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Selective α-glucosidase substrates and inhibitors containing short aromatic peptidyl moieties

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ABSTRACT

We constructed a library of sugar-dipeptide conjugate to find out the best complementary against hydrophobic pocket of α-glucosidase. The best substrate showed 150-fold improved K_m value relative *p*-acetaminophenyl-α-D-glucopyranoside for α-glucosidase from *Bacillus stearothermophillus*. Using information from the complementary, we synthesized sp-WY and β-Glc-sp-WY, which selectivity inhibited the cognate enzyme.

α-Glucosidases comprise one of the most abundant families of enzymes found in various living systems, ranging from microorganism to more evolved animals and plants. Because these enzymes are essential for maintaining catabolic metabolism, they are potentially important targets for treating pathogenic features of organisms. Many types of inhibitors of members of this family of enzymes exist, including even commercially available chemotherapeutic agents for the treatment of human metabolic disease.[1] Some of the inhibitors of α-glucosidases contain several monosaccharide rings and multiple hydroxyl functional groups,[2] which lead to strong nanomolar binding affinities at carbohydrate binding sites. However, substances of this type generally lack specificity in which they inhibit a range of isozymes.

Other inhibitors of α-glucosidases, which do not contain carbohydrate structures and polyhydroxyl substitution, derive from natural sources.[3] In the family of non-carbohydrate type inhibitors, aromatic residues are frequently observed to be the key pharmacophores. Consequently, substances of this type display noncompetitive or mixed type inhibition owing to the fact that they partially share binding sites with carbohydrate substrates. In the early reported crystal structures of endo-α-glucosidases, roomy binding sites (+1 and +2 region) have been observed, which are composed not only of hydrophilic amino acid side chains but also hydrophobic[4] and aromatic[5] groups of amino acids that interact with aromatic substituents of the inhibitors through π–π interactions. Furthermore, since these regions are distant from the conserved catalytic core carboxylate residues, different amino acid residues are frequently located at distant sites in various isozymes.[6] Thus, by properly introducing aromatic moieties into inhibitors that can be recognized by residues outside of the carbohydrate binding region, it might be possible to create selectivity in inhibitor binding to different isozymes of α-glucosidases.

For the most part, typical carbohydrate containing inhibitors have been designed to target carbohydrate binding sites of α-glucosidases and, as a result, they bind competitively with substrates of the enzymes. However, several substrates have been designed that contain simple chromogenic groups covalently attached to the glycosidic centers in carbohydrates.[7] Typical chromogenic groups incorporate aromatic residues, such as nitrophenol and coumarin, which change color or fluorescence intensity when the glycosidic bond is cleaved during the enzyme catalyzed hydrolysis reactions. Thus, sufficient space must be available (+1 and +2 region) to accommodate these chromogenic moieties in the active sites of α-glucosidases. This portion of the enzyme active sites, however, has seldom been targeted in the design of inhibitors for α-glucosidases and their isozymes.[8]

We hypothesized that inhibitors, which incorporate appropriate aromatic residues that interact with groups in the non-carbohydrate recognizing areas of these enzymes, might display binding selectively to the target enzymes. Consequently, we felt that a study involving construction and evaluation of libraries of various aromatic ring containing substances that can bind to the non-carbohydrate region, might uncover selective α-glucosidase substrates and inhibitors. Below, we describe the results of an investigation in which a library of peptidyl glucose substrates was prepared and their activities against various α-glucosidases were carried out.

Substrates prepared in the initial library were designed to contain tethered aromatic amino acid groups on opposite sides of carbohydrate moieties. Since the plan was to have substrate

