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Inhibition of Human Intestinal α-Glucosidases by Calystegines

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Supporting Information

ABSTRACT: Calystegines are polyhydroxylated nortropane alkaloids found in Convolvulaceae, Solanaceae, and other plant families. These plants produce common fruits and vegetables. The calystegine structures resemble sugars and suggest interaction with enzymes of carbohydrate metabolism. Maltase and sucrase are α-glucosidases contributing to human carbohydrate degradation in the small intestine. Inhibition of these enzymes by orally administered drugs is one option for treatment of diabetes mellitus type 2. In this study, inhibition of maltase and sucrase by calystegines A_3 and B_2 purified from potatoes was investigated. In silico docking studies confirmed binding of both calystegines to the active sites of the enzymes. Calystegine A_3 showed low in vitro enzyme inhibition; calystegine B_2 inhibited mainly sucrose activity. Both compounds were not transported by Caco-2 cells indicating low systemic availability. Vegetables rich in calystegine B_2 should be further investigated as possible components of a diet preventing a steep increase in blood glucose after a carbohydrate-rich meal.

KEYWORDS: *Solanum tuberosum, Solanaceae, potato, human digestive α-glucosidase inhibition, maltase, sucrase, Caco-2 cell culture, calystegine A_3, calystegine B_2*

INTRODUCTION

Calystegines are nortropane alkaloids found in Convolvulaceae, Solanaceae, and further unrelated plant families like Brassicaceae, Erythroxylaceae, and Moraceae.[1] The nortropane skeleton carries three to five hydroxyl groups and thus resembles monosaccharides (Figure 1). Like other nitrogen-containing glycosidase inhibitors,[2] calystegines interact with various glycosidases. Strong inhibition was first found with β-glucosidase from sweet almonds and with a fungal α-galactosidase.[3] Later, mammalian enzymes, e.g., bovine liver β-galactosidase and pig kidney trehalase, were observed to be inhibited by calystegines.[4] Several alkaloidal glycosidase inhibitors were identified in forage plants, which caused potential lethal intoxications in farm animals.[5−7] Typical lesions were observed in neural and glandular tissues after the hydoxylated alkaloids had been absorbed from the feed into the blood of the animals.[8] The *Ipomoea* species that were investigated contain calystegines but also swainsonine (**1**), an alkaloidal glycosidase inhibitor with potent toxicity. Calystegines alone have rarely been investigated for their toxicity. In experimental mice after injection they proved rather nontoxic on whole animals; however, histological examination revealed liver cell alterations with high doses (140 mg/kg/day) of calystegine A_3 (**2**).[9] Swainsonine, in contrast, exhibited mouse toxicity, although mice as rodents are less sensitive toward glycosidase inhibitor toxicity and demand higher doses than ruminants like sheep and goats.[9]

Various fruits and vegetables contain calystegines in micromolar concentrations. Potato tubers are rich in calystegines A_3 and B_2 (**3**), the content depending on the cultivar and the part of the tuber that was analyzed.[10,11] Vegetables from Solanaceae such as sweet peppers (*Capsicum annuum*) and eggplant (*Solanum melongena*) and from Convolvulaceae, e.g., sweet potatoes (*Ipomoea batatas*) contain predominantly calystegine B_2.[12] These plants are consumed in many parts of the world; in the case of potatoes they are a major source of dietary carbohydrates. If calystegines act on intestinal glycosidases, it is conceivable that these vegetables in the human diet inhibit or retard carbohydrate digestion to a moderate extent.

Maltase and sucrase are major carbohydrate digesting α-glucosidases in the mammalian small intestine. Food starch is degraded in the human body by amylases from saliva and from the pancreas, and the breakdown products, maltose (**4**), isomaltose, and dextrins with a chain length of 2−8 glucose units, are further cleaved into monomers by glucoamylase, maltase, and isomaltase activities. Sucrose (**5**), a major sweetener of human food, is a α-glucoside of fructose and cleaved by intestinal sucrase. These four enzyme activities are localized in two heterodimeric proteins: maltase−glucoamylase and sucrase−isomaltase. They are bound to the cell membrane of intestinal brush border cells with the active sites directed to the intestinal lumen. Maltase and sucrase are grouped into glycosidase family 31 based on their amino acid sequences.[13] Recently, the N-terminal moieties of both proteins were crystallized after heterologous expression in insect cells[14,15] allowing insight into substrate and inhibitor binding to these enzyme subunits. As the velocity of these α-glucosidases is rate-limiting for carbohydrate digestion,[16] the inhibition of the

