The Mechanism of the Human Intestinal Sucrase Action

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Summary: Highly purified sucrase accepts as a substrate α-anomers within D-glucopyranosides. Hydrolysis of sucrose and palatinose proceeds with the net retention of configuration at the C-1 of D-glucopyranose. The fission of the glycosidic bond takes place between the C-1 of the D-glucose ring and the glycosidic oxygen; therefore D-fructose is released as β-D-fructofuranose from sucrose. During hydrolysis, transglycosylation takes place and a few oligosaccharides are formed and in the presence of methanol α-methyl-D-glucopyranoside is also produced. Non-stoichiometric amounts of D-glucose and D-fructose are produced from sucrose, turanose and palatinose and less D-glucose than expected is present. Formation of sucrose from D-glucose and D-fructose was not observed.

Intestinal disaccharidases play an important role in the metabolism and transport of sugars. Recently a few of them have been very well described (1, 2, 3) and the mechanism of action precisely investigated (4, 5, 6, 7). Human intestinal sucrase is believed to exist in a stabile complex with isomaltase (8, 9, 10) which contains up to 52% covalently bound saccharide, the exact composition of which varies with blood group (11). In this paper we report studies on the stereospecificity, steric course of the catalysis and the mechanism of fission of the glucosidic bond. The role of the enzyme-bound sugar in the catalysis is not considered here.

Materials and methods

Sucrase was extracted from human small intestine by papain solubilization and purified by ammonium sulfate fractionation and affinity chromatography on Sephadex G-200 (3). The final preparation was electrophoretically homogenous and had about equal activity with sucrose and isomaltose. Specific activity was 11 as assayed with sucrose (3). All sugars, glucosides, $^{2}H_2O$ (99.7 atom %), $H_2{}^{18}O$ (21.35 atom %) and other reagents used in this work are commercially

available. Carbohydrate analyses were performed by GLC (12) and by a coupled GLC-MS system (13, 14). Saccharides and glucosides were simply and quantitatively converted to per-O-trimethylsilyl-ethers which were readily analyzed. The silylation (15) of water containing samples was performed in the following manner: 5 or 10 microliters of sample were diluted with 100 microliters of dimethylformamide (containing xylitol as an internal standard) and after a few seconds, 1 milliliter of the silylating reagent was added (2:1:1 v/v, pyridine:trimethylchlorosilane:hexamethyldisilazane). Finally samples were left at room temperature for 30 min to complete the silylation. A Perkin-Elmer gas chromatograph Model 990, equipped with the GC data system PEP-1 was used. The carrier gas was nitrogen at flow rate of 38 ml / min. Columns were 2 m long and 2 mm i. diameter. The separation of D-fructose (one peak) α- and β-D-glucopyranose, sucrose (and other substrates) as well as produced oligosaccharides was achieved on 10% SE-54 column under the following temperature program: 8 min isothermal hold at 160^{o} followed by 3^{o} / min increase up to 280^{o} and a final hold of ca. 60 min, if necessary, to release oligosaccharides. A 1% SE-30 column was used to achieve the separation of α-methyl-D-glucopyranoside from other saccharides under a 24 min isothermal hold at 140^{o} followed by 3^{o} / min increase up 200^{o}. The 20 eV mass spectra of per-O-trimethylsilyl-ethers of saccharides were recorded with a gas chromatography-mass spectrometry apparatus LKB 9000 equipped with a 1.8 m long 1% SE-30 column. Helium was used as a carrier gas at flow rate of 18 ml / min. The separation of monosaccharides was performed at 160^{o} isothermal hold and for di- and other saccharides with an additional temperature increase of 3^{o} / min up to 200^{o}. Enzymatic incubations were performed on a micro scale in capillary pipettes or micro test tubes and carried out at 37^{o}. In order to have comparable results as well as to decrease simultaneous anomerization of produced monosaccharides, all enzymatic experiments were done in twice-distilled water instead of buffer and with enzyme previously dialyzed against water. All

comparable activity experiments with different saccharides were performed as following: 0.24 or 0.37 units of dialyzed enzyme (as assayed by GC and calculated with respect to free D-fructose) were placed in test tubes and lyophilized. The reaction was started by the addition of 10, 20 or 40 microliters of 0.27 M sugar solution and carried out for 10 or 30 min. Incubations with cellobiose, gentiobiose, melibiose, trehalose, α- and β-methyl-D-glucopyranosides, lactulose, raffinose, melezitose, 3-nitrophenyl-β-D-glucopyranoside were extended up to 90 min. During the reaction, 5 or 10 microliter samples were taken for the sugar analysis. Poorly soluble saccharides as for example 4-nitrophenyl-α-D-glucopyranoside were used as their saturated solutions. All other enzymatic experiments (sucrase anomerization effect on α-and β-D-glucose, steric course of the hydrolysis of sucrose or palatinose, reactions in $^{2}H_{2}O$, $H_{2}{}^{18}O$) were performed in the same manner as above, using 0.37 or 0.74 units of dialyzed and lyophilized sucrase.

