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Modeling of cooked starch digestion process using recombinant human pancreatic α-amylase and maltase-glucoamylase for in vitro evaluation of α-glucosidase inhibitors

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ABSTRACT

In human, digestion of cooked starch mainly involves breaking down of α-amylase to α-limit dextrins and small linear malto-oligosaccharides, which are in turn hydrolyzed to glucose by the gut mucosal maltase-glucoamylase (MGAM). Human pancreatic α-amylase (HPA), amino- and carboxyl-terminal portions of MGAM (ntMGAM and ctMGAM) catalyze the hydrolysis of α-D-(1,4) glycosidic linkages in starch, playing a crucial role in the production of glucose in the human lumen. Accordingly, these enzymes are effective drug targets for the treatments of type 2 diabetes and obesity. In this study, a Plackett–Burman based statistical screening procedure was adopted to determine the most critical factors affecting cooked starch digestion by the combination of HPA, ctMGAM and ntMGAM. Six factors were tested and experimental results showed that pH and temperature were the major influencing factors, with optimal pH and temperature at 6.0 and 50 °C, respectively. Surprisingly, ntMGAM had no significant contribution to the glucose production from starch digestion compared to the HPA and ctMGAM. The optimal proportion of HPA and ctMGAM in a starch digestion system was further determined by response surface methodology. Results showed a maximum starch digestion (88.05%) within 0.5 h when used HPA:ctMGAM=1:9 (U). The inhibitory effects of various inhibitors on the cooked starch digestion by HPA_1/$ctMGAM_9$ were evaluated by determining their half maximal inhibitory concentration (IC_{50}) values. Acarviostatin II03 showed the highest inhibitory activity, with 67 times higher potency than acarbose. Moreover, acarviostatin II03 could significantly depress postprandial blood glucose levels in mice, better than that by acarbose. These findings suggest that our in vitro enzymatic system can simulate in vivo starch digestion process, and thus can be used to screen and evaluate α-glucosidase inhibitors.

1. Introduction

Starch constitutes the main source of energy for humans. Ingestion of starch in the human diet has received particular attention in recent years due to its nutritional value. The imbalance between energy consumption and dietary intake of energy-rich foods has a direct consequence in the development of metabolic diseases, such as obesity and diabetes.[1–3] Such adverse consequences have led to the study of enzyme/substrate or enzyme/inhibitor interactions in the starch digestion process.

Starch presents in two main molecular structures: mostly linear α-1,4-D-glucopyranose linked polymers known as amyloses, and those with a mixture of α-1,4- and α-1,6-branched a-D-glucopyranose linkages, known as amylopectins.[4] To generate dietary glucose from starchy foods, salivary and pancreatic α-amylase and four intestinal mucosal α-glucosidases are employed.[5] α-Amylase (enzyme class EC 3.2.1.1.) belongs to the glycoside hydrolase family 13 (GH13) and catalyzes the hydrolysis of α-(1,4) glycosidic linkages in starch.[6,7] It does not hydrolyze α-1,6- linkages, certain neighboring α-1,4-linkages and branch linkages with α-1,6 branched oligosaccharides (α-limit dextrins, αLDx).[8,9] In human, cooked starch digestion is mainly carried out by human pancreatic α-amylase (HPA) produced in the pancreas and excreted into the lumen.[10] The resulting hydrolysates, α-1,4-linked oligosaccharides and αLDx, are

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eventually hydrolyzed into glucose by maltase-glucoamylase (MGAM; EC 3.2.1.20 and 3.2.1.3) and sucrase-isomaltase (SI; EC 3.2.148 and 3.2.10) in the lumen.[11,12]

Human gut mucosal MGAM consists of two glycoside hydrolase family 31 (GH31) catalytic subunits, the N-terminal subunit (ntMGAM) and C-terminal subunit (ctMGAM).[13,14] The two subunits of human MGAM share high sequence identity (40%) and show similar digestion activities against α-1,4-linked substrates.[12] However, ntMGAM and ctMGAM have evolved different substrate specificities and inhibitor tolerance. ctMGAM has higher affinity for longer maltose oligosaccharides compared to the ntMGAM.[15,16] Structural studies revealed major differences in the active sites between ctMGAM and ntMGAM. The active site of ctMGAM has an extra segment of 21 amino acids compared to the ntMGAM. The extra residues positioned near the opening of catalytic site make ctMGAM likely to form more glucose binding subsites, and more flexible and adaptable active cleft.[17]