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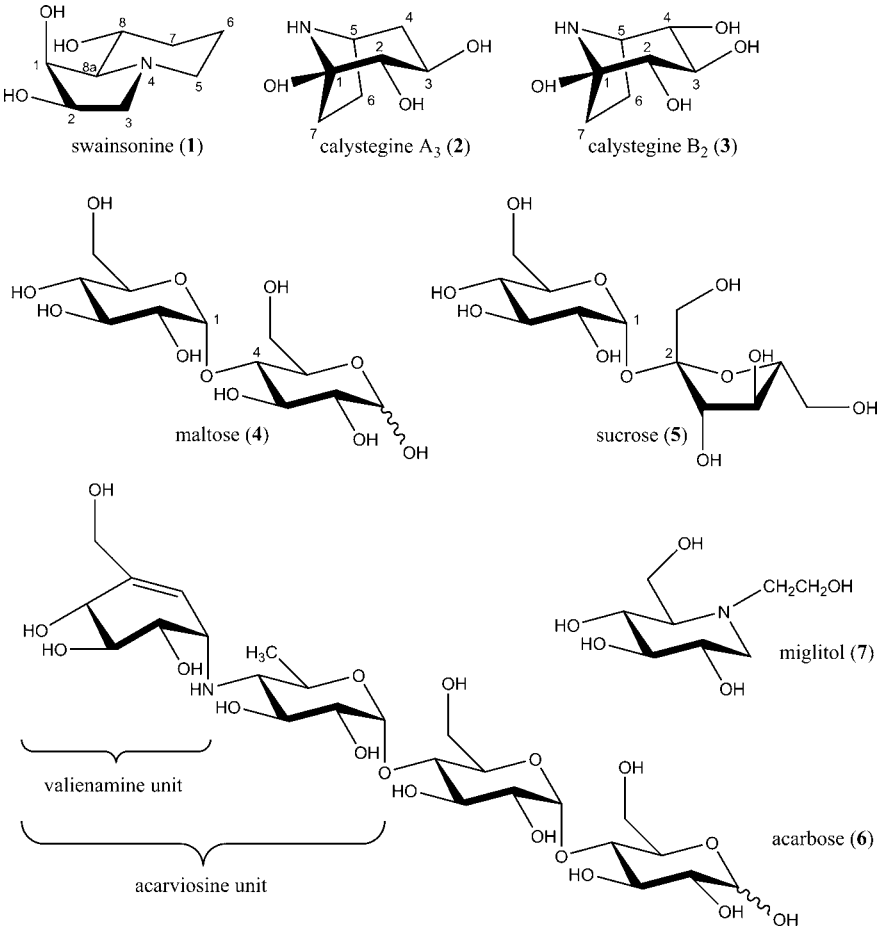

Figure 1. Structures of substrates and inhibitors.

enzyme activities has become an important option for the treatment of diabetes. In diabetic patients short-term elevated blood glucose levels after a carbohydrate-rich meal exert long-term detrimental micro- and macrovascular, renal, and coronary damages.[17−19] Cases of type 2 diabetes are rising worldwide, and this type of diabetes is distinguished from the autoimmune type 1 by a correlation with nutrition and life style habits. Type 2 diabetes, in contrast to the sudden onset of type 1 diabetes, develops slowly in most patients. It is often preceded by a prediabetes status called metabolic syndrome that is characterized by obesity, high blood pressure, and increased blood glucose levels after a meal due to less insulin sensitivity of the tissues. For these patients, clearly, a steep rise in blood glucose is adverse and should be avoided by prudent food selection, e.g., a preference for carbohydrate sources that are digested slowly. Glycosidase inhibitors are applied medicinally to help control postprandial blood glucose levels.[20] The drugs acarbose (**6**) and miglitol (**7**) predominantly inhibit maltase−glucoamylase and sucrase−isomaltase; they retard carbohydrate absorption and support mitigation of blood glucose peaks. In traditional Chinese medicine, the extract from mulberry leaves is used orally for prevention and treatment of diabetes. Mulberry extract contains glycosidase inhibitors and lowers postprandial blood glucose.[21]

The goal of this study is the assessment of potential effects of calystegines from food on carbohydrate digestion. Calystegines A_3 and B_2 are the major calystegines of several foods and vegetables. They could fit into the active sites of maltase and sucrase similarly to acarbose. Digestive enzymes in vitro vary considerably in their sensitivity toward glycosidase inhibitors depending on their origin, e.g., human or animal, and on the way of preparation, native or heterologously expressed.[16,22−24] Therefore, we chose to work with human enzymes prepared from human Caco-2 cell cultures that serve as a model for intestinal cell transport and have been used for measuring digestive enzyme inhibition.[25,26] Caco-2 cells were also applied to study transport of calystegines, which would be a prerequisite for systemic intoxication.

