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Aglycon specificity profiling of α-glucosidases using synthetic probes

Wataru Hakamata,[a,*] Makoto Muroi,[a] Kazunari Kadokura,[a] Toshiyuki Nishio,[b] Tadatake Oku,[b] Atsuo Kimura,[c] Seiya Chiba[c] and Akira Takatsuki[a]

[a]*Animal and Cellular Systems Laboratory, The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan*
[b]*Department of Biological Chemistry, College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa-shi, Kanagawa 252-8510, Japan*
[c]*Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Nishi-9, Kita-9, Kita-ku, Sapporo, Hokkaido 060-8589, Japan*

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Abstract—We designed and synthesized hydrogen bond based probes **1**–**8** with the exception of known glycosidase inhibition mechanisms, and aglycon specificity of 11 different sources of α-glucosidases were investigated using their probes. Probe **4** (2,6-anhydro-1-deoxy-1-[(1-oxopentyl-5-hydroxy)amino]-D-*glycero*-D-*ido*-heptitol) showed a potent inhibition of *S. cerevisiae* α-glucosidase among all α-glucosidases. Probe **4** was found to be a competitive inhibitor for *S. cerevisiae* α-glucosidase with K_i 0.13 mM.
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α-Glucosidases (EC 3.2.1.20) are also exo-acting carbohydrases, catalyzing the release of α-D-glucopyranose from the non-reducing ends of various substrates,[1] and on the basis of amino acid sequence similarities, α-glucosidases are classified into two families, family 13 and family 31.[2] Endoplasmic reticulum (ER) processing α-glucosidases, α-glucosidase I (EC 3.2.1.106), and α-glucosidase II (EC 3.2.1.84), are key enzymes in the biosynthesis of asparagine-linked oligosaccharides that catalyze the first processing event after the transfer of Glc3Man9GlcNAc2 to proteins. These enzymes are a target for inhibition by anti-viral agents that interfere with the formation of essential glycoproteins required in viral assembly, secretion, and infectivity.[3] Many papers reported that inhibitors of α-glucosidases are potential therapeutics for the treatment of such diseases as viral diseases, cancer, and diabetes.[3a,4] However, lots of screenings of α-glucosidase inhibitors were not used enzymes from target tissues or organs. We think that the substrate specificities of three kinds of α-glucosidases (family 13, family 31, and ER processing α-glucosidases) are different. Therefore, aglycon specificity profiling of α-glucosidases were an important approach for the research of α-glucosidase inhibitors. Chemically modified substrates are effective methods in the study of substrate specificity profiling. We have already applied this approach to family 13 and family 31 α-glucosidases,[5–7] α-galactosidases,[6,8] α-mannosidases,[6,9] and ER processing α-glucosidases[10] using partially substituted monosaccharide. We have reported that the presence of a C-2 hydroxyl group of glycon is not essential for the hydrolysis action of family 31 α-glucosidases and ER processing α-glucosidase II, and family 13 α-glucosidases and ER processing α-glucosidase I do necessarily need all of the hydroxyl groups of the glycon for hydrolyzing activity.

In a previous paper, we described the design and synthesis of 1-amino-2,6-anhydro-1-deoxy-D-*glycero*-D-*ido*-heptitol derivatives **1**–**8** as synthetic probes[10] (Fig. 1). These probes do not have the specific functional groups for glycosidase inhibition, electrostatic interactions (e.g., deoxynojirimycine), transition state mimetic structure (e.g., D-gluconolactone), and covalent bond formation with the enzyme catalytic site (e.g., conduritol B

Keywords: α-Glucosidase; ER processing α-glucosidase; Inhibitor; Aglycon specificity.
* Corresponding author at present address: Division of Organic Chemistry, National Institute of Health Sciences (NIHS), 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. Fax: +81 3 3707 6950; e-mail: hakamata@nihs.go.jp

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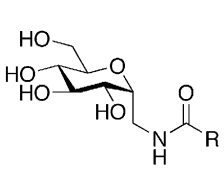


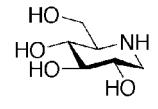
1: R=CH_2OH
2: R=$(CH_2)_2OH$
3: R=$(CH_2)_3OH$
4: R=$(CH_2)_4OH$
5: R=$(CH_2)_5OH$
6: R=Me
7: R=Ph
8: R=$C(CH_3)_3$

Figure 1. Chemical structure of probes **1**–**8**.

epoxide). The structures of α-glucosidase inhibitors were summarized in Figure 2. We think that with the elimination of the above functional groups from probes, the hydrogen bond formations is expressed clearly. In this paper, we report the inhibition of family 13 α-glucosidases (*Saccharomyces* (*S.*) *cerevisiae*, *Bacillus* (*B.*) *stearothermophilus*, and honeybee isozyme I, II, and III (HBGase I, II, and III)), family 31 enzymes (rice, sugar beet, flint corn, and *Aspergillus* (*A.*) *niger*), ER processing α-glucosidase I and II from rat liver microsome, and other glycosidases (almond β-glucosidase, jack beans α-mannosidase, snail β-mannosidase, *Moltierella* (*M.*) *vinacea* α-galactosidase, and jack beans β-galactosidase) against the probes **1**–**8**, and their aglycon specificity profiling is discussed.

