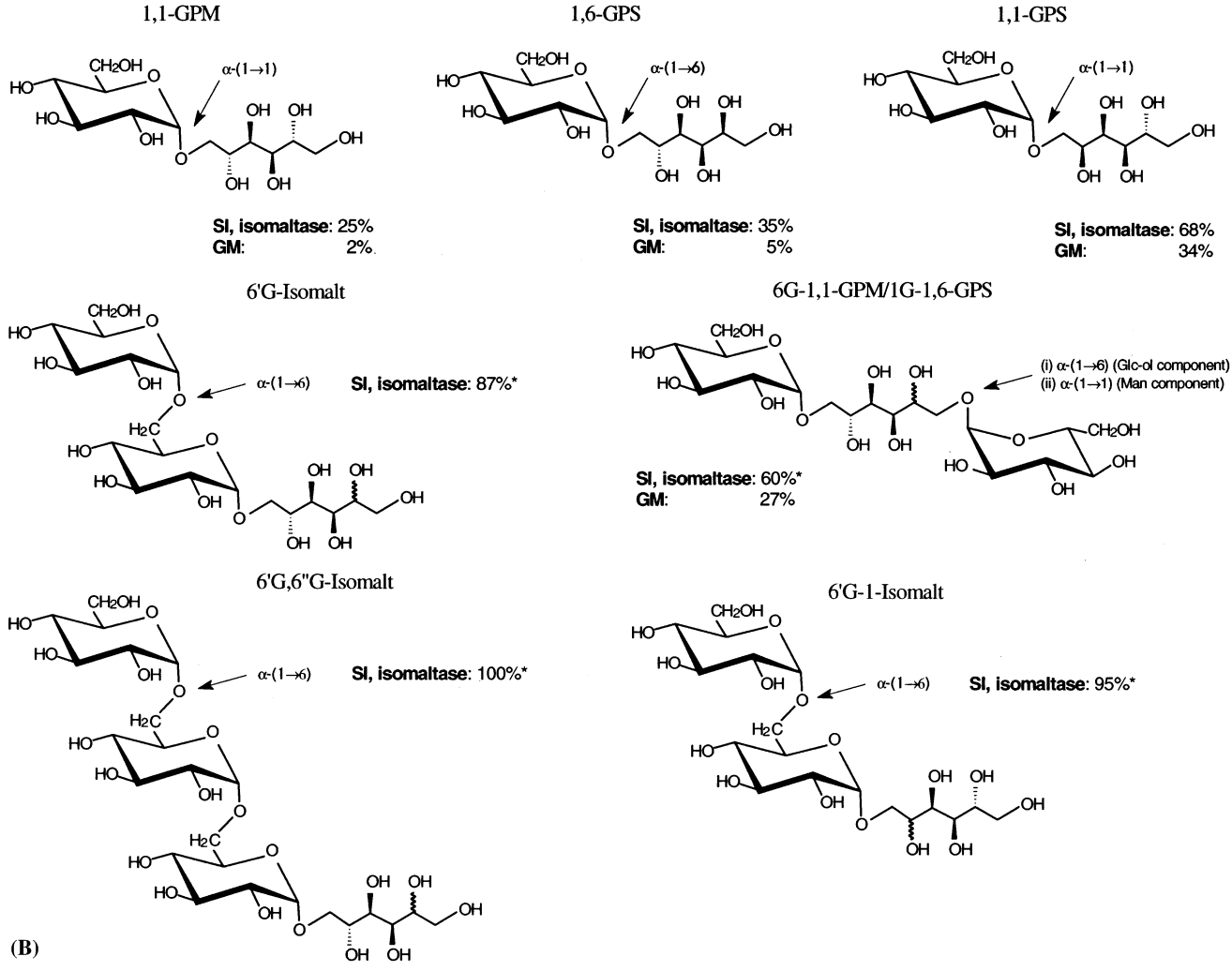


Fig. 1. (*Continued*)

hols (Fig. 1(B)), by the isolated intestinal α-glucosidases was determined and compared with the cleavage of maltose.

2. Results

The amount of enzyme in the incubation assay was chosen to give a maltase activity of 0.7 U/mL for SI and GM, leading to 90 and 95% hydrolysis of maltose, respectively, after 2 h of incubation (Fig. 2).

An overview of the percentage rates of hydrolysis for the oligosaccharides, reduced disaccharides and reduced oligosaccharides, expressed by the decrease of the substrates (for abbreviations see Table 1), is shown in Figs. 2 and 3. SI had the capability of hydrolyzing all of the sugars and reduced sugars tested, whereas GM only cleaved 1G-Pala, 6G-1,1-GPM/1G-1,6-GPS and 1,1-GPS. In spite of the maximum cleavages of 62, 27 and 35%, the rates of hydrolysis of these compounds were comparatively low.

With the exception of Isomele, the oligosaccharides containing α-(1→6) and α-(1→1) glycosidic bonds were cleaved by SI to an extent of 92–100% (Fig. 2(A)). However, the progress curves compared with that of Mal indicate a lower velocity for the hydrolysis of the oligosaccharides. After 2 h of reaction, the rates of hydrolysis were determined to be 11, 27, 45, 60 and 69% for Isomele, 6′G-Tre, 6′G-Pala, 6′G,6″G-Pala, and 1G-Pala, respectively.

For the reduced oligosaccharides, with the exception of 6G-1,1-GPM/1G-1,6-GPS, the rates of hydrolysis by SI (Fig. 3(A)) were similar to those of the corresponding oligosac-

charides (Fig. 2(A)). The rate of hydrolysis of 6G-1,1-GPM/1G-1,6-GPS was nearly below 50% compared with that of the analogous 1G-Pala. This relationship was also observed for the action of GM (Fig. 2(B) and Fig. 3(B)).

The reduced disaccharides 1,1-GPM, 1,6-GPS and 1,1-GPS were hydrolyzed at a slower rate by SI than the reduced oligosaccharides and Mal (Fig. 3). The best activity was found for 1,1-GPS, being more than twice as high as that of 1,6-GPS. Furthermore, 1,6-GPS was clearly a better substrate for SI than 1,1-GPM. GM only hydrolyzed 1,1-GPS.