MATERIAL AND METHODS

Material. The Caco-2 cell line was obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). Cell culture reagents were purchased from PAA Laboratories GmbH (Cölbe, Germany), except fetal bovine serum (Biochrom AG, Berlin, Germany). Permeability test buffer components were provided by Sigma-Aldrich Chemical GmbH (Taufkirchen, Germany), except MES, HEPES, and Tris (Carl Roth GmbH, Karlsruhe, Germany). D-[^{14}C]Mannitol solution (1.89 mM, specific radioactivity 53 mCi/mmol, radioactivity concentration 100 μCi/mL) was obtained from Moravek Biochemicals (Brea, CA, U.S.A.). Sweet almond β-glucosidase (EC 3.2.1.21) was obtained from Sigma. Enzyme substrates, products, and inhibitors were from the following: maltose, glucose (Merck, Darmstadt, Germany), sucrose, fructose (Roth, Karlsruhe, Germany), acarbose (Bayer, Leverkusen, Germany), *p*-nitrophenyl β-D-glucoside (Diagonal GmbH & Co. KG, Münster, Germany). All other chemicals, including material and reagents for calystegine purification and derivatization, were from Merck (Darmstadt, Germany), except silylation reagents (AppliChem GmbH, Darmstadt, Germany). Calystegines A_3 and B_2 were extracted

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and purified from the potato sprouts (Rosara cultivar) as described elsewhere.[4,27] Briefly, lyophilized potato sprouts were extracted with water−methanol mixture (1:1). Methanol was evaporated, and calystegines were retained from the extract on a strong cation-exchange column. After elution with 0.5 NH_4OH, the calystegines were separated from contaminants, mostly amino acids, by an anion-exchange column eluted with water. A weak cation-exchange column then served to separate calystegine A_3 from calystegine B_2. Substrates and inhibitors were dissolved in bidistilled water.

Molecular Modeling and Docking Studies. All ligands to be docked were constructed with the SYBYL version 8.0 (Tripos International, St. Louis, MO, U.S.A.) modeling suite and energy-minimized with the TRIPOS force field[28] using Gasteiger charges.[29,30] The X-ray structures of the N-terminal domain of sucrase−isomaltase (3LPP)[15] and of human intestinal maltase−glucoamylase (3L4X and 2QMJ)[14] were downloaded from the protein database (PDB at Research Collaboratory for Structural Bioinformatics, RCSB). All hydrogen atoms were added by the 3d-protonate module of Molecular Operating Environment (MOE) version 2007.09 (Chemical Computing Group Inc., Montreal, QC, Canada). Subsequently, partial charges were assigned by using Gasteiger charges. All ligands were docked to the active site of the proteins using standard settings of Genetic Optimized Ligand Docking (GOLD) (Genetic Optimized Ligand Docking, Cambridge Crystallographic Data Centre)[31−33] allowing a maximum output of 30 docking arrangements. The active sites were defined by a radius of 15 Å around the $O_{\delta 2}$ atoms of Asp443 (3L4X) and Asp472 (3LPP). Those with the best docking score (Table 1) were optimized with the Tripos force field. For this purpose only those amino acid residues in the direct interaction contact to the ligands were considered to be flexible, whereas all other atoms of the protein were fixed. The resulting interaction energies were calculated by subtraction of the energies of the empty enzymes and the isolated ligands from the energy of the complex. Solvent effects were not included.

Table 1. GOLD Docking Scores and Interaction Energies[a]

	maltase		sucrase	
compd	GOLD score	interaction energy [kcal/mol]	GOLD score	interaction energy [kcal/mol]
acarbose	64.5	n.d.	56.3	n.d.
calystegine A_3	51.3	−49.1	52.7	−55.0
calystegine B_2	48.9	−48.7	49.4	−53.3

[a]GOLD scores have no unit and reflect fitness of the ligands. Higher values indicate higher affinities of the ligands to the protein. More negative interaction energy indicates higher affinity.

Cell Culture. Caco-2 cells were cultivated in 75 cm^2 culture flasks (Greiner, Frickenhausen, Germany) with Eagle's minimum essential medium supplemented with 10% fetal bovine serum, 1% nonessential amino acid solution, and gentamicin (50 μg/mL), in 5% CO_2−95% air atmosphere at 37 °C. Medium was replaced on day 1 after seeding and thereafter every 2 days. Cell density for cultivation was 0.8×10^6 per flask; after 4 days 80−90% confluence was reached.[34] The confluent cell layers were dispersed by trypsinization and resuspended in medium and subcultured in 175 cm^2 culture flasks for enzyme assays. Flasks were used for enzyme α-glucosidase measurements 7 days after subcultivation, because sucrase and maltase activity increase during cultivation.[35,36] Total cell number in assay flasks was 7×10^6 (cell density 40 000/cm^2). Transport assays were performed in plates with six Transwell permeable inserts of polycarbonate microporous membrane (4.67 cm^2, pore size 3 μm, Corning Life Sciences, Schiphol-Rijk, The Netherlands). Cell suspensions were placed on the insert membranes at a density of 43 000−44 000 cells/cm^2 and cultivated for 21 days. Medium was changed on day 1 after subculture, then every 2 days and on the day before the experiment.

Cell Extract Preparation. Caco-2 cells cultured for 7 days were washed twice with ice-cold 20 mM phosphate buffer pH 6.0,[37] scraped with rubber cell scrapers, resuspended, and homogenized in 8−10 mL of buffer per flask. The cells were lysed by freezing at −80 °C and thawing at 37 °C twice, then pulled twice through 24 G cannulas (Braun-Sterican, Melsungen, Germany). The homogenized cell suspension was centrifuged 30 min at 12 000*g* and 4 °C. Yield of soluble protein after centrifugation was 4.6 mg of protein per flask in a volume of 450 μg/mL.[38] Enzyme activity was assayed using the supernatant.