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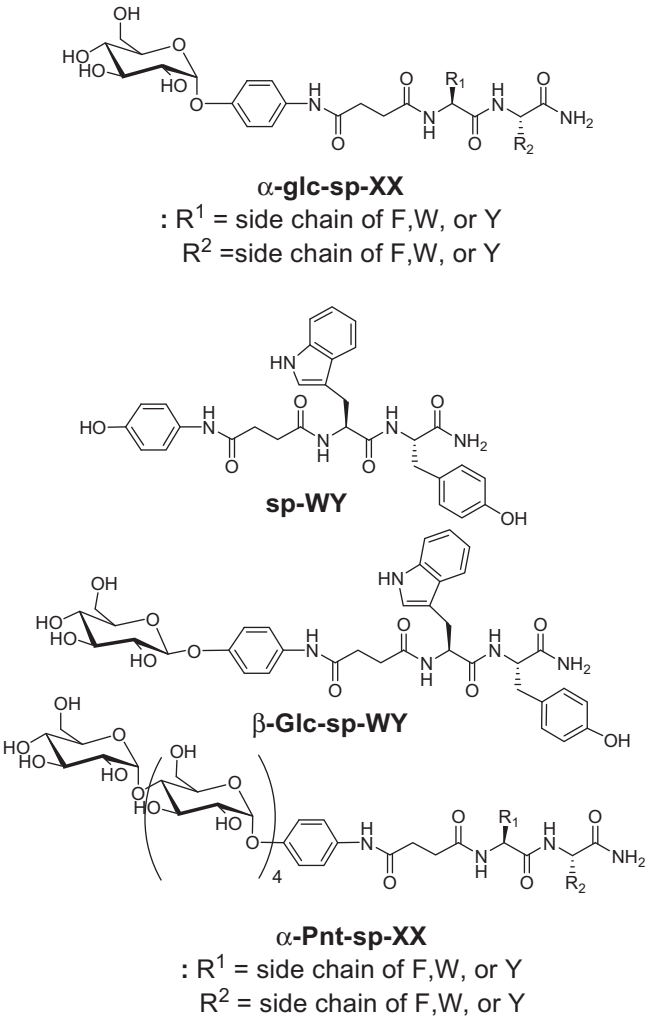
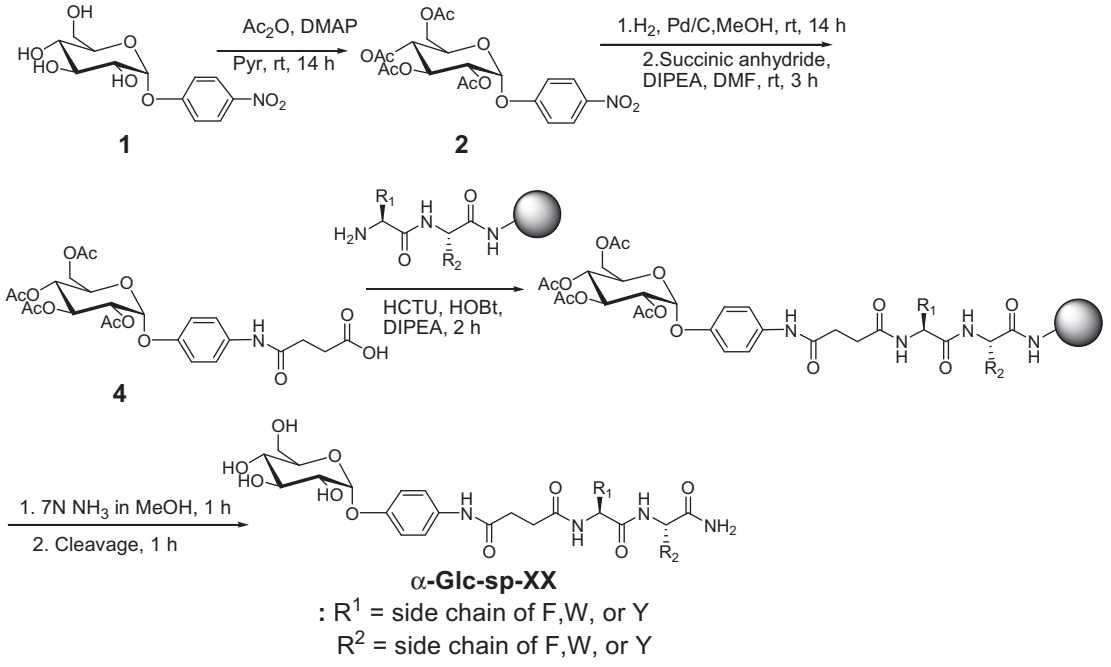