The values of % inhibition and IC_{50} were summarized in Table 1. Probe **4** indicated specific inhibitions of *S. cerevisiae* (IC_{50} = 55.5 μM) and *B. stearothermophilus* (IC_{50} = 415 μM) α-glucosidases. Probe **7** inhibited α-glucosidase from *S. cerevisiae* (IC_{50} = 449 μM). HBGase I was inhibited by probe **1** (IC_{50} = 851 μM). Family 13 α-glucosidases and ER processing α-glucosidases were inhibited by the specific probes. On the other hand, family 31 α-glucosidases were broadly inhibited by probes **1**–**8**. All probes did not inhibit β-glucosidase, α- and β-mannosidases, and α- and β-galactosidases at a 5-fold concentration. These facts indicated that aglycon specificities of α-glucosidases differed greatly among family 13 α-glucosidases, family 31 α-glucosidases, and ER processing α-glucosidases. Moreover, each aglycon specificity of family 13 α-glucosidases is different in spite of the highly conserved amino acid sequences in the catalytic site.[2f] Therefore, it is better to use enzymes of target tissues or organs for screening of agents for viral diseases, cancer, and diabetes.

In the kinetic studies on the inhibitions of **4** and **7** and hydrolysis of *p*-nitrophenyl α-D-glucopyranoside (PNP α-Glc) by *S. cerevisiae* and *B.stearothermophilus* α-glucosidases, the values of K_i and K_m (mM) were calculated



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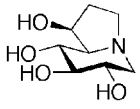

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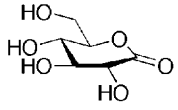
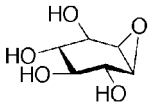


D-gluconolactone



conduritol B epoxide

Figure 2. Chemical structure of α-glucosidase inhibitors.

Table 1. Inhibitory activities of probes **1**–**8** against glycosidases[11]

Enzyme source	% Inhibition (IC_{50})							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Family 13 α-glucosidase								
S. cerevisiae	<1.0	21.1	<1.0	**100 (55.5 μM)**	<1.0	<1.0	67.4 (449 μM)	6.1
B. stearothermophilus	<1.0	<1.0	<1.0	100 (415 μM)	<1.0	<1.0	<1.0	<1.0
HBGase I	52.3 (851 μM)	<1.0	<1.0	37.5	<1.0	10.4	4.6	<1.0
HBGase II	4.4	2.7	3.6	21.4	4.4	<1.0	12.3	<1.0
HBGase III	<1.0	3.2	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Family 31 α-glucosidase								
Rice	10.7	8.5	7.6	18.3	26.0	21.8	16.0	3.8
Sugar beet	6.9	1.7	3.6	3.1	11.9	8.8	9.8	3.2
Flint corn	29.1	14.1	18.5	37.0	44.6	31.0	49.2	5.6
A. niger	6.6	2.6	<1.0	6.8	<1.0	23.3	14.0	1.2
ER processing α-glucosidase								
Glucosidase I	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	18.2	<1.0
Glucosidase II	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	5.9	<1.0
β-Glucosidase	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
α-Mannosidase	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
β-Mannosidase	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
α-Galactosidase	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
β-Galactosidase	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

Probe concentrations (family 13 and 31 α-glucosidases: 1 μmol/mL, ER processing α-glucosidases: 2 μmol/mL, β-glucosidase, mannosidases, and galactosidases:5 μmol/ml) Substrate (family 13 and 3l α-glucosidases, ER processing α-glucosidase II: PNP α-Glc, ER processing α-glucosidases I: [^{3}H] labeled vesicular stomatitis virus glycoprotein, β-glucosidase: PNP β-Glc, α-mannosidase: PNP α-Man, β-mannosidase: PNP β-Man, α-galactosidase: PNP α-Gal, β-galactosidase: PNP β-Gal).

