Fig. 5B), results in an inactive protein. Proline is added in the middle of an α-helix and likely disrupts the helix and associated secondary structure. The mutation is 21.2 Å away from the catalytic nucleophile, but it is in the interior of the protein where proline is found only infrequently because this residue typically lies on the surface of globular proteins. The disorder of the active site caused by this mutation likely affects function and causes the inactivity of the protein.

In addition to the specifics of inhibitor-enzyme interactions, there are some important questions that can be addressed by future structural analyses. The roles of portions of the enzyme module structures that are distal to the active sites have not been investigated. In particular, the structure identified a β-sheet region, a fold that has been associated with both carbohydrate-binding modules of microbial glycoside hydrolases and protein–protein interactions. Furthermore, although studies of individual modules have been critical in studying their characteristics, MGAM and SI both exist physiologically as dienzyme complexes. How do the modules interact within the intact complex? To what extent is this interaction important for their activities? Finally, what is the basis for the altered activity or trafficking of mutated SI domains in patients with CSID? Structural studies of intact MGAM and SI enzymes can contribute to answering these questions.

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Starch Digestion and Patients With Congenital Sucrease-Isomaltase Deficiency

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Starch is the major carbohydrate storage type found in plant seeds and tubers in a semicrystalline form. Starch is consumed by humans as a major glucose source of dietary energy and it can supply as much as 70% to 80% of the calories in the overall average human diet (1–3). Starch has 2 main molecular structures: amylose, which consists of long linear chains of glucose associated by α-1,4 glucosidic linkages and occasional branching with α-1,6 linkages, and amylopectin consisting of relatively short α-1,4 bound glucose chains of variable length with a relatively high content of α-1,6 branching chains (Fig. 1, top left). Amylopectin, in particular, is an extremely large molecule containing approximately 1 million glucose units that lead to a complexity of branched structures that differs among genetic backgrounds (4–6). The proportion of amylose versus amylopectin, the average length of α-1,4-linked linear chains, and the frequency of α-1,6 branching vary considerably among starches. Such differences lead to variation in the digestion rate and production of α-amylose digestion products (7–9). These products, known as α-limit dextrins (Fig. 1, bottom), can modulate catalytic activities of the mucosal α-glucosidases.

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A new view on the human diet and evolution proposes that evidence of cooking up to 1.8 million years ago (10), and its accompanying gelatinization of starch and cereals, increased energy availability in prehistoric humans and was an evolutionary force for the development of modern humans. The importance of extracting maximum glucose from starchy foods for our ancestors cannot be overstated. What on cursory glance seems like a redundancy of enzymes to digest starch to glucose instead is an efficient, if not elegant, system for obtaining energy for the body.

PROCESS OF STARCH DIGESTION
In humans, the digestion of starch occurs by enzymatic hydrolysis during its transit through the gastrointestinal tract and requires the participation of 6 different α-glucosidic activities. Two luminal α-1,4 endoglucosidases, namely salivary and pancreatic α-amylases, hydrolyze linear unbranched starch segments with >5 glucose residues, releasing oligomers with from 2 (maltose, the simplest glucose oligomer) to 5 glucose residues, but with minimal production of free glucose (Fig. 1, top middle and right) (11). The segments containing α-1,6-linked branches are resistant to these α-amylase activities. To attain the effective release of free glucose, linear and branched glucose oligomers resulting from α-amylase digestion must be further hydrolyzed by 4 exohydrolases present in the mucosal epithelial cells of the small intestine (Fig. 1, bottom). These hydrolases comprise sucrase-isomaltase (SI) and maltase-glucoamylase (MGAM) complexes with C- and N-terminal enzymes, each composed of 2 α-glucosidases containing catalytic sites that act on the nonreducing ends of linear and branched glucose oligomers, with substantial release of free glucose monomers (12,13). All 4 enzymes have α-1,4 glucosidic activity and 1 enzyme, isomaltase, has substantial α-1,6 glucosidic activity that cleaves the linkages present in the branch points of α-limit dextrins. Individual MGAM and SI subunits share high sequence identity, approximately 40% to 60%. C-terminal enzymes have approximately 60% sequence identity, as do the N-terminal enzymes; however, sequence identity was relatively lower (approximately 40%) between C- and N-terminal α-glucosidases (Fig. 2) (14–16).

![Figure 1. Schematic representation on the digestion process of starch, beginning with starch branched molecule (top left), digestion by α-amylase (top middle), susceptibility of segments of the branched molecule to α-amylase (top right) (red = nondigestible, blue = digestible, purple = slowly digestible, magenta and orange = poorly or nondigestible), digestion of α-amylase products by mucosal intestinal enzymes showing variability in digestion rates and kinetics to produce glucose (bottom). Amy = α-amylase, C = glucoamylase (C-terminal), M = maltase (N-terminal), S = sucrase (C-terminal), I = isomaltase (N-terminal).](image-url)
values calculated for sucrase and MGAM and SI protein complexes linked to the trans-membrane domains (TMD) via the O-glycosylated linkage. Percentages among mucosal α-glucosidases represent sequence identity. SGLT1 = sodium dependent glucose transporter 1; GLUT5 = glucose transporter 5.

