J. Appl. Glycosci., **53**, 149–154 (2006)

Proceedings of the Symposium on Amylases and Related Enzymes, 2005

N-Linked Oligosaccharide Processing Enzymes as Molecular Targets for Drug Discovery

(Received December 1, 2005)

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Abstract: *N*-Linked oligosaccharide processing enzymes are key enzymes in the biosynthesis of *N*-linked oligosaccharides. These enzymes are a molecular target for inhibition by anti-viral agents that interfere with the formation of essential glycoproteins required in viral assembly, secretion and infectivity. We think that the molecular recognition of three kinds of glucosidases (family 13 and family 31 α-glucosidases and endoplasmic reticulum glucosidases) are different. Therefore, glycon and aglycon specificity profiling of glucosidases was an important approach for the research of glucosidase inhibitors. We carried out the profiling of glucosidases using small molecules as a probe. Moreover, we designed and synthesized three types of glucosidase inhibitors. These compounds were evaluated with regard to their ability to inhibit glucosidases *in vitro*, and were also tested in a cell culture system. We found some compounds having glucosidase inhibitory activity and anti-viral activity.

Key words: α-glucosidase, ER glucosidase, inhibitor, anti-viral activity

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,[1,2] and on the basis of amino acid sequence similarities, α-glucosidases are classified into two families, family 13 and family 31.[3,4] Endoplasmic reticulum (ER) glucosidases, glucosidase I (EC 3.2.1.106) and 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 $Glc_3Man_9GlcNAc_2$ 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.[5] Many papers reported that inhibitors of α-glucosidases are potential therapeutics for the treatment of such diseases as viral diseases, cancer and diabetes.[5,6] However, many screenings of α-glucosidase inhibitors did not use enzymes from target tissues or organs. We think that the molecular recognitions of three kinds of glucosidases (family 13, family 31 α-glucosidases and ER glucosidases) are different. Therefore, the glycon and aglycon specificity profiling of glucosidases has been an important approach for the research of glucosidase inhibitors.

In this research, we first describe the glycon and aglycon specificity profiling of glucosidases using small molecules as probes. Next, compounds designed and synthesized as glucosidase inhibitor candidates were evaluated with regard to their ability to inhibit three kinds of glucosidases. Finally, the glucosidase inhibitor candidates were tested for their anti-viral activities in a cell culture system.

Glycon specificity profiling of glucosidases using chemically modified substrates.

Chemically modified substrates are effective methods in the study of substrate specificity profiling. We have applied this approach to family 13 and family 31 α-glucosidases,[7–10] ER glucosidases,[11,12] α-galactosidases[8,13] and α-mannosidases[8,14] using partially substituted monosaccharides. We used all of the monodeoxy analogs of *p*-nitrophenyl α-D-glucopyranoside (PNP α-Glc) **1**–**4** (Fig. 1) as chemically modified substrates for glycon specificity profiling. We investigated the hydrolytic activities of family 13 and family 31 α-glucosidases and ER glucosidase II of PNP α-Glc and its deoxy derivatives **1**–**4**, and checked the inhibitory activities of ER glucosidase I of PNP α-Glc and probes **1**–**4**, so that PNP α-Glc was not a substrate for ER glucosidase I. These results are shown in Table 1.[11,12] Clearly, of the four deoxy derivatives of PNP α-Glc **1**–**4**, family 31 α-glucosidases and ER glucosidase II hydrolyzed the 2-deoxy glucopyranoside (**1**); its activity with **1** appeared to be substantially higher than that with PNP α-Glc. Kinetic studies of the hydrolysis of PNP α-Glc, **1** and **2** were also carried out (Table 2).[9,11] The V_{max}/K_m or k_{cat}/K_m values of family 31 α-glucosidases

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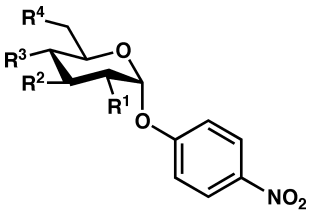
and ER glucosidase II for **1** was about twice as great as PNP α-Glc, which indicated that probe **1** was a good substrate for the enzymes. These reaction velocities to probe **1** increased to 3–28 fold that of PNP α-Glc. PNP Glc and probes **1**–**4** inhibited ER glucosidase I by 56.2, 71.7, 18.5, 22.2 and 32.3% at 5 mM, respectively. These results also indicated that ER glucosidase II might have properties similar to those found in family 31 α-glucosidases.

Aglycon specificity profiling and inhibition of glucosidases using heptitol derivatives.