Maltase and Sucrase Assay. Linearity of enzyme activities[39] was confirmed for the protein concentrations between 120 and 490 μg/mL and up to 120 min. The amount of protein applied in the standard assay was 200 μg. Amounts of 25 μL of substrate and 50 μL of double-distilled water or inhibitor solution were mixed, and the assay was started by adding 450 μL of cell extract. Samples were incubated for 60 min at 37 °C and 600 rpm. The reaction was stopped by heating the samples for 10 min at 95 °C and 24 rpm. Flocculated proteins were centrifuged for 10 min at 12 000*g* and 4 °C. Substrate concentrations in the assay were 0.5, 5, 25, 50, and 75 mM, inhibitors were acarbose 5 μM, calystegine A_3, and calystegine B_2, both 119 and 476 μM. Blank samples were prepared with inactivated protein extract (10 min at 95 °C) in the assay mixture. Control experiments contained the substrates with 20 mM phosphate buffer instead of protein extract. With 25 mM substrate, proteins inactivated by heating showed low maltase activity (3.5% of the active enzyme) but surprisingly relatively high sucrase activity considering the inactivation (9% of the active enzyme).

Enzyme Product Analysis. The supernatants after centrifugation of the maltase/sucrase assay samples were used for product analysis by gas chromatography (GC). Lyophilized samples were silylated with 20 μL of hexamethyldisilazane and 5 μL of trimethylchlorosilane in 15 μL of pyridine for 30 min at 60 °C. Azobenzene in *n*-hexane was used as an internal standard. Total silylation volume amounted to 125 μL. GC separation was performed on a HP Agilent 6890 GC (Agilent, Santa Clara, CA, U.S.A.) gas chromatograph with 3 μL split injection in a split ratio of 20:1. The column used was a 30 m × 0.25 mm i.d., 0.25 μm film thickness, FS-Supreme-5 with the carrier gas helium (1 mL/min constant flow), injection temperature of 250 °C, detector temperature of 310 °C, and a temperature program: 160 °C, 5 °C/min up to 240 °C, then 10 °C/min up to 300 °C. The run time was 26 min, including 2 min hold time after injection and 2 min post run at the end. Quantitation was done using the FID signal for sugars and calystegines as well as the FID/NPD (flame ionization detector/nitrogen/phosphorous detector) signal ratio for peak purity of calystegines.[40] Glucose content in the enzyme solution was measured to be below 1 μg/mL and did not interfere with product measurements. Calibration curves were made for calystegines, sugar substrates, and products, each 0.01−50 mg/mL. Injection volume was 3 μL. The quantitation limit for sugars was 0.8 μg/mL silylated sample corresponding to 2.4 ng of sugar per injection; for calystegines the quantitation limit was 10 μg/mL silylated mixture. Linearity was ensured up to 4 mg of sugar/mL of silylated sample (12 μg of sugar/injection) and 0.6 mg of calystegine/mL of silylated sample. The GC sample volumes of the assay samples were chosen so that they fitted to this range. Maltase and sucrase activities were calculated by glucose concentration converted from maltose or sucrose and were indicated as picokatal (pkat) per mg of protein. Enzyme kinetic parameters of maltase and sucrase (K_m and V_{max}; K_i of calystegines) were calculated by SigmaPlot 10.0 program and the enzyme kinetics 1.3 Module (Systat Software Inc., San Jose, CA, U.S.A.) and given as means ± standard errors of 3−6 assay samples. K_i values for calystegines were calculated using specific enzyme activities with both calystegine concentrations (119 and 476 μM). Since acarbose was used as inhibition control in only one concentration, 5 μM, the K_i value for acarbose was calculated with the equation for single inhibitor concentration using the kinetic parameters obtained by SigmaPlot. Therefore, standard errors for K_i of acarbose are not available. Inhibition type for calystegines was competitive.

Calystegine Transport and Caco-2 Cell Permeability Experiments. Transepithelial electrical resistance (TEER) of cell monolayers