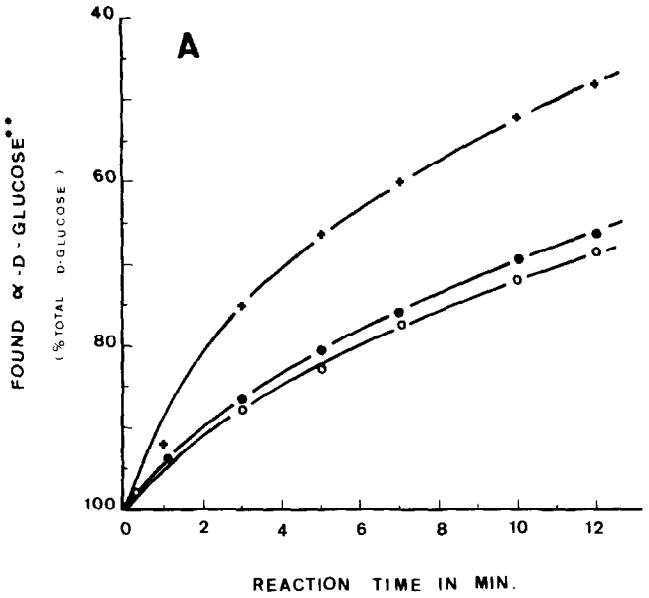
Results

From our kinetic data obtained with highly purified sucrase, we calculated the relative rates of catalysis for the following substrates: maltose (2.00) > isomaltose (1.10) > sucrose (1.00) > turanose (0.34) > palatinose (0.26) > 4-nitrophenyl-α-D-glucopyranoside (0.20) > α-methyl-D-glucopyranoside (0.018) > melezitose (0.002). The hydrolysis is accelerated by a factor of 1.27 when the reaction was carried out in sodium chloride or sodium maleate buffer pH 6.8 (both 33 mM). Trehalose, 3-nitrophenyl-β-D-glucopyranoside, cellobiose, gentiobiose, melibiose, lactulose and raffinose were not hydrolyzed. Also we found that the reaction of transglycosylation occurs concomitantly with enzymatic hydrolysis; the rate of transglycosylation is in the range of 5 to 15% of the rate of hydrolysis and is strongly dependent on the nature and concentration of substrate. It was also easily observed that, during hydrolysis, non-stoichiometric amounts of D-glucose and D-fructose are produced from

sucrose, turanose and palatinose with up to 15% less D-glucose than D-fructose being formed. On the other hand, no sucrose was observed to be formed from D-glucose and D-fructose when incubated with sucrase (0.37 units of sucrase in 0.4 M D-glucose and D-fructose) even over a period of 6 hrs. The enzymatic hydrolysis of sucrose in the presence of 20% (v/v) methanol leads to the formation of α-methyl-D-glucopyranoside amounting to 14% of the free D-glucose. Fig. 1 shows the steric course of sucrose and palatinose hydrolysis, and on extrapolation to zero-time, it is apparent that α-D-glucopyranose is the only form to be released from sucrose or palatinose. Sodium chloride as an activator of sucrase has no visible effect on the steric course of the reaction. When sucrose hydrolysis by sucrase was carried out in $H_2{}^{18}O$, it was found that after 2 or 5 min of the reaction, 21 atom % of ^{18}O is present in α- and β-D-glucopyranose and no ^{18}O was incorporated into D-fructose. Control experiments with $H_2{}^{18}O$ and α-D-glucose show no detectable exchange of oxygen into mutarotated sugars after 5 or 10 min at 37° (confirming earlier results (16)) and sucrase has no effect on it. The enzymatic hydrolysis of sucrose carried out in ^2H_2O gave no visible deuterium incorporation into D-glucose or D-fructose as detected by GC-MS.

Discussion

The above results show that the sucrase has high stereospecificity toward substrate and accepts only α-substituted D-glucopyranosides. On the other hand, the enzyme has no special requirement for the aglycone part of the substrate. In order to localize the site of the glycosidic bond cleavage in sucrose, enzymatic hydrolysis was carried out in $H_2{}^{18}O$. GC-MS analysis of the reaction products showed the incorporation of one equivalent of ^{18}O at the C-1 of α-D-glucopyranose. Therefore the fission of the glycosidic bond takes place between the C-1 of the α-D-glucopyranoside ring and the glycosidic oxygen releasing β-D-fructofuranose. In the course of the reaction,