α-GLUCOSIDASES OF THE GASTROINTESTINAL TRACT

α-Amylase

Salivary and pancreatic α-amylases are synthesized as approximately 78 kDa and are coded by the genes AMY1 and AMY2, respectively, located in the human chromosome 1. The typical human haploid genome contains 2 copies of AMY2 (AMY2 A and B) (17), but the presence of multiple copies of the segment containing both genes, AMY1 and 2, is common among normal human populations and seems to be associated with the amounts of starch ingested by particular human ethnic groups, suggesting that multiple copies of amylase genes is an adaptive feature for efficient digestion of high starch diets (18). The human α-amylases have specificity for the α-1,4 linked straight-chain regions of α-glucosyl polysaccharides. Human α-amylases have maximal specificity for the interior links, and the active sites bind 5 consecutive glucose residues at specific subsites, cleaving between the second and third subsites to form 2 smaller polymers (Fig. 1, top middle and right). Products that are smaller than the linear maltopentaose are unable to bind at all subsites, have low affinity for the active site of α-amylases, and the productive cleavage of these smaller oligosaccharides by α-amylases is markedly hampered. In addition, α-1,6 branching linkages interfere with the activity of α-amylases and may contribute to a slow digestion fraction observed in the hydrolysis products of starch. The sequential action of α-amylases promotes the release of linear glucose oligomers with 2 to 5 glucose residues together with larger and highly branched molecules, usually termed α-limit dextrins, as the main final products of luminal starch digestion.

MGAM

Human MGAM contains 1857 amino acid residues, which after glycosylation and insertion in the apical membrane, displays a total molecular weight of close to 335 kDa (19,20). The cDNA sequences for the human enzyme have revealed the presence of 2 α-glucosidases in the mature protein (ct-MGAM and nt-MGAM), which display high sequence identity to the respective α-glucosidases of the SI complex and contain 1 potential active site (WIDMNE) in each. MGAM was originally described as 2 relatively thermostable maltase activities present in the human intestinal mucosa, and experimental evidence shows the existence of at least 2 subunits in the MGAM complex (21,22). Research has demonstrated that substantial differences in catalytic properties exist between the N- and C-terminal α-glucosidases of the enzyme (19,20). The C-terminal α-glucosidase shows a faster glucoseamylase activity than the N-terminal α-glucosidase, and experiences substrate inhibition in the 3 to 5 series of glucose oligomers (maltriose to maltopentaose) (Fig. 1, bottom middle). In contrast, the N-terminal α-glucosidase displays slower catalytic α-glucogenesis than the C-terminal α-glucosidase, but it shows much lower substrate inhibitory effect by the same glucose oligomers (11,23). In addition, clear differences of up to 2 orders of magnitude were observed in their degree of susceptibility to inhibition by acarbose, with the C-terminal α-glucosidase being more sensitive than the N-terminal α-glucosidase (24). The human MGAM gene (National Center for Biotechnology Information reference sequence NM_004668.2) is located in chromosome 7 (7q34). The human and mouse genomic projects have shown that the genomic region coding for MGAM of most mammalian species contains paralogous replications (4,5) of the 3’ segment coding for the C-terminal α-glucosidase of MGAM, each with potential for its transcription and alternative splicing. The recombinant mouse and human MGAM cDNA sequences show that the corresponding mRNAs are spliced alternatively in the segment corresponding to exons 22 to 44. In addition, the respective recombinant C-terminal proteins display variations in catalytic properties. These observations suggest that MGAM may be considered as a family of closely related proteins rather than a single unimodal protein and suggest that the catalytic properties of MGAM molecules may display variations depending on developmental stage, nutritional status, or diet. Its individual role in starch digestion, therefore, may require independent analysis and determination of the relative proportions of at least the most prominent splicing isoforms.

SI

The gene coding for the human SI complex is located on chromosome 3q16, producing a protein with a predicted size of nearly 210 kDa (25,26). The mature SI is a complex composed of 2 α-glucosidases, sucrase and isomaltase (National Center for Biotechnology Information reference sequence NP_001302.2). After synthesis, the fully active proenzyme is transported and inserted in the apical membrane of the enterocytes through its N-terminus (27). Subsequently, SI is subjected to extracellular processing by pancreatic proteolytic enzymes in the intestinal lumen cleaving the complex and generating free sucrase and membrane-bound isomaltase subunits (28). The cleaved molecules remain associated to each other through noncovalent interactions. Both α-glucosidases display considerable α-glucosidic activity on starch-derived glucose oligomers. Human SI complex has an overall contribution of 60% to 80% of the total human intestinal maltase activity because of the amount of protein in the small intestine (29,30); however, the apparent Km values calculated for sucrase and
isomaltase in association is at least 10 times higher than the apparent K_m values calculated for the same activities of ct-MGAM (glucoamylase). The α-glucosidic activities of SI are, therefore, predominant in the human intestine but are a much slower glucose producer from glucose oligomers than is MGAM. The α-1,6 glucosidic linkage of the branched α-limit dextrans is hydrolyzed by the isomaltase of the SI complex. Although these branched linkages may contribute to the generation of “slow-digesting” products of starch digestion, SI displays approximately 50% as much debranching (isomaltase) activity as its maltase activity, which provides enough activity to cope with the total branched linkages that may be present during the starch digestive process (Fig. 1, bottom right).