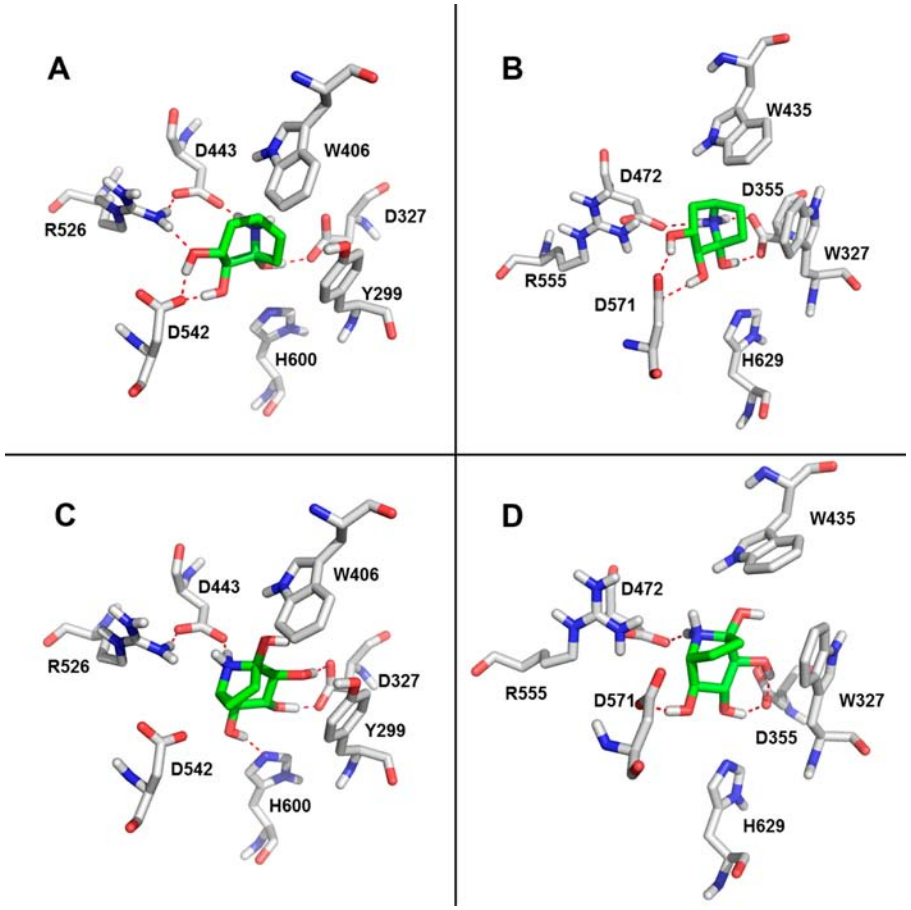

Figure 2. Docking arrangements of calystegines in the active centers of maltase and sucrase. Dotted red lines represent hydrogen bonds connecting calystegine hydroxyl and amino groups with amino acid residues. (A) Calystegine A_3 and maltase: five H-bonds between C_1OH and D327, C_2OH and C_3OH and D542, C_3OH and R526, and NH_2^+ and D443. (B) Calystegine A_3 and sucrase: five H-bonds between C_1OH and D355, C_2OH and D571, C_3OH and D571, NH_2^+ and D355, and NH_2^+ and D472. (C) Calystegine B_2 and maltase: three H-bonds between C_2OH and D327, C_3OH and D327, and NH_2^+ and D443; H-bond between C_4OH and H600, H-bond between C_1OH and NH and W406 (distance 2.4 Å, not indicated). (D) Calystegine B_2 and sucrase: four H-bonds between C_2OH and D355, C_3OH and D355, C_4OH and D571, NH_2^+ and D472; H-bond between C_1OH and NH and W435 (distance 2.5 Å, not indicated).

on Transwell inserts was checked on the day of the experiment using a Millicell ERS ohmmeter (Millipore Co., Bedford, Billerica, MA, U.S.A.). TEER was 913 ± 69 Ω·cm^2. Cell culture medium was removed, and the membranes were rinsed two times with donor buffer (37 °C). Donor buffer was 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM glucose, and 25 mM MES/Tris, pH 6.0. Acceptor compartment buffer was the same, only 25 mM HEPES/Tris was set to pH 7.5. The experiment started by filling the donor (luminal) compartments with 2.5 and 5 mM calystegine A_3 and B_2 solution in donor buffer, each in triplicate. Samples were collected from donor and acceptor compartments. Donor samples (100 μL) were taken after 0 and 120 min. Acceptor compartment samples (200 μL) were taken after 10, 30, 60, and 120 min. Three assays were prepared for each calystegine concentration. All samples were replaced immediately after the sampling by corresponding compartment solutions. During the experiment, multiwell Transwell chambers were kept in the incubator at 37 °C and 150 rpm. After 120 min, TEER was again measured to confirm the integrity of the monolayers. Calystegine B_2 in the acceptor buffer was measured enzymatically by the inhibition of sweet almond β-glucosidase cleavage of *p*-nitrophenyglucoside, based on competitive inhibition. β-Glucosidase activity was measured by the colorimetric glucose assay TRINDER (Sigma-Aldrich, Taufkirchen, Germany; principle: glucose oxidase, peroxidase, aminoantipyrine, *p*-hydroxybenzene sulfonate) using small assay volumes in a microplate reader (Infinite 200 Pro, Tecan, Männedorf, Switzerland). Detection limit was 30 ng of calystegines B_2/mL of sample. Calystegine A_3 was analyzed by GC.