The reaction mixtures of oligosaccharides and the oligosaccharide alditols gave varying intermediates and monomeric end products. The time courses of the substrates and all products of the enzymatic hydrolysis are shown in Figs. 4–7 (see Table 1 for abbreviations).

The hydrolysis of Isomele by SI (Fig. 4(A)) resulted in the initial formation of Pala and Glc. After 3 h of incubation, the increase of the Pala concentration was reduced, and Fru was released. No sucrose was detected. The incubation of 6′G-Pala (Fig. 4(B)) also led to the production of Pala and Glc, but at a much higher rate than from Isomele. After 3 h, the concentration of Pala remained constant, and Fru was released. A very small amount of Iso-DP2 was detectable. The time course of the reaction of 6′G-Tre, and the formation of intermediate products, was qualitatively similar to the hydrolysis of 6′G-Pala (Fig. 4(C)). 6′G,6″G-Pala was cleaved by SI leading to the production of 6′G-Pala and Glc (Fig. 4(D)). As the intermediate trisaccharide was a substrate itself, it accumulated only for the first 2 h. After this lag phase, the hydrolysis of 6′G-Pala predominated.

1G-Pala was hydrolyzed by both SI and GM (Fig. 5(A and B)). The SI hydrolysis pattern was similar to those of 6′G-Pala and 6′G-Tre. The hydrolysis by GM was slow, giving increasing amounts of Pala, because GM had no activity on this disaccharide.

As mentioned above, the action of SI on 6′G-Isomalt, 6′G,6″G-Isomalt and 6′G-1-Isomalt resembled the action on the corresponding oligosaccharides. Intermediate disaccharide or trisaccharide alcohols were formed, which, as well as the substrates, were mixtures of isomers containing Man and Glc-ol components. The end products were Glc, Man and Glc-ol. When 6′G-Isomalt was hydrolyzed by SI (Fig. 6(A)), 1,1-GPM and 1,6-GPS appeared in a 2:3 ratio, in accordance with the composition of the initial substrate. During incubation, the concentration of the

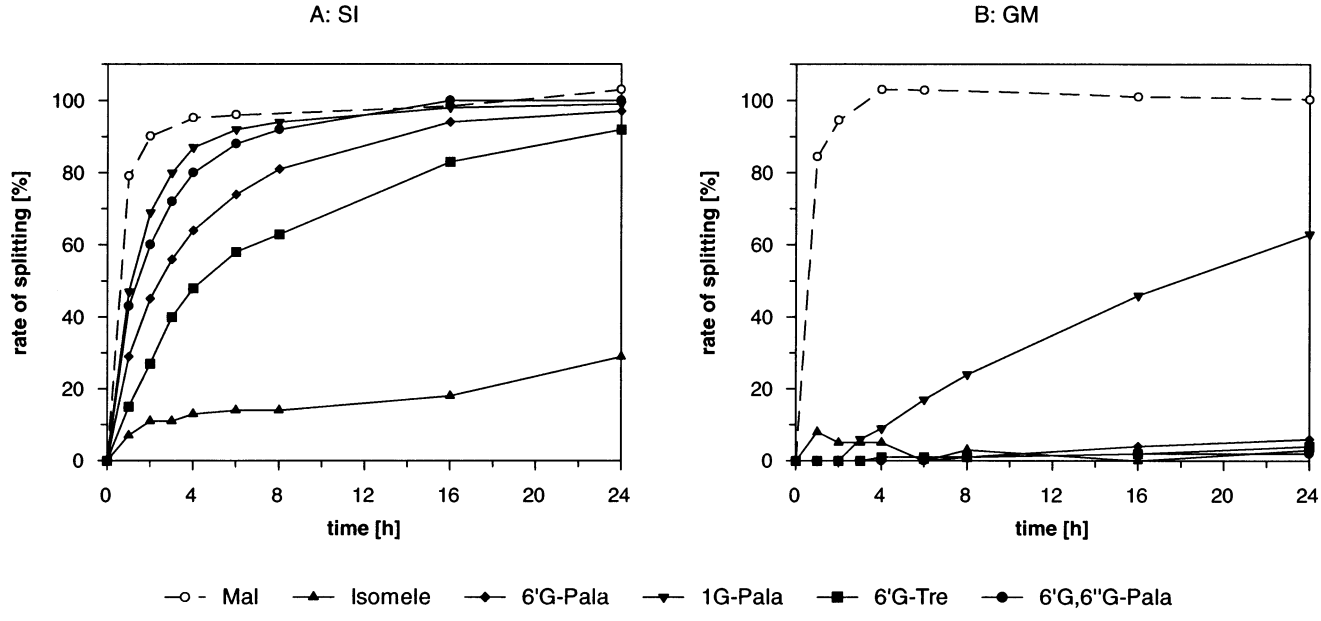


Fig. 2. Percentage rates of hydrolysis of Isomele, 6′G-Pala, 1G-Pala, 6′G-Tre and 6′G,6″G-Pala (for abbreviations see Table 1) by SI (A) and GM (B) compared with the splitting of Mal.

Table 1
Nomenclature for di- and oligosaccharides and their reduced forms and abbreviations used in this paper

Systematic name	Abbreviation
D-Glucose	Glc
D-Fructose	Fru
D-Mannitol	Man
D-Glucitol	Glc-ol
6-*O*-α-D-Glucopyranosyl-D-fructofuranose (isomaltulose, palatinose)	Pala
6-*O*-α-D-Glucopyranosyl-D-glucopyranose (isomaltose)	Iso-DP2
1-*O*-α-D-Glucopyranosyl-D-fructopyranose (trehalulose)	Tre
4-*O*-α-D-Glucopyranosyl-D-glucopyranose (maltose)	Mal
α-D-Glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-D-glucopyranose (isomaltotriose)	Iso-DP3
1-*O*-α-D-Glucopyranosyl-D-mannitol	1,1-GPM
6-*O*-α-D-Glucopyranosyl-D-glucitol	1,6-GPS
1-*O*-α-D-Glucopyranosyl-D-glucitol	1,1-GPS
α-D-Glucopyranosyl-(1→6)-β-fructofuranosyl-α-D-glucopyranoside (isomelezitose)	Isomele
α-D-Glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-D-fructofuranose	6′G-Pala
1,6-Di-*O*-α-D-glucopyranosyl-D-fructofuranose	1G-Pala
α-D-Glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→1)-D-fructopyranose	6′G-Tre
α-D-Glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-gluco-pyranosyl-(1→6)-D-fructofuranose	6′G,6″G-Pala
α-D-Glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→1)-D-mannitol and α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-D-glucitol	6′G-Isomalt
1,6-Di-*O*-α-D-glucopyranosyl-D-mannitol and 1,6-di-*O*-α-D-glucopyranosyl-D-glucitol	6G-1,1-GPM/1G-1,6-GPS
α-D-Glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→1)-D-mannitol and α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→1)-D-glucitol	6′G-1-Isomalt
α-D-Glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-gluco-pyranosyl-(1→1)-D-mannitol and α-D-glucopyranosyl-(1→6)-α-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-D-glucitol	6′G,6″G-Isomalt