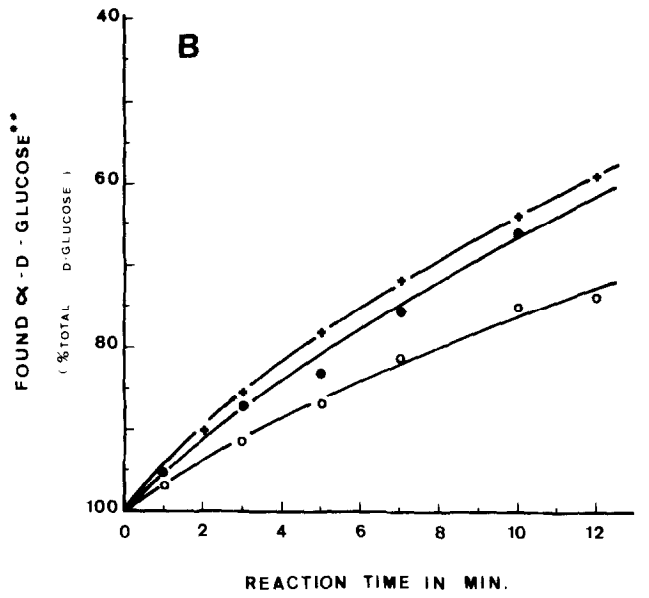


Figure No. 1

A. Effect of sucrase and sodium chloride on anomerisation of α-D-glucose* (0.27 M) in water at 37° (control experiments).
--o-- α-D-glucose only
--+-- α-D-glucose and o.37 units of sucrase
--●-- α-D-glucose in 33 mM NaCl

B. Form of D-glucose released during hydrolysis catalyzed by human sucrase at 37°.
--o-- 0.27 M sucrose and 0.37 units of sucrase in water
--+-- 0.27 M palatinose and 0.37 units of sucrase in water
--●-- 0.27 M sucrose and 0.37 units of sucrase in 33mM NaCl

* crystalline α-D-glucose contained 1% of its β-form
** the rest of assayed D-glucose was in β-form

the D-glucopyranose moiety is also released in its α-form, as is seen in Fig. 1. The net retention of configuration at the C-1 of the D-glucopyranoside ring is confirmed also by the formation of α-methyl-D-glucopyranoside in the presence of methanol in the reaction medium. After taking into account the facts known about chemical acid-catalyzed hydrolysis of glucosides (see Rev. 1) and our stereochemical results, we propose a simple model of enzymatic hydrolysis catalyzed by sucrase (see Fig. 2). Our recent results do not allow one to distinguish which of the two possible mechanisms takes place, since the stereochemical outcome is the same in both cases, assuming that in the case of the S_N1 mechanism, during the substitution step the α-D-glucopyranoside ring is sterically controlled by the surface of sucrase and the substitution proceeds from one side only. The proposed mechanisms explain also the forma-

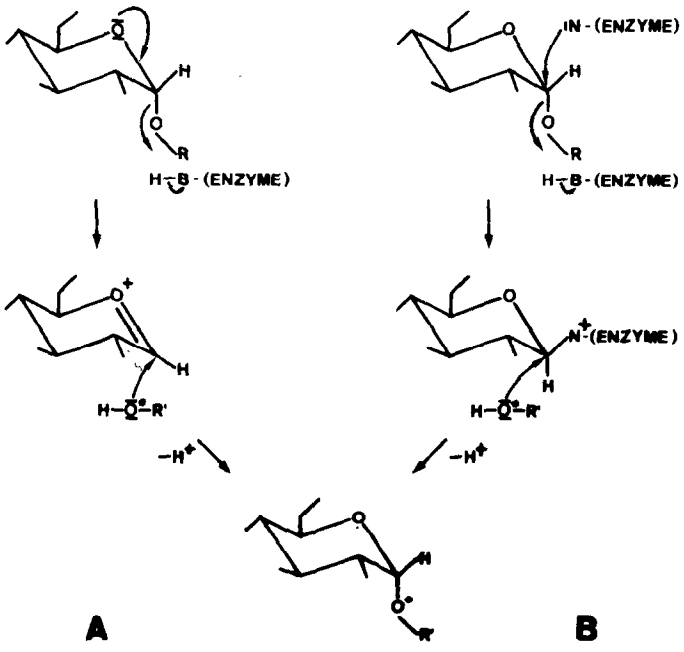


Figure No. 2

Possible mechanisms of human intestinal sucrase action:
A = S_N1 type, B = S_N2 type (using a nucleophilic group on the enzyme).
R = aglycone part in substrate,
R' = aglycone part in product = H-, CH_3-, sugar-.

tion of a few oligosaccharides and α-methyl-D-glucopyranoside. The cyclic oxonium ion (or the intermediate in the S_N2 type) is not captured exclusively by water; there is a competition between nucleophiles like water, alcohols, sugars and so on, present in the medium. Furthermore, this step depends on the nucleophilicity of the participants and their concentrations; therefore free α-D-glucose is released or a new α-D-glucopyranoside is formed. On the basis of the substrate specificity studies, observed α-D-transglucosylation, net retention of configuration of liberated products and mechanistic arguments that the α-D-glucopyranosidic ring is critically important for the catalysis, we propose that human intestinal sucrase should be classified as an α-D-glucopyranosidase.

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