Caco-2 cell monolayer integrity was also monitored by measuring the transepithelial flux of D-[^{14}C]mannitol (10 μM) included in donor calystegine solutions for 120 min. Samples of 100 μL from three assays were mixed with 2.8 mL of Rotiszint eco plus scintillation cocktail (Roth, Karlsruhe, Germany) and measured in a liquid scintillation spectrometer (Tri-Carb-2100 TR, Perkin-Elmer, Waltham, MA, U.S.A.), 5 min per sample.

RESULTS AND DISCUSSION

In Silico Docking of Calystegines to Maltase and Sucrase. As a first approach to a possible inhibition of intestinal α-glucosidases, docking of calystegines to protein models of maltase and sucrase was examined. The crystal structures of N-terminal parts of human sucrase−isomaltase (3LPP) and maltase−glucoamylase (3L4X, 2QMJ) are available. Docking of acarbose to maltase resulted in a position for the ligand coinciding with that of the resolved crystal structure (2QMJ; RMSD 1.24 Å) and confirmed the validity of the docking procedure. Docking positions for calystegines A_3 and B_2 with the best scores in the active sites of maltase and sucrase were selected (Figure 2). Maltase and sucrase are retaining glycosidases[13] implying that a catalytic acid−base and a nucleophile residue in the enzyme attack the glycosidic bond

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in a two-step mechanism. Each step inverts the configuration of the anomeric glycoside oxygen and leads to an overall retention of the oxygen configuration.[41] The formation of a salt bridge was observed in all docking arrangements between the protonated amino groups of the calystegines and the aspartate side chains of catalytic nucleophiles (D472 in sucrase, D443 in maltase). Calystegines are further positioned by hydrogen bonds. Hydroxyl groups of calystegine A_3 bind to side chains of the acid−base-catalytic aspartate residues (D542 and D327 in maltase, D571 and D355 in sucrase). Hydroxyl groups of calystegine B_2 bind to the same aspartate residues, to histidine 600 in maltase, and possibly, to the NH group of tryptophan 406 in maltase and tryptophan 435 in sucrase. GOLD-scoring values for calystegines largely coincide (Table 1). Due to the larger size of acarbose and more interactions with the proteins in comparison to calystegines, docking scores for acarbose in maltase and sucrase are higher. Interaction energies for both calystegines indicate higher affinity (more negative values) for sucrase than for maltase. Since the only major difference in the active site is the replacement of tyrosine 299 in maltase by tryptophan 327 in sucrase, hydrophobic interaction of the tryptophan side chain with the ethylene bridge in the calystegines (Figure 3) is likely responsible for the better

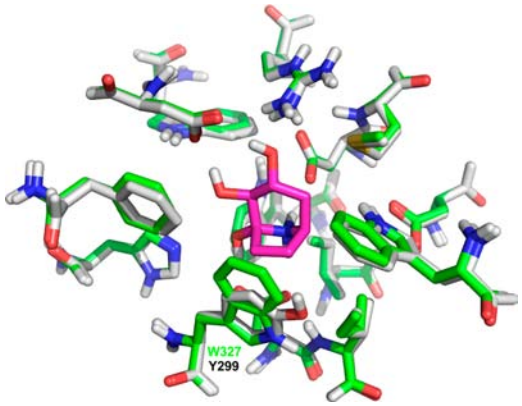

Figure 3. Superposition of the active sites of maltase (PDB 3L4X, gray carbon atoms) and sucrase (PDB 3LPP, green carbon atoms) with calystegine A_3 as ligand (magenta). The only difference, tyrosine 299 in maltase replaced by tryptophan 327 in sucrose, is indicated.

interaction energies in sucrase. In summary, the docking studies support the potential of calystegines to be inhibitors of maltase and sucrase.

Enzyme Inhibition. Maltase and sucrase activities were determined in the supernatant of homogenized Caco-2 cells. Attempts to enrich the membrane-bound glycosidases by ultracentrifugation yielded nonreproducible total activities, indicating that the enzyme proteins suffered from the calcium-containing centrifugation medium or the resuspension procedures. Activity measurements in the supernatant were reproducible (Table 2). Both activities showed substrate saturation and were suitable to Michaelis−Menten kinetic calculations (Figure 4), although maltase activity resulted from four different active sites, i.e., from both subunits of each, MGAM (maltase−glucoamylase), and SI (sucrase−isomaltase).[22] Sucrase activity is exerted by one enzyme species only, the C-terminal subunit of SI. K_m values published for human maltase activity are similar to our findings (7.3 mM); in the lysate from human intestinal mucosa K_m of 10.7 mM for maltose was reported,[16] while the N-terminal maltase moiety of human MGAM heterologously expressed in *Drosophila* S2 cell system showed K_m of 4.6 mM.[42] A K_m value for sucrose on human sucrase has not been published. The inhibitory effect of acarbose on maltase and sucrase was confirmed. K_i values for acarbose showed that maltase is more susceptible to acarbose inhibition than sucrase (K_i of 4.4 vs 7.8 μM) (Table 2). With higher substrate concentrations, sucrase was inhibited more strongly by acarbose than maltase; at 50 mM substrate, sucrase decreased to approximately one-third of the activity without inhibitor, while the total maltase activities still was at 76% of the corresponding noninhibited enzyme (Figure 4). Maltase sensitivity to acarbose appears highly variable, depending on the source of enzyme, native, precipitated, or heterologously expressed, and on the subunit(s) tested[23] and ranged from K_i of 0.84 μM in human intestinal mucosa lysate[43] to K_i of 62 μM for the heterologously expressed N-terminal subunit of human MGAM.[42] Sucrase sensitivity for acarbose from immunoprecipitated human intestinal mucosa was reported to be lower with a K_i of 5.3 mM.