disaccharide alcohols increased for 6 h, followed by a stagnation period. At the same time, the production of Man and Glc-ol was observed, but compared with the release of Fru from the corresponding oligosaccharide, the hexitols were released at a much slower rate. 6′G,6″G-Isomalt also consisted of Man and Glc-ol components in a ratio of 2:3. The same ratio was observed for the intermediates of SI hydrolysis, the sum of the components being given in Fig. 6(C). The first hydrolytic reaction produced 6′G-Isomalt, which itself is a substrate of SI (see Fig. 6(A)). Initially, the trisaccharide accumulated, but after 3 h, hydrolysis of the intermediate to Isomalt and Glc predominated. Early in the reaction of 6′G-1-Isomalt with SI, the rates of release of 1,1-GPM and 1,1-GPS essentially corresponded to the Glc release (Fig. 6(B)), indicating that Man and Glc-ol components of the substrate were cleaved to nearly the same extent, as was observed for 6′G-Isomalt and 6′G,6″G-Isomalt. In the later periods of the reaction, the concentrations of the intermediates 1,1-GPM and 1,1-GPS diverged, whereas 1,1-GPM and 1,6-GPS ran parallel in the case of 6′G-Isomalt (Fig. 6(A)). Thus, a different activity of SI with regard to the intermediate disaccharide alcohols was indicated, agreeing with the results reported in Fig. 3.

As mentioned above for the analogous trisaccharide, 6G-1,1-GPM/1G-1,6-GPS was hydrolyzed by both SI and GM. The Glc-ol and Man components of this substrate were not hydrolyzed to the same extent: SI favored 1G-1,6-GPS, as indicated by higher concentrations of the intermediate 1,6-GPS compared with 1,1-GPM. Also, GM hydrolyzed only the Glc-ol component of the substrate.

3. Discussion

The rates of hydrolysis of disaccharide alcohols by SI and GM were calculated from the D-glucose released, and it was found to be in agreement with the consumption of the substrate. However, for oligosaccharides and

oligosaccharide alcohols, a complete description of the degradation requires a time-course study including the substrates as well as all intermediates and products. Furthermore, analysis of the various intermediates gives insight into the specific breakdown of the different glycosidic bonds.

SI has the capacity to hydrolyze all the substrates tested, but the rates of hydrolysis differ. Comparing the rate of hydrolysis for Isomele with those for 6′G-Pala, 1G-Pala, 6′G-Tre and 6′G,6″G-Pala, Isomele is the slowest to be hydrolyzed (Fig. 2). Isomele contains two nonreducing ends, and it could be expected that both the α,β-(1→2) and the α-(1→6) glycosidic bond would be hydrolyzed. In fact, only the α,β-(1→2) bond was hydrolyzed, since Pala was the only disaccharide intermediate to be observed (Figs. 1 and 3).

Considering the different substrate specificities of the active sites of the SI complex (see Section 1), it was assumed that the α,β-(1→2) bond of Isomele was hydrolyzed at the active site of sucrase, and that the cleavage of the other oligosaccharides as well as of the oligo- and disaccharide alcohols tested, all saccharides containing α-(1→6) and/or α-(1→1) glycosidic linkages, occurred at the active site of the isomaltase subunit. The different binding sites and the low rate of Isomele hydrolysis indicate a different binding mode for the oligomeric structures at both active sites of the SI complex. The active site of the sucrase subunit contains only two subsites and, thus, can bind only two monomeric parts of a substrate with positive affinity, providing a possible explanation for the reduced activity on Isomele compared with sucrose. The rate of hydrolysis was 29% for Isomele (Fig. 3) compared with 86% for sucrose under the same conditions (data not shown). Contrary to the sucrase activity, the active site of isomaltase seems to contain other binding sites in addition to the binding sites for the monomeric glycon and aglycon moieties, as the tri- and