Figure 1. Structures of inhibitors and substrate libraries. Synthesises of these compounds are described in Supplementary data.

structures that are complementary with amino acid side chains in the hydrophobic pockets of the enzymes, only three aromatic amino acids (Trp, Phe, and Tyr) were used as building blocks. The nitro group of *p*-nitrophenyl-α-D-glucopyranoside was reduced to produce the corresponding aniline derivative, which enabled introduction of a succinamide side chain required to make the carbohydrate suitable for solid phase peptide synthesis. Fmoc-protected aromatic amino acids were used to make nine dimeric peptides that were employed in reactions with 2,3,4,6-*O*-tetraacetyl-(*p*-succinyl)anilino-α-D-glucopyranoside to produce the corresponding protected dipeptidyl-α-D-glucopyranosides. Protecting group removal followed by cleavage from the solid support afforded the peptidyl substrates (α-Glc-sp-XX, Fig. 1) that were purified by HPLC, giving 30–40% isolated yields for each substrate (Scheme 1).

α-Glucosidases from *Bacillus stearothermophillus*, rice, and rat intestine were used to assay the substrate activities of the peptidyl substrate library. For initial screening, equimolar mixtures of individual members of the substrate library along with the typical non-peptidyl substrate, *p*-nitrophenyl-α-D-glucopyranoside (*p*NP-α-D-Glc) were incubated with each enzyme while monitoring the production of the chromogenic product, *p*-nitrophenol.[9] These assays led to interesting results. Firstly, the rates of formation of *p*-nitrophenol were observed to be low for every sample relative to that of the non-peptidyl substrate alone (Fig. 2). Secondly, the peptidyl substrates caused a greater reduction in the activity of the *B. stearothermophillus* α-glucosidase as compared to those of the rice and rat intestine enzymes as judged by the more greatly reduced, peptidyl substrate induced rates of production of *p*-nitrophenol (Fig. 2). These findings suggest that differences exist in the chromogenic recognition regions of enzymes, which lead to the differences in substrate susceptibilities. More importantly, the peptidyl substrates that brought about the greatest reduction in the rates differed in all three of the α-glucosidases. For example, α-Glc-sp-WY caused the largest decrease in *p*-nitrophenol production for the α-glucosidase from *B. stearothermophillus*, whereas the peptidyl substrates α-Glc-sp-YF and α-Glc-sp-FF had the largest effect on catalysis by the respective rice and rat intestine enzymes. The observations indicate that the peptidyl substrates are competitive inhibitors of all of the glucosidases owing to the fact that they



Scheme 1. Synthesis of peptidyl glucose library.

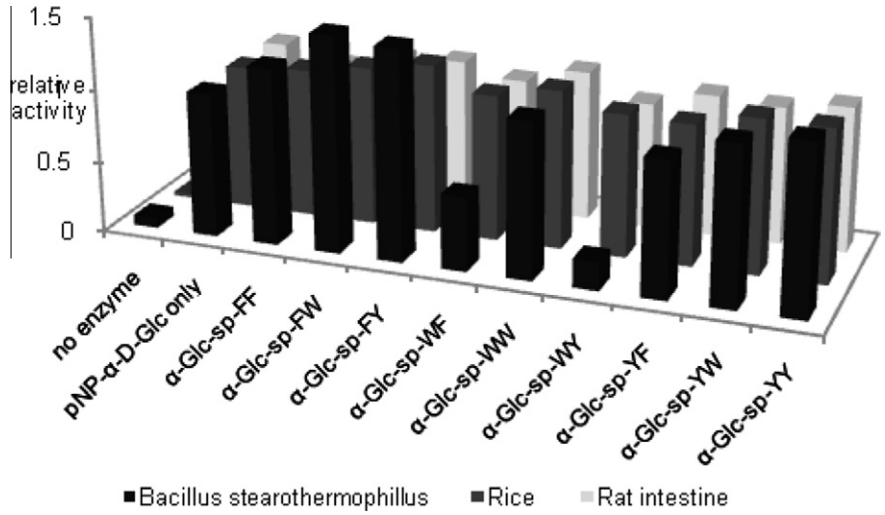


Figure 2. Initial screening of the substrate library (α-Glc-sp-XX) using *p*-nitrophenyl-α-D-glucose (*p*NP-α-D-Glc) as a competitive substrate.

contain structural features that are complementary to then on-carbohydrate recognizing regions of these enzymes. Owing to the significantly large effects observed on the rates of *p*-nitrophenol formation and the selectivity among substrates, the α-glucosidase from *B. stearothermophillus* was chosen for further study.