Inhibition of pancreatic α-amylase and/or intestinal α-glucosidase activity is currently applied to the treatment of type 2 diabetics to adjust glucose levels in the blood stream.[18,19] Acarbose, voglibose, miglitol and 1-deoxynojirimycin (DNJ) are commercially available α-glucosidase inhibitors with pseudo-carbohydrate structures, in which the oxygens are replaced by nitrogens (Fig. 1).[20–22] Acarviostatins are a series of amylose mimetics with a repeating pseudotrisaccharide unit (Fig. 1) and they have been found to possess potent inhibitory activities against α-amylase/glucosidase.[23,24]

In recent years, statistical approaches such as Plackett–Burman (PB) design and response surface methodology (RSM) have widely been applied in biochemical process optimization.[25–28] The PB design allows the screening of major factors from a large number of variables that affect the biochemical process. RSM is a collection of statistical and mathematical techniques useful for the improvement and optimization of complex processes. The main advantage of RSM is its ability to reduce the number of experimental trials needed to evaluate multiple parameters and their interactions, to obtain statistically acceptable results.[29,30] It has been successfully used to optimize process variables.[31,32]

Although the individual roles of these catalytic units (HPA, ntMGAM and ctMGAM) are relatively well studied, the combinatorial roles of the HPA and MGAM complexes in starch digestion process have not been investigated in depth. In this study, the optimal condition of in vitro cooked starch digestion by the human pancreatic α-amylase and maltase-glucoamylase was determined using PB and RSM. Using this model, we further evaluated the effects of various inhibitors on the cooked starch digestion process.

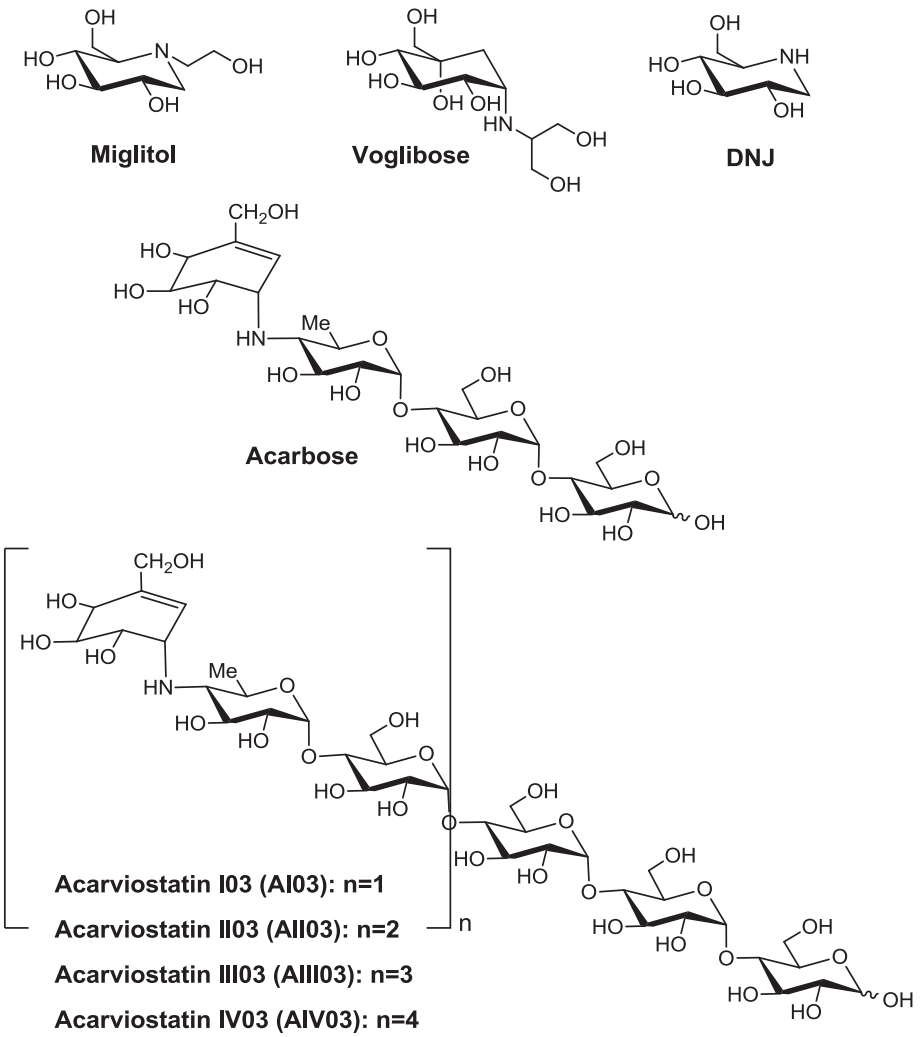

Fig. 1. Structures of inhibitors of α-amylase and α-glucosidase: miglitol, voglibose, DNJ, acarbose, AI03, AII03, AIII03 and AIV03.