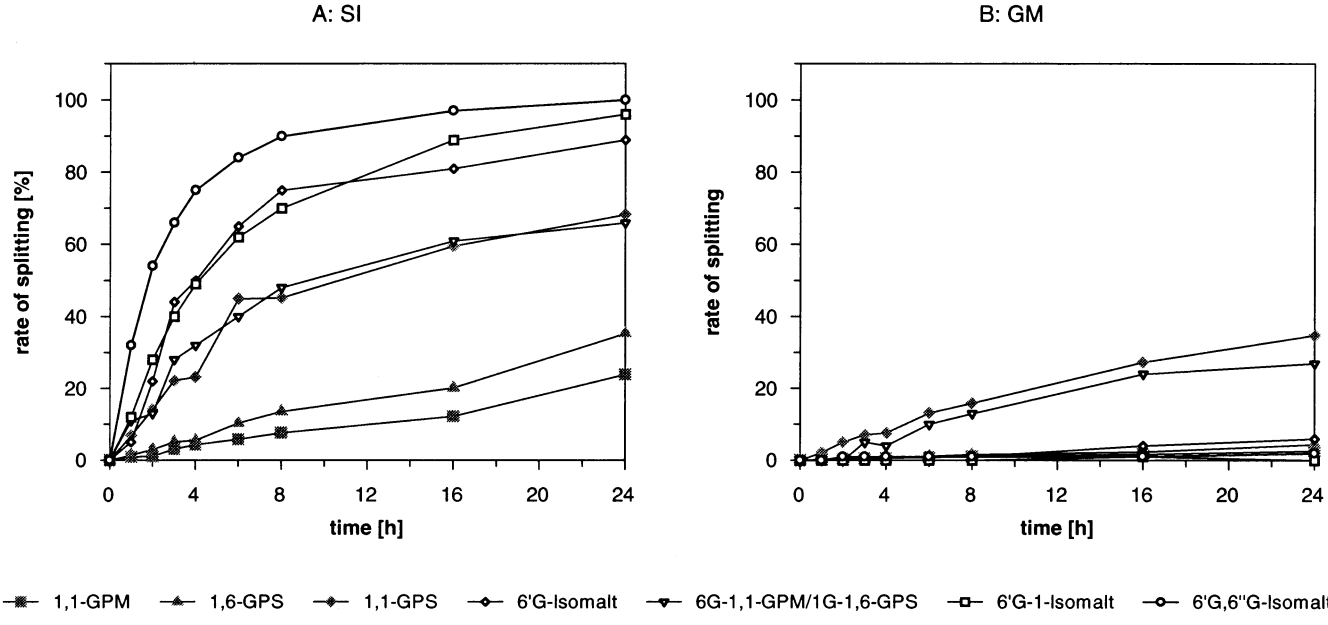


Fig. 3. Percentage rates of hydrolysis of 1,1-GPM, 1,6-GPS, 1,1-GPS, 6′G-Isomalt, 6G-1,1-GPM/1G-1,6-GPS, 6′G-1-Isomalt 6G′,6″G-Isomalt (for abbreviations see Table 1) by SI (A) and GM (B).

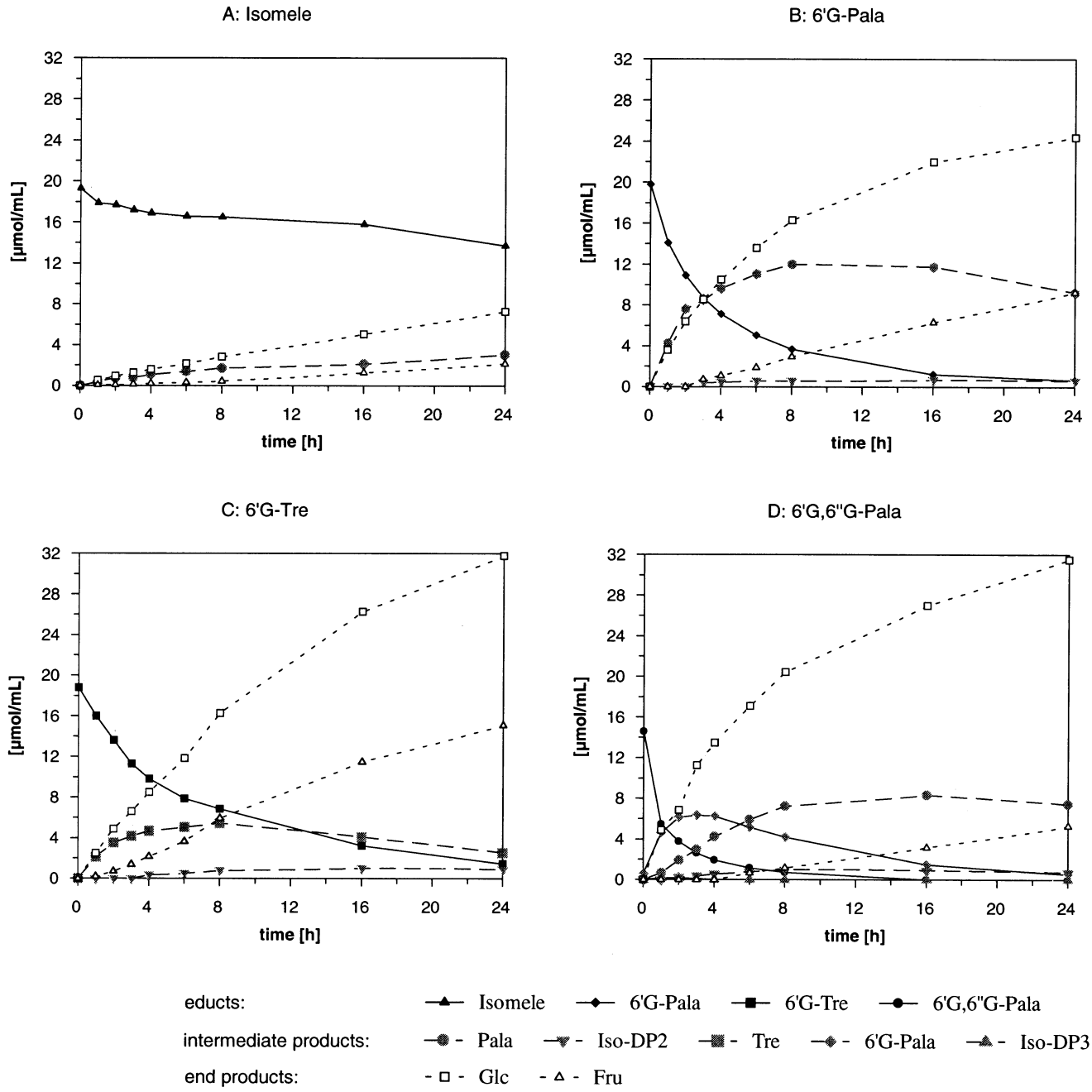


Fig. 4. Time course of substrates (continuous lines), intermediates (long dashed lines) and end products (short dashed lines) for tests of (A) Isomele, (B) 6′G-Pala, (C)6′G-Tre and (D) 6′G,6″G-Pala (for abbreviations see Table 1) with SI over a period of 24 h.

tetrasaccharides tested can be efficiently hydrolyzed by this subunit. Assuming a minimum of four subsites at the active site of isomaltase [9,11], this hypothesis would explain the higher rate of hydrolysis for 6′G,6″G-Pala compared with 6′G-Pala. Heymann et al. [2] thus suggested that the active site of isomaltase of human small intestinal SI could contain four subsites in contrast to only two subsites for the active site of sucrase.