Two possible reasons exist for the reduced production of *p*-nitrophenol. The first is that the selected peptidyl substrates are competitive inhibitors of the glucosidase catalyzed reaction of the chromogenic substrate. Another possibility is that the products of hydrolysis of the peptidyl substrates might inhibit the activity of these enzymes. Both explanations suggest that the complementary structures of each the glucosidases are recognized by peptidyl residues in the substrates. Among the first generation substrates explored, α-Glc-sp-WY displayed the most profound inhibitory effect. The K_m value of this substrate was determined and compared with those with *p*-nitrophenyl- and *p*-acetaminophenyl-α-D-glucopyranoside. As expected, the K_m value of α-Glc-sp-WY was about 90-fold and 150-fold less than that of the two non-peptidyl substrates (Table 1). This finding demonstrates that the peptidyl part of α-Glc-sp-WY is well-recognized by the complementary region of the cognate enzyme.

We next designed selective glucosidase inhibitors using the information gained from the above studies. The first question we probed was if the non-carbohydrate part of the substrate might itself be a glucosidase inhibitor. Indeed, studies showed that sp-WY (Fig. 1) was a low millimolar (K_i = 1.4 mM) inhibitor of the *Bacillus* α-glucosidase and that it did not inhibit the other α-glucosidases. A second generation inhibitor, in which the peptide side chain of α-Glc-sp-WY was directly bonded to the carbohydrate backbone via non-hydrolysable β-glycosidic linkage, was studied.[9] The assay results showed that the α-glucosidase from *B. stearothermophillus* was completely inhibited by β-Glc-sp-WY (Fig. 1), which displayed a high affinity for the enzyme (K_i = 110 μM, Fig. 3). Importantly, β-Glc-sp-WY was observed to display almost no inhibition against the activities of the other isozymes (Table 2).[9] The data suggest that the role played by the carbohydrate group of the inhibitor is merely to anchor the inhibitor to the active site and, moreover, that it does not govern binding selectivity of the inhibitors.

Owing to the fact that they are categorized as members of the α-glucosidase family[10] and are important targets for dietary and medical purposes, salivary and pancreatic α-amylases were explored next. Since they are more accessible compared to the corresponding pyranosides, *p*-nitrophenyl-α-D-pentaosides were used to synthesize a 9 peptidyl substrates (α-Pnt-sp-XX, Fig. 1) library to probe the effects on α-amylase activities.[9] An equimolar mixture of each peptidyl substrate and *p*-nitrophenyl-α-D-pentaoside was

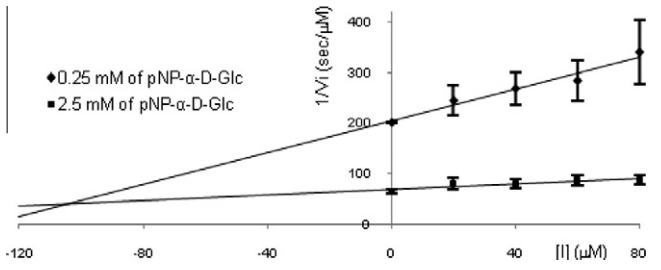


Figure 3. Dixon plot for inhibition by β-Glc-sp-WY of the α-glucosidase from *Bacillus stearothermophillus*. Each data point was triplicated and averaged.