Both calystegine A_3 and B_2 inhibited sucrase and maltase activities. Sucrase (K_i of 55 μM for calystegine B_2, K_i of 227 μM for calystegine A_3) was affected more severely than maltase (K_i of 582 μM for calystegine B_2; no K_i for calystegine A_3 was calculable because of weak inhibition and high V_{max}), supporting the results of interaction energy obtained from docking (Table 1). The slight differences between calystegine A_3 and B_2 interaction energy on the sucrase pointed to calystegine A_3 as the stronger inhibitor, which was not confirmed by the in vitro data. Such small calculated differences must consequently be rated as insignificant. Overall inhibition of maltase by calystegines is weak, and it is conceivable that the maltase activities in each of the two α-glycosidase subunits in the Caco-2 cell wall were affected differentially. Different inhibitory activity of acarbose on various maltase preparations supports this assumption. The mode of inhibition by calystegine A_3 and B_2 and by acarbose was competitive judging by enzyme kinetics nonlinear fit parameters of both enzymes.

Table 2. Maltase and Sucrase Inhibition by Calystegines A_3 and B_2[a]

	maltase				sucrase			
	V_{max} [pkat/mg]	K_m(maltose) [mM]	R^2 [b]	K_i [μM]	V_{max} [pkat/mg]	K_m(sucrose) [mM]	R^2 [b]	K_i [μM]
no inhibitor	827 ± 12	7.3 ± 0.5	0.9938		412 ± 23	11.1 ± 2.5	0.9570	
acarbose 5 μM	725 ± 11	15.6 ± 0.8[c]	0.9980	4.4	166 ± 11	18.2 ± 3.9[c]	0.9678	7.8
calystegine A_3 476 μM	939 ± 23	11.1 ± 1.1[c]	0.9913	*d*	298 ± 7	12.5 ± 1.1[c]	0.9939	227 ± 47
calystegine B_2 476 μM	1016 ± 22	25.4 ± 1.5[c]	0.9980	582 ± 144	236 ± 8	25.4 ± 2.3[c]	0.9948	55 ± 12

[a]Kinetic parameters are given as means ± standard error (n = 3−6). [b]Correlation coefficient, goodness of fit between single values and the kinetic parameters derived from the activity curve. [c]Apparent K_m values for inhibited enzyme. [d]Test for inhibition models by SigmaPlot failed, as V_{max} was higher than maltase V_{max} without inhibitor (Figure 4).

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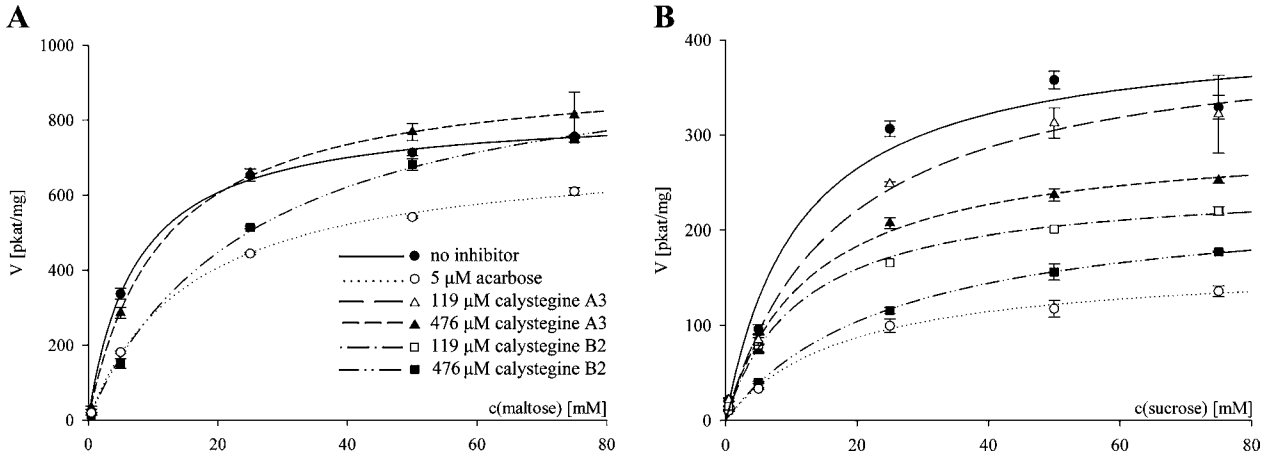

Figure 4. Enzyme activities of maltase (A) and sucrase (B) in Michaelis−Menten plots. Maltase activity in presence of 119 μM calystegines A_3 and B_2 (not shown) was indistinguishable from noninhibited activity. Error bars on the data points show standard error resulting from $n = 3-6$.