Our results for pig intestinal enzymes indicate that the subsite model is also valid for certain oligosaccharide substrates containing D-glucose, D-fructose, D-glucitol and D-mannitol residues. Intermediate Pala in the experiments with 6′G-Pala and 6′G,6″G-Pala was hydrolyzed at only a slow rate, because the affinity of subsite 2 for the fructosyl residue is reduced compared with the glucosyl residue. Pala released from Isomele first has to leave

the active site of the sucrose subunit and move to the isomaltase subunit, leading to an even lower rate of hydrolysis.

A comparison of the structures of 6′G-Pala, 1G-Pala and 6′G-Tre (Fig. 1) shows the influence of the position of the D-fructose residue on the activity of SI (Fig. 2(A)). The enzyme hydrolyzed 1G-Pala faster than the two other trisaccharides, though it consists of a 'Pala part' and a 'Tre part', which were hydrolyzed by a negligible amount only in the experiments with 6′G-Pala and 6′G-Tre (see Fig. 4(B and C)). Furthermore, the beginning of the 'aglycon' moiety of 1G-Pala is a fructofuranosyl ring, whereas it is a glucopyranosyl ring in 6′G-Pala and 6′G-Tre. During hydrolysis of glucosyloligosaccharides, the aglycon part occupies subsite 2, the subsite with the highest affinity for D-glucose residues [2]. Consequently, a decrease of the activity should result when D-fructose is accommodated at this subsite. So, the highest activity found on 1G-Pala is clearly in contradiction with the occupation of subsite 2 by the D-fructose residue. Apparently, different binding sites exist for glucopyranoside and fructofuranoside aglycon moieties of oligosaccharides, as described by Heymann [7] for the SI-catalyzed hydrolysis of dimeric substrates.

Due to the lack of specificity for α-(1→6) glycosidic bonds, GM hydrolyzed only 1G-Pala, and none of the other oligosaccharides (Fig. 2(B)). When the extent of hydrolysis had reached 60%, contamination by SI was excluded as a cause of this conversion. The accumulation of Pala and negligible concentrations of monosaccharides (Glc and Fru; Fig. 5) reflect that the 'Tre part' of the trisaccharide was exclusively hydrolyzed. Agreeing with an earlier subsite model for the active sites of GM and glucoamylases of different origins [5,7,9–13] based on the interaction with linear maltooligosaccharides, our results for 1G-Pala indicate that the subsite model is not limited to those simple α-(1→4)-linked oligosaccharides. Furthermore, the hydrolysis of 1G-Pala by GM shows activity towards α-(1→1) glycosidic linkages as well as affinity to the enzyme for fructosyl residues. Although the activity on α-(1→1) glycosidic bonds was clearly less than on α-(1→4) linkages, further evidence for this specificity was provided by the results found for 6G-1,1-GPM/1G-1,1-GPS and 1,1-GPS. Affinity for fructosyl units agrees with our recent results [14] on the competitive inhibition of maltose hydrolysis by L-ribulose, which indicated a low affinity of GM for furanoside structures.

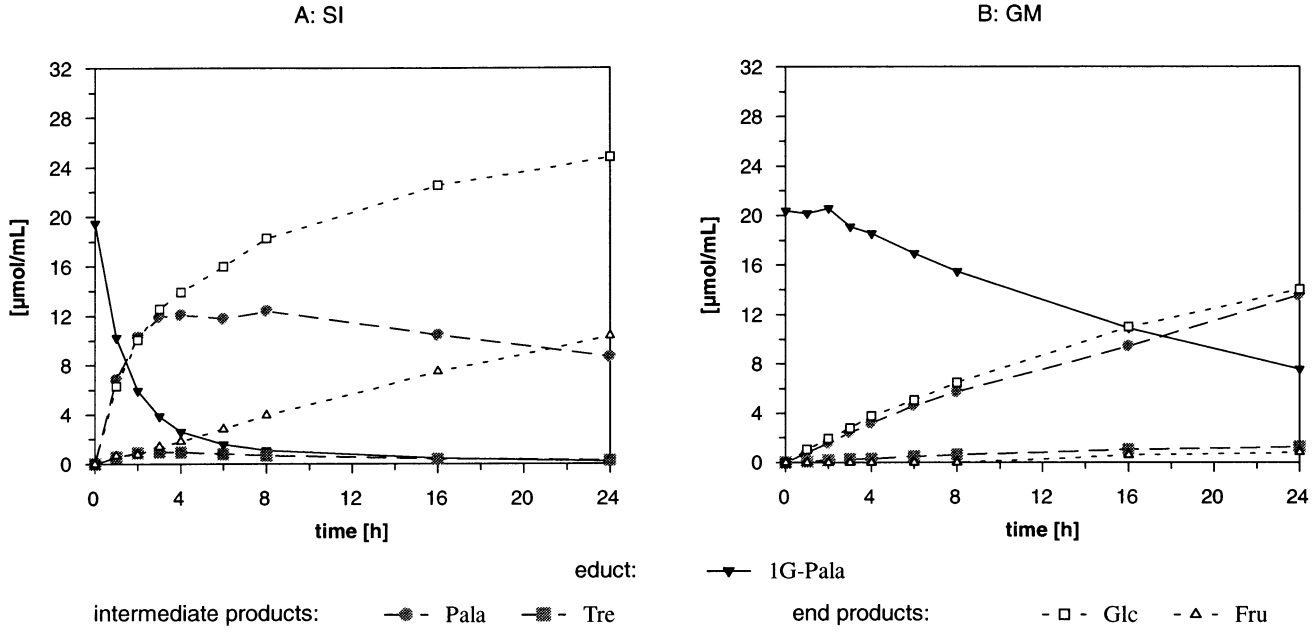


Fig. 5. Time course of substrates (continuous lines), intermediates (long dashed lines) and end products (short dashed lines) for tests of 1G-Pala (for abbreviations see Table 1) with SI (A) and GM (B) over a period of 24 h.