Table 1
Enzymatic constants of α-glucosidase from *Bacillus stearothermophillus* with various substrates[a]

	*p*NP-α-D-Glc	*p*-Acetaminophenyl-α-D-glucopyranoside[b]	α-Glc-sp-WY
K_m (mM)	1.2	1.9	0.013
v_{max} (μM s^{-1})	0.019	0.0015	0.00025
k_{cat} (s^{-1})	9.5	0.76	0.13
k_{cat}/K_m(mM^{-1} s^{-1})	7.6	0.40	10

[a] K_m value was calculated by Michaelis–Menten kinetics. Measurement of each value was triplicated and averaged.
[b] The structure and synthesis of this compound were described in Supplementary data.

Table 2
Comparison of inhibitory effects between the selected peptides and known inhibitors using various α-glucosidases[a]

	sp-WY (μM)	β-Glc-sp-WY (μM)	Acarbose (μM)
Bacillus stearothermophillus	1400	110	0.34
Rice	NC[b]	NC	4.2
Rat intestine	NC	NC	3.5

[a] K_i value was calculated by Dixon plot. Measurement of each value was triplicated and averaged.
[b] NC, the constants could not be calculated because of barely detectable product trace or little difference from those in the absence of the inhibitor.

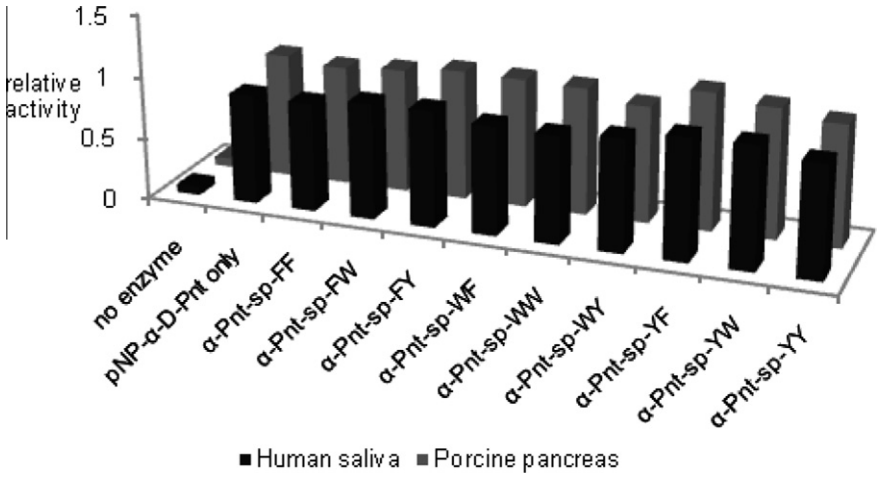


Figure 4. Initial screening of the substrate library (α-Pnt-sp-XX) using *p*-nitrophenyl-α-D-pentaoside (*p*NP-α-D-Pnt) as a competitive substrate and various γ-amylases. Each data point was triplicated and averaged.

incubated with each enzyme.[9] Reduced rates of formation of *p*-nitrophenol were observed in each case in comparison with that of the non-peptidyl chromogenic substrate alone. However, the rate reductions were not as large as those seen in studies of the α-glucosidases (Fig. 4). Certain peptidyl substrates and their corresponding hydrolysis products showed the same selectivity for both α-amylases. Even though the peptidyl substrates have a reduced impact on the retardation of both α-amylases compared to those for α-glucosidases, the observation show that strategy we have devised might be general. The lower inhibition seen with the α-amylase substrates could be a consequence of the fact that peptidyl moieties in these substances are small relative to the large pentaoside unit. If this is the case, increasing the size of the non-carbohydrate component and/or reducing the size of the carbohydrate group[11] could lead to more affective and specific substrates for the α-amylases.