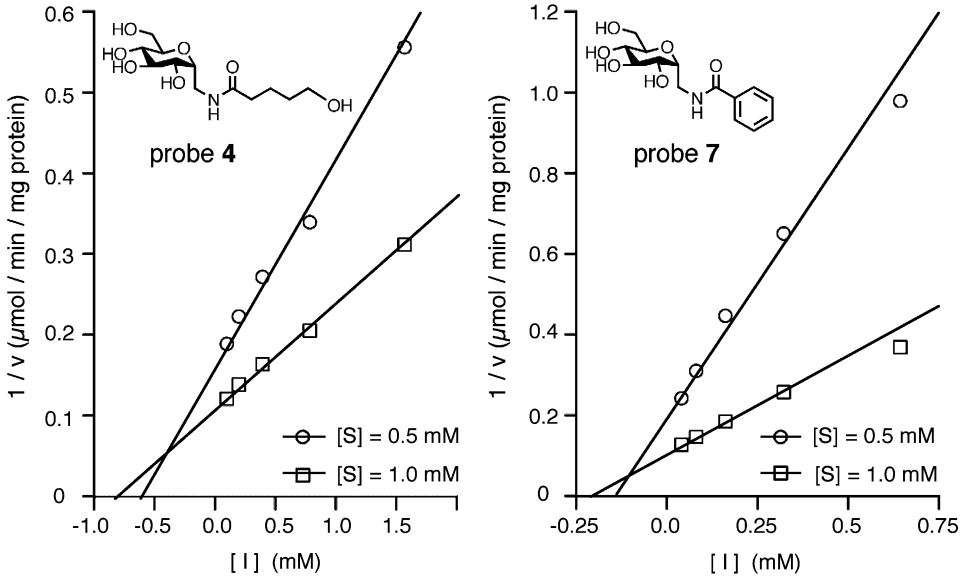


Figure 3. Dixon plots of the inhibition of *S. cerevisiae* α-glucosidase by probes **4** and **7**.

from Dixon plots (Figs. 3 and 4) and Michaelis–Menten plots, respectively, and these values and inhibition types were summarized in Table 2. Probes **4** and **7** were competitive type inhibitors of *S. cerevisiae* enzyme (K_i = 0.13 and 0.50 mM). Probe **4** was a mixed type inhibitor of *B. stearothermophilus* enzyme (K_i = 0.58 mM). The affinities of **4** against both enzymes were higher than PNP α-Glc as substrate. These results indicated that probe **4** formed a specific hydrogen bond between the primary hydroxyl group of aglycon moiety and *S. cerevisiae* enzyme, and that probe **7**, with a terminal phenyl group, formed a hydrophobic interaction with *S. cerevisiae* enzyme. These results were compared with other typical α-glucosidase inhibitors. The K_i values of deoxynojirimycine, castanospermine (see Fig. 2), D-gluconolactone, conduritol B epoxide, and probe **4** against *S. cerevisiae* α-glucosidase were 0.013 mM,[17] >1.5 mM,[17] 2.0 mM,[18] ~25 mM,[17] and 0.13 mM, respectively. From this comparison, the combination of the designed hydrogen bond formation and the other static binding factors (electrostatic interaction, transition state mimetic conformation, and covalent bond formation), it may be possible to become a potent and specific α-glucosidase inhibitor. Further studies using new probes (in which the –C–O–C– group of glycon moiety is replaced by a –C–NH–C– group) should be done to develop new potent and specific α-glucosidase inhibitors. The discovery of α-glucosidase inhibitors may help us to understand the roles of oligosaccharides of glycoproteins and glycolipids in cellular functions, and pharmaceutical applications.

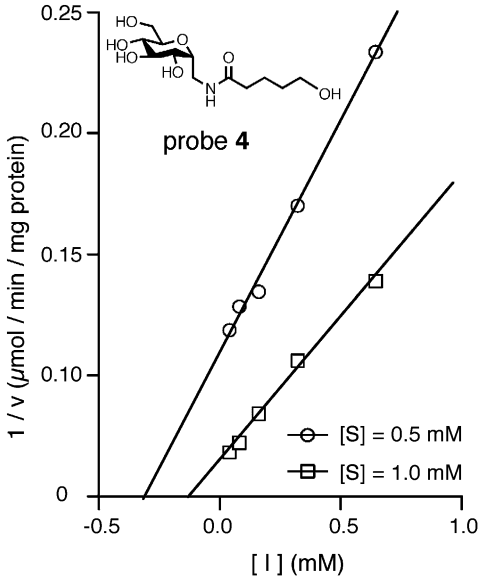


Figure 4. Dixon plot of the inhibition of *B. stearothermophilus* α-glucosidase by probe **4**.

Table 2. Kinetic studies of the inhibition of α-glucosidases

Probe	*S. cerevisiae*		*B. stearothermophilus*	
	K_i (mM)	Inhibition type	K_i (mM)	Inhibition type
4	0.13	Competitive	0.58	Mixed
7	0.50	Competitive	—	—
PNP α-Glc	0.35[a]	—	1.16[a]	—

[a] K_m value.

Acknowledgements

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11. Enzyme preparation: α-Glucosidases from *S. cerevisiae*, *B. stearothermophilus*, and rice (Sigma–Aldrich Inc.), β-glucosidases from almond (Sigma–Aldrich Inc.), α-mannosidases from jack beans (Wako Pure Chemical Ind.), β-mannosidases from snail (Sigma–Aldrich Inc.), α-galactosidases from *M. vinacea* (Seikagaku Ind.), and β-galactosidases from jack beans (Seikagaku Ind.) were used in this study. α-Glucosidases from honeybee,[12] sugar beet,[13] flint corn,[14] *A. niger*[15] and rat liver microsome processing α-glucosidases[10,16] were purified as reported in the previous paper. The inhibition assays of glucosidases,[5] ER processing α-glucosidases,[10] mannosidases,[9] and galactosidases[8] were assayed as described previously.
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