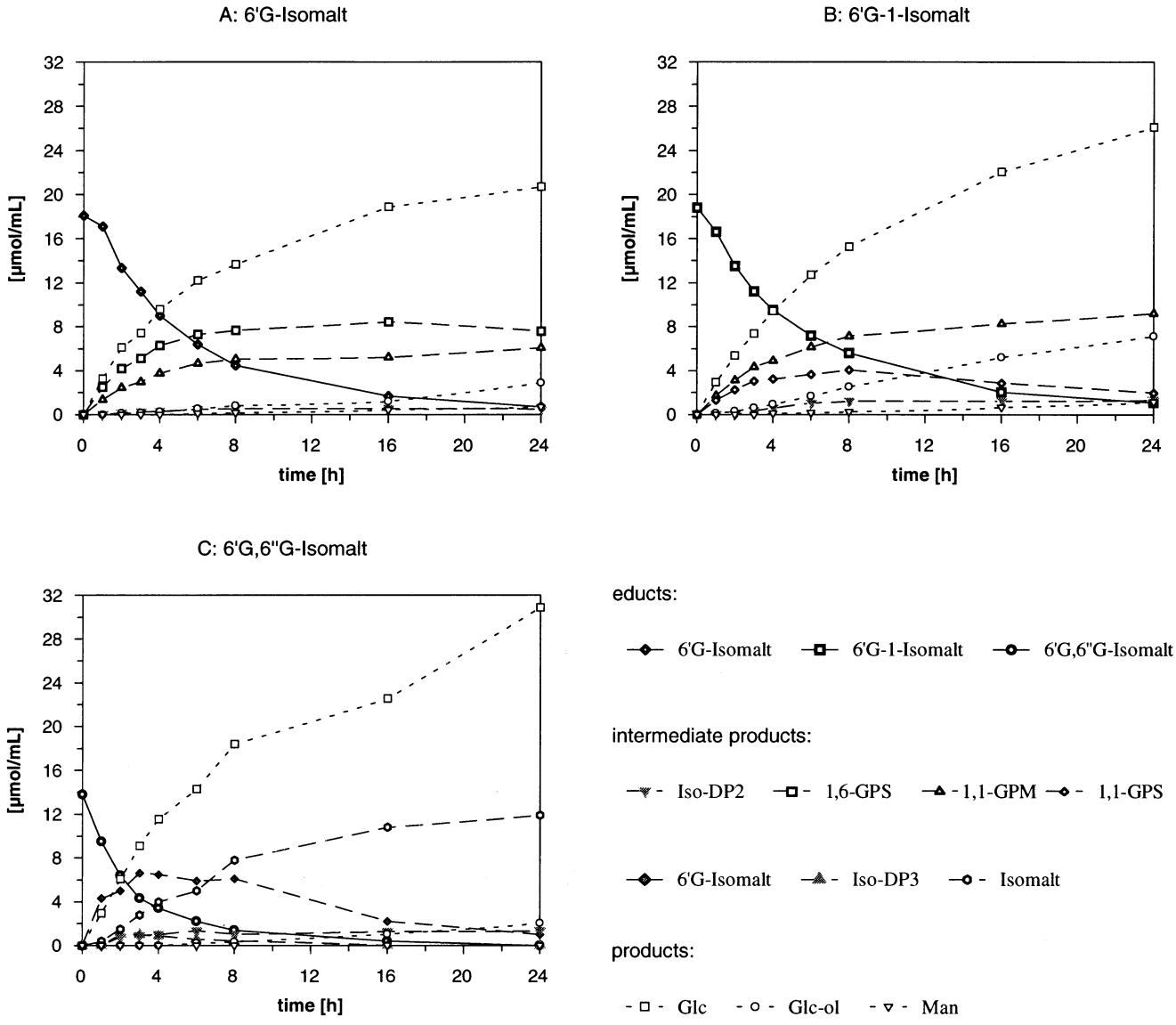


Fig. 6. Time course of substrates (continuous lines), intermediates (roughly dashed lines) and end products (finely dashed lines) for tests of (A) 6′G-Isomalt, (B) 6′G-1-Isomalt and (C) 6G′, 6″G-Isomalt (for abbreviations see Table 1) with SI over a period of 24 h.

The activity of SI towards 6′G-Isomalt, 6′G,6″G-Isomalt and 6′G-1-Isomalt and the corresponding oligosaccharides was at the same level (Figs. 2 and 3), indicating that hydrogenation of the reducing end of oligosaccharides having a minimum of three monomeric units has no influence on hydrolysis. The hydrolytic capacity of SI, however, was decreased for 6G-1,1-GPM/1G-1,6-GPS and the disaccharide alcohols, having the hexitol unit corresponding to the aglycon binding site of the enzyme. In agreement with the high affinity of subsite 2 for glucopyranosydic structures (see above), hydrolysis was found to be favored for substrates with cyclic sugar residues that interact with the aglycon binding site.

In steady-state kinetic experiments, carried out with crude homogenates of human intestinal mucosa, the K_M values of 1,1-GPM and 1,6-GPS were found to be much higher than the value for maltose (Mal: 2.7 mM; 1,1-GPM: 11.4 mM; 1,6-GPS: 7.7 mM), and the relative rates of hydrolysis for Mal, 1,1-GPM and 1,6-GPS are ‘100:4:2’ [15]. This agrees with the reduced capacity for hydrolysis compared with Mal found in our experiments. For the isomer 1,1-GPS, no kinetic data were re-

ported. Our results demonstrate that 1,1-GPS was hydrolyzed by SI to a much lower extent than maltose, but with a notably higher rate than both of the other disaccharide alcohols. Similarly, only 1,1-GPS was hydrolyzed by GM.