In summary, by carrying out assays, we were able to explore substrates that have peptidyl structural components that are complementary to the hydrophobic pocket of α-glucosidases. The best substrate, α-glucose-spacer-Trp-Tyr (α-Glc-sp-WY) showed a 150-fold lesser K_m value (13 μM) relative to that of *p*-acetaminophenyl-α-D-glucopyranoside (1.9 mM) with the α-glucosidase from *B. stearothermophillus*. A substrate lacking the carbohydrate component, sp-WY, was observed to be only a moderate inhibitor (1.4 mM) of the *B. stearothermophillus* glucosidase but it possessed high selectivity for this enzyme in comparison to other isozymes. With the goal of making a more potent inhibitor, this non-carbohydrate structure was tethered to a glucose moiety via a β-glycoside linkage. The resulting substance, β-Glc-sp-WY was an exceptionally moderate but selective inhibitor (K_i = 110 μM) of the *Bacillus* glucosidase. Moreover, α-glucosidases from other origins were not inhibited by this substance, a result which suggests that the peptidyl moiety is essential for selectivity. This substance is much more selective against the cognate enzyme than commercially available inhibitor, acarbose. This effort has demonstrated that a strategy employing aromatic peptides as an additive recognition tool is viable for the generation of specific glucosidase inhibitors and that it might be generally applied to the design of inhibitors of any glucosidase.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.062.

References and notes

1. Van de Laar, F. A.; Lucassen, P. L.; Akkermans, R. P.; Van de Lisdonk, E. H.; Rutten, G. E.; Van Weel, C. *Diabetes Care* **2005**, *28*, 154.
2. (a) Jacob, G. S. *Curr, Opin. Struct. Biol.* **1995**, *5*, 605; (b) Scott, L. J.; Spencer, C. M. *Drugs* **2000**, *59*, 521.
3. (a) Lee, D. S.; Lee, S. H. *FEBS Lett.* **2001**, *501*, 84; (b) Yoshikawa, M.; Murakami, T.; Shimada, H.; Matsuda, H.; Yamahara, J.; Tanabe, G.; Muraoka, O. *Tetrahedron Lett.* **1997**, *38*, 8367; (c) de Melo, E. B.; Gomes, A. S.; Carvalho, I. *Tetrahedron* **2006**, *62*, 10277; (d) Gao, H.; Kawabata, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 812.
4. (a) Ramasubbu, N.; Paloth, V.; Luo, Y.; Brayer, G. D.; Levine, M. *J. Acta Crystallogr. Sect. D* **1996**, *52*, 435; (b) Ramasubbu, N.; Ragunath, C.; Mishra, P. J. *J. Mol. Biol.* **2003**, *325*, 1061; (c) Mishra, P. J.; Ragunath, C.; Ramasubbu, N. *Biochem. Biophys. Res. Commun.* **2002**, *292*, 468; (d) Qian, M.; Haser, R.; Buisson, G.; Duee, E.; Payan, F. *Biochemistry* **1994**, *24*, 6284; (e) Kadziola, A.; Sogaard, M.; Svensson, B.; Haser, R. J. *J. Mol. Biol.* **1998**, *278*, 205.
5. Watanabe, K.; Hata, Y.; Kizaki, H.; Katsube, Y.; Suzuki, Y. *J. Mol. Biol.* **1997**, *269*, 142.
6. (a) Kitamura, M.; Okuyama, M.; Tanzawa, F.; Mori, H.; Kitago, Y.; Watanabe, N.; Kimura, A.; Tanaka, I.; Yao, M. *J. Biol. Chem.* **2008**, *283*, 36328; (b) Brumshtein, B.; Greenblatt, H. M.; Butters, T. D.; Shaaltiel, Y.; Aviezer, D.; Silman, I.; Futerman, A. H.; Sussman, J. L. *J. Biol. Chem.* **2007**, *282*, 29052.
7. Chapdelaine, P.; Tremblay, R. R.; Dube, J. Y. *Clin. Chem.* **1978**, *24*, 208.
8. Kandra, L.; Zajácz, A.; Remenyik, J.; Gyémánt, G. *Biochem. Biophys. Res. Commun.* **2005**, *334*, 824.
9. See Supplementary data.
10. EC number of α-amylase from *Human saliva* Type XIII-A is 3.2.1.1 and EC number of α-amylase from *Porcine pancreas* Type VI-B is 3.2.1.1. And EC number of α-glucosidase from *Bacillus stearothremophillus* is 3.2.1.20.
11. Maurus, R.; Begum, A.; Kuo, H. H.; Racaza, A.; Numao, S.; Andersen, C.; Tams, J. W.; Vind, J.; Overall, C. M.; Withers, S. G.; Brayer, G. D. *Protein Sci.* **2005**, *14*, 743.