The relevance of these results for food digestion was estimated by the calystegine content in vegetables and potato cultivars. Maltase activity on the whole as measured here appears rather unaffected. Sucrase becomes the default α-glucosidase by the luminal "starch brake" of maltase observed after a starch-rich meal.[23] Potatoes contain predominantly calystegine B_2, which can amount to up to 57 mg/kg fresh potato flesh without peel.[10] When whole tubers were analyzed, 239 mg/kg dry mass of calystegine B_2 were found.[44] With portions of 200 g of those potatoes, the calystegine B_2 concentration in an assumed stomach volume of 1 L after the meal[45] reaches 55−65 μM. The concentrations are similar to the K_i of calystegine B_2 for sucrase, indicating a noteworthy inhibition of sucrose digestion. When the stomach content is emptied into the small intestine and further diluted, inhibition is still possible. It has to be tested whether maltose hydrolysis by sucrase is equally inhibited by calystegine B_2. Some other vegetables consumed worldwide are also rich in calystegines. Sweet potatoes (*I. batatas*), sweet peppers (*C. annuum*), and eggplant (*S. melongena*) contain 19, 37, and 73 mg of calystegine B_2 per kg of fresh weight.[12] Portions of 200 g of those vegetables would result in 22−83 μM calystegine B_2 in the stomach, again concentrations that are estimated to seriously inhibit sucrase.

Calystegine Transport through Epithelial Cells. Considering the high calystegine concentrations that may be present in the intestine after a potato-rich meal, possible absorption of the compounds was investigated. Concern over calystegine neurological toxicity arose mostly from farm animal intoxications[44,46] observed with plants, e.g., *Ipomoea* and *Solanum* species that contained other alkaloidal toxins in addition to calystegines. Caco-2 cells were used as model for intestinal transport. Calystegines A_3 and B_2 in concentrations of 2.5 and 5 mM were applied to the donor solution of a confluent cell monolayer in Transwell chambers. Transport of D-[^{14}C]mannitol applied together with 5 mM calystegine A_3 and B_2 for control of interruption of cell confluence by calystegines was below that of the D-[^{14}C]mannitol control (0.5%/h/cm^2). Lack of an increased mannitol transport by calystegines showed that Caco-2 cell monolayers remained intact and were not damaged by calystegine within 2 h at 37 °C. The donor compartments maintained 100% (±10%) of their initially supplied calystegine concentration for 2 h. In acceptor compartments calystegine B_2 was additionally analyzed using the β-glucosidase assay because the detection limit of this method (30 ng/mL acceptor buffer) was ca. 5 times lower than the GC detection limit. Concentrations of ca. 1 μM in the acceptor compartment could be detected. The concentration of calystegine B_2 measured in acceptor compartments after 2 h was below 0.1% of the initial donor concentration. From the almost complete calystegine retention in the donor compartments and the negligible transepithelial flux we conclude that calystegines are not transported across confluent Caco-2 cell monolayers. Judging from the calystegine structures, the hydrophilicity will impede simple diffusion across cell membranes. In summary, Caco-2 cells did not transport calystegines, and absorption of calystegines into the intestinal mucosa and across the intestinal barrier into the blood appears negligible.

Our results indicate that calystegines can affect the extent and the velocity of intestinal glucose absorption, and they may influence postprandial levels of blood glucose. This might be important for the role of potatoes in dietary recommendations that aim at avoiding obesity and type 2 diabetes. Literature data for the glycemic index (GI) for meals containing potatoes vary considerably. The glycemic index quantitates the effect of foods on blood glucose relative to the effect of the same quantity of standard food, mostly glucose, in healthy individuals. The rise in blood glucose after pure glucose ingestion during 2 h is set as a GI of 100. GI of boiled potatoes were reported between 56 and 101, the variation being attributed to the way of preparation, the maturity of the tubers,[47] and the potato cultivar.[48] It is conceivable that also calystegine levels influence the GI of potato dishes. Young and small potatoes were reported to cause lower GI, which was tentatively explained by a different starch texture.[48] Young and small potatoes equally contain a higher total content of calystegines, as most of the calystegines are localized in the outer two millimeters of the tuber and the ratio of surface to core is higher in small potatoes.[49] Before calystegine-rich vegetables and potatoes are recommended as foods contributing to the management of GI, studies with human volunteers and clinical studies are necessary.

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■ ASSOCIATED CONTENT

Supporting Information

Figure S1, gas chromatagrams of maltose, glucose, and calystegine B_2 after silylation. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

K_i, enzyme−inhibitor complex dissociation constant; K_m, Michaelis−Menten constant; V_{max}, enzyme maximum specific activity; GI, glycemic index; GOLD, Genetic Optimized Ligand Docking (docking program)

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