The disaccharide alcohols differ from maltose by the glycosidic linkage and by the structure of the aglycon part. The α-(1→6) and α-(1→1) glycosidic bonds of 1,6-GPS, 1,1-GPM and 1,1-GPS correspond to each other, because in both cases the glycon is linked with a terminal carbon atom of the hexitol chain. Therefore, it was concluded that the different activities of SI and GM are influenced by the conformation of the alditol chains. 1,1-GPM consists of a D-mannitol part adopting a nearly planar zigzag conformation, extending the α-(1→6) glycosidic bond and comprising a total of 10 atoms from the terminal O-6 to C-2′ of the D-glucose part [16,17]. In 1,6-GPS, the nearly planar chain of the D-hexitol comprises only eight atoms (from O-2′ to C-2). Due to unfavorable 1,3-interactions, the molecule rotates about the C-2–C-3 bond through 120° and attaches the terminal hydroxymethyl group of the D-glucitol in a 67° bend [17–19]. The D-hexitol chains of 1,1-GPM and 1,6-GPS thus meet the 'alditol rules' of Jeffrey and Kim [20]: 'the carbon chain of an alditol adopts the extended, planar zigzag conformation when the configurations at alternate carbon centers are different, and is bent and non-planar when they are the same'. Also based on these rules, the conformation of the D-glucitol part of 1,1-GPS results from a rotation about the C-2–C-3 bond through 120° [19], producing two extended-chain por-

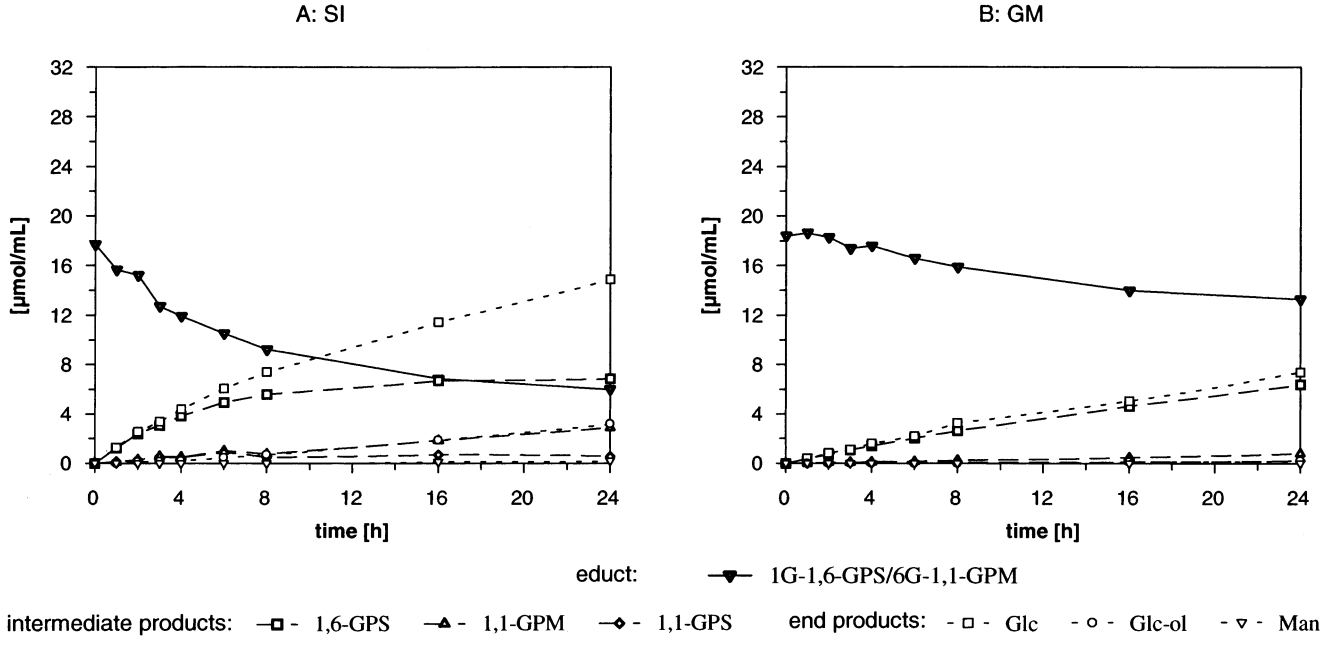
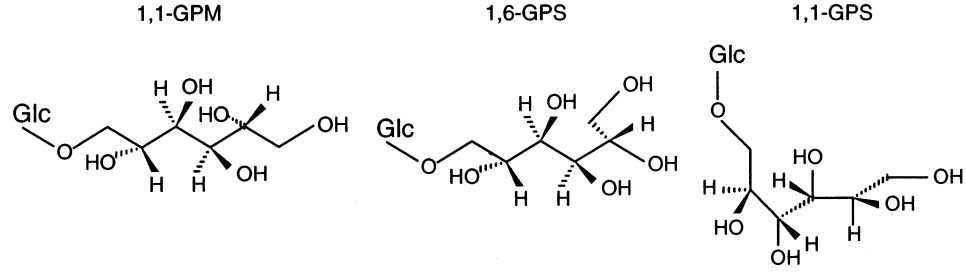


Fig. 7. Time course of substrates (continuous lines), intermediates (long dashed lines) and end products (short dashed lines) for tests of 6G-1,1-GPM/1G-1,6-GPS (for abbreviations see Table 1) with SI (A) and GM (B) over a period of 24 h.



Scheme 1. Conformation of the alditol chains in 1,1-GPM, 1,6-GPS and 1,1-GPS (for abbreviations see Table 1) according to Immel and Lichtenthaler [17], Munir et al. [18], and Lichtenthaler and Lindner [19].

tions crossing each other (from C-1 to O-3 and from O-2 to O-6). With these conformations (Scheme 1), the aglycon parts of 1,6-GPS and 1,1-GPS show increasing deviations from the planar zigzag chain of the D-mannitol moiety of 1,1-GPM. So, it can be concluded that the rate of hydrolysis by SI, and in part by GM, increases with the deviation of the alditol chain from the extended linear conformation. Since SI and GM bind the aglycon part of their substrates via hydrogen bonds [21], these interactions will decrease with variation in the configurations of the hydroxyl groups of the relevant residue from those of the structure of the natural substrate. Amongst the disaccharide alcohols, the variation from a cyclic structure of the aglycon is highest for 1,1-GPM. The compound cannot be hydrolyzed by GM and is hydrolyzed only to a low extent by SI. The bend in the D-glucitol chain of 1,6-GPS seems to improve the interaction of the hydroxyl groups with the active site of isomaltase, whereas the bend in the middle of the hexitol chain, as realized for 1,1-GPS, is most favorable for this kind of interaction for both SI and GM.