Production of glucose from dietary starch depends on the orchestrated activities of SI and MGAM. Rates of substrate digestion depend on their specific interaction with 4 mucusol α-glucosidases. Control of glucogenesis can be obtained both through types of available substrates and inhibitory effects of oligomers on the different α-glucosidases.

GLYCEMIC RESPONSE DURING STARCH DIGESTION

Digestion of starch in the small intestine can occur rapidly, slowly, or not at all, and accordingly it has been nutritionally categorized in vitro as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). In the in vitro Englyst et al (31) assay, RDS is digested within 20 minutes and correlated to the high glycemic index in that it leads to a sudden increase in the blood glucose level. In contrast, SDS is hydrolyzed at a much slower rate, and in the in vitro method is measured as glucose released from 20 to 120 minutes. RS, unlike RDS and SDS, cannot be digested in the small intestine and is used as dietary fiber in the large intestine (31, 32). SDS has drawn interest because foods containing SDS are considered to have a glycemic index. Low glycemic index foods including SDS provide a moderated post-prandial glucose response and extended glucose release and the possibility of reducing the risk of common chronic diet-related metabolic diseases (eg, type 2 diabetes mellitus, obesity, cardiovascular diseases) (33–38).

CSID ISSUES

In patients with congenital sucrase-isomaltase deficiency (CSID), the only α-glucosidases present are the 2 MGAM subunits, commonly termed maltase and glucoamylase. Although these enzymes have the function of digesting α-amylase digestion products of starch to glucose, it is the full complement of MGAM and SI that efficiently accomplishes starch digestion. For starch digestion, that which is most missing in patients with CSID, is the debranching activity of isomaltase that is responsible for removing branches of the α-limit dextrans to linear maltooligosaccharides for rapid digestion by MGAM. Also missing is the abundance of the SI enzyme complex and its proposed role in slower digestion of starch-degradation products. The well-known sucrose malabsorption in CSID is often coupled with a problem of starch malabsorption. Undigested starch and sucrose molecules can also cause chronic diseases of the colon (eg, chronic osmotic diarrhea, abdominal pain) (39, 40).

Possibilities of Increasing Starch Digestion by MGAM

One strategy for reducing starch malabsorption in patients with CSID would be to find ways to increase starch digestibility by MGAM so that undigested starch does not cause abdominal distress. A recent finding by our group shows that glucoamylase, also referred to as ct-MGAM, has high hydrolytic activity toward native starch molecules, so much so that it has been proposed to assist α-amylase in digesting starch (41). Starchy foods designed to be better digested by ct-MGAM would thus result in more complete digestion and glucose absorption in the small intestine. Such foods may include those with dispersed starch molecules that are found in well-gelatinized foods that include shear processes to break apart swollen starch granules (eg, purées, puddings, porridges). Maltoollextrins, which are partially hydrolyzed starch-based products, would also likely be well digested by the MGAM enzymes, as would maltooligosaccharides (smaller breakdown products of starch).

Could Slowly Digestible Starchy Foods Be Better for Patients With CSID?

Another strategy for reducing abdominal distress experienced by some patients with CSID after consumption of starchy foods may be to consume slowly digestible, low-glycemic-response starchy foods. The reasoning here is that such starchy digest slowly in the small intestine and can slow gastric emptying and motility of food (40), thus slowing starch delivery to the small intestine, where it would be better able to digest it, and that starch which enters the colon would do so in smaller amounts and during a longer post-prandial period. As a result, bloating and osmotic effects of maltooligosaccharides in the bowel that can cause diarrhea would be reduced.

Slowly digestible starchy foods can be found in a number of foods that have slowly digestible matrices (eg, al dente pasta, some whole-grain foods) and slowly digestible starch types (eg, moderately higher amylose cereals, partially gelatinized) (42). Consumption of these foods would represent a somewhat restrictive diet, which in the case of consumption of sucrose has been alleviated by supplementation with sacrosidase (43, 44). Still, there is a fairly wide range of foods with slow digestible starch property. Other approaches that may be less restrictive in achieving slow starch digestion would include the addition of glucoamylase as a supplement or partial inhibition of α-amylase or MGAM, and disaccharides or maltooligosaccharides with α-linkages other than the 1,4 and 1,6 linkages found in starch molecules (eg, kojibiose, nigerose, isomaltulose).

REFERENCES


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**8th Starch Digestion Consortium Workshop**

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**Frequency of Sucrase Deficiency in Mucosal Biopsies**

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Carbohydrates, sugars, and starches are an important source of energy, especially for the brain, which is completely dependent on glucose for energy (1). The US Department of Agriculture recommends that carbohydrates provide 45% to 65% of daily energy units (2) and the dietary reference intakes set the adequate

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