The findings for the experiments with 6G-1,1-GPM/1G-1,6-GPS (SI favoring 1G-1,6-GPS or GM selecting the Glc-ol component only, respectively) can be explained by the fact that the bonds cleaved correspond to those linkages of 1,1-GPM and 1,1-GPS (see Fig. 1). Referring to the subsite model described for GM and for the isomaltase subunit of SI, 6G-1,1-GPM/1G-1,6-GPS should be hydrolyzed to a higher extent than 1,1-GPS. The results given in Fig. 3, however, show identical rates of hydrolysis for both substrates, suggesting that subsite 3 was not occupied during hydrolysis of 6G-1,1-GPM/1G-1,6-GPS, presumably because the bend in the D-glucitol chain creates a distance between subsite 3 and the relevant residue.

Based on their experiments with linear isomalto- and maltooligosaccharides, Heymann et al. [2] postulated a similar binding mode of linear glucosyloligosaccharides to human small intestinal GM and to the isomaltase subunit of SI. Our results, which demonstrate the analogy between the hydrolysis of 1G-Pala by GM (representing the exception amongst the tri- and tetrasaccharides tested) and by isomaltase (that dominates the hydrolysis of all other oligosaccharides) may be explained if a similar binding mechanism is valid for GM and SI from pig intestine. The parallels found for the hydrolysis of 1,1-GPS and 6G-1,1-GPM/1G-1,6-GPS by GM and isomaltase offer further evidence for this conclusion.

4. Experimental

Isomele was isolated from the product solution of sucrose isomerization to isomaltulose, using immobilized cells of *Protaminobacter rubrum* (CBS 574.77). 6G-Pala, 6′G,6″G-Pala, and 6′G-Tre were obtained by incubating sucrose as the glucosyl donor with isomaltulose or trehalulose, in the presence of dextransucrase. 1G-Pala was obtained by reacting sucrose and isomaltulose in the presence of inulinase (SP230). After isolation from the reaction solutions by gel permeation chromatography on Fractogel HW40S (TOSOHAAS) and rechromatography on the same column, the tri- and tetrasaccharides were purified to minimum purity of 95%. The oligosaccharide alcohols were prepared by reducing the purified oligosaccharides (redissolved in a 1:1 mixture of water and MeOH) with $NaBH_4$ (at 35 °C for 3 h; afterwards pH adjustment to 6.5 by adding 0.1 M HCl). After neutralization, the oligosaccharide alditols were isolated from the reaction by Fractogel HW40S column chromatography. 6′G-Isomalt, 6G-1,1-GPM/1G-1,6-GPS and 6′G,6″G-Isomalt were isomeric mixtures of Man and Glc-ol components in a ratio of 2:3; the composition of 6′G-1-Isomalt concerning Man and Glc-ol components was not known. 1,1-GPM and 1,6-GPS were obtained by fractional crystallization of isomalt followed by fractional recrystallization. For 1,1-GPS, the starting material was trehalulose, which was first catalytically hydrogenated with an H_2–Pt catalyst. 1,1-GPM was removed from the product mixture by fractional crystallization, and afterwards, 1,1-GPS was purified by cation-exchange chromatograpy in the Ca^{2+}-form resin. In all cases, the purity of the polyols was over 95%.

Hexokinase (yeast), glucose-6-phosphate dehydrogenase (*Leuconostoc mesenteroides*), papain (*Carica papaya*), ATP, NAD and triethanolamine hydrochloride were from Boehringer Mannheim. Ammonium sulfate was obtained from Serva. All other biochemicals were purchased from E. Merck. DEAE-cellulose (DE52) was from Whatman, and a prepacked HiLoad 16/60 Superdex 200 column was purchased from Pharmacia Biotech.

SI and GM were isolated from pig intestinal mucosa by treatment with papain, and purified by a procedure containing fractionation with ammonium sulfate, followed by ion-exchange chromatography on DEAE-cellulose and gel filtration on Superdex 200 [6,7,22–24]. The enzyme preparations achieved yields of 45% for SI and 31% for GM, respectively, related to the activities after papain solubilization. Expressed by sucrase activity for SI and by maltase activity for GM, the specific activities were 15.3 and 8.2 U/mg. For SI, the maltase to sucrase quotient was 0.7. For GM, the sucrase activity could be reduced to 1.3% of the sum of maltase and sucrase activities.

Protein concentrations were estimated according to Bradford [25]. The purity of the enzyme preparations was demonstrated by SDS gel electrophoresis according to Davis [26] and Laemmli [27].

Oligosaccharides and oligosaccharide alditols were incubated with the enzymes for 24 h at 37 °C (substrate concentration: 15–20 mM in 100 mM triethanolamine buffer, pH 7, in a final assay volume of 1.25 mL; enzyme dosage: 0.7 U/mL maltase activity for SI and GM, determined by detecting the rate of liberated Glc using the hexokinase/Glc-6-P dehydrogenase method [28]; one unit of activity is defined as the rate of hydrolysis of 1 μmol of substrate per minute at 37 °C at saturating substrate concentration. Samples of the reaction mixture (0.12 mL) were removed after 0, 1, 2, 3, 4, 6, 8, 16 and 24 h, and the reaction was stopped by heating at 95 °C for 2 min. To determine the rate of hydrolysis, the concentration of liberated Glc was measured at room temperature by the hexokinase/Glc-6-P dehydrogenase endpoint method [28]. Furthermore, substrates and all products of enzymatic hydrolysis were detected by HPAEC (column: Carbopac PA1, 4 mm × 250 mm, anion exchanger; two columns in series in the case of reduced oligosaccharides; pre-column: Carbopac PA1, 4 mm × 50 mm, anion exchanger; eluent: 0.22 M NaOH with a 0–20% gradient of 0.5 M sodium acetate in 15 min for Isomele and all non-reduced forms of oligosaccharides or 0.12 M NaOH with a 1–33% gradient of 0.5 sodium acetate in 25 min for all reduced oligosaccharides; flow-rate: 1 mL/h; sample: 0.020 mL; temperature: 25 °C; detection: pulsed amperometric detector, gold electrode, potentials E and pulse time settings t of the detector are $E_1 = 0.05$ V and $t_1 = 480$ ms, $E_2 = 0.60$ V and $t_2 = 300$ ms, and $E_3 = -0.60$ V and $t_3 = 240$ ms).

Acknowledgements

The authors thank A. Pantke and H. Dattge for their technical assistance with the HPAEC-analyses.

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