For aglycon specificity profiling, we designed and synthesized eight probes, **5**–**12**, including 1-amino-2, 6-anhydro-1-deoxy-D-*glycero*-D-*ido*-heptitol, which might mimic to a great extent the topography of α-D-glucopyranoside and modified aglycon of α-glucopyranoside (Fig. 2).[11] These probes do not have the specific functional groups for glycosidase inhibition, electrostatic interactions (*e.g.* 1-deoxynojirimycine), transition state mimetic structure (*e.g.* D-gluconolactone), or covalent bond formation with the enzyme catalytic site (*e.g.* conduritol B epoxide). The structures of α-glucosidase inhibitors are summarized in Fig. 3. We investigated the inhibitory activities of family 13 and family 31 α-glucosidases, ER glucosidases, and other glycosidases (β-glucosidase, α- and β-mannosidase, α- and β-galactosidase) against probes



1: R^1=H, R^2=R^3=R^4=OH
2: R^1=R^3=R^4=OH, R^2=H
3: R^1=R^2=R^4=OH, R^3=H
4: R^1=R^2=R^3=OH, R^4=H

Fig. 1. Chemical structure of glycon profiling probes **1**–**4**.

5–**12**, and their aglycon specificity profiling was discussed. The values of the % inhibition and IC_{50} are summarized in Table 3.[12] Probe **8** indicated specific inhibitions of *Saccharomyces* (*S.*) *cerevisiae* (IC_{50}=55.5 μM) and *Bacillus* (*B.*) *stearothermophilus* (IC_{50}=415 μM) α-glucosidases. Probe **11** inhibited α-glucosidase from *S. cerevisiae* (IC_{50}=449 μM). Honey bee isozyme I (HBG I) was inhibited by probe **5** (IC_{50}=851 μM). Family 13 α-glucosidases and ER glucosidases were inhibited by the specific probes. On the other hand, family 31 α-glucosidases were broadly inhibited by probes **5**–**12**. All probes did not inhibit β-glucosidase, α- or β-mannosidases, or α- or 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 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.[15] In the kinetic studies on the inhibitions of **8** and **11** and the hydrolysis of PNP α-Glc by *S. cerevisiae* and *B. stearothermophilus* α-glucosidases, the values of K_i and K_m (mM) were calculated from Dixon plots and Michaelis-Menten plots, respectively, and these values and inhibition types are summarized in Table 4.[12] Probes **8** and **11** were competitive type inhibitors of the *S. cerevisiae* enzyme (K_i=0.13 mM and 0.50 mM). Probe **8** was a mixed type inhibitor of *B. stearothermophilus* enzyme (K_i=0.58 mM). The affinities of **8** against both enzymes were higher than PNP α-Glc as a substrate. These results indicated that probe **8** formed a specific hydrogen bond between the primary hydroxyl group of aglycon moiety and *S. cerevisiae* enzyme, and that probe **11**, with a terminal phenyl group, formed a hydrophobic interaction with the *S. cerevisiae* enzyme.

Inhibition of α-glucosidase by reactive oxygen species.

The reactive oxygen species (ROS) generated com-

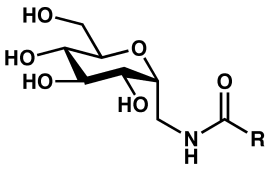
Table 1. Hydrolytic activities and inhibitory activities of probes **1**–**4** against glucosidases.[11,12]

Enzyme source	Relative rate of hydrolysis (%) / % Inhibition				
	PNP α-Glc	PNP 2D α-Glc (**1**)	PNP 3D α-Glc (**2**)	PNP 4D α-Glc (**3**)	PNP 6D α-Glc (**4**)
ER Processing glucosidase					
Rat microsome					
Glucosidase I	− / 56.2	− / 71.7	− / 18.5	− / 22.2	− / 32.3
Glucosidase II	100 / HD	189 / HD	− / −	− / −	− / −
	Relative rate of hydrolysis (%)				
α-Glucosidase family 13					
S. cerevisiae	100	−	−	−	−
B. stearothermophilus	100	−	−	−	−
Honey bee I	100	−	−	−	−
Honey bee II	100	−	−	−	−
Honey bee III	100	−	−	−	−
α-Glucosidase family 31					
Rice	100	175	−	−	−
Sugar beet	100	244	−	−	−
Flint corn	100	231	3.7	−	−
A. niger	100	259	11.9	−	−

Relative rate of hydrolysis was expressed by comparison with the amount of *p*-nitrophenol that was released from PNP α-Glc, which was taken as 100%. Assay of glucosidase I inhibitory activities used [^{3}H] glucose-labeled VSV glycoprotein as a substrate. −, Hydrolytic or inhibitory activity was not detected, HD, Hydrolyzing activity was observed.

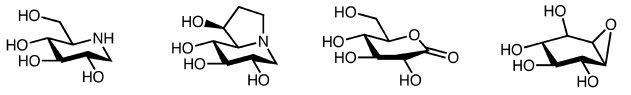
Table 2. Kinetic study of hydrolysis of family 31 α-glucosidases and ER glucosidase II.[9,11]

Enzyme / Substrate	K_m (mM)	V_{max} (μmol/min/U)	V_{max}/K_m
ER glucosidase II			
PNP α-Glc	0.92	1.12	1.23
PNP 2D α-Glc (**1**)	0.76	3.44	4.53
Enzyme / Substrate	K_m (mM)	k_{cat} (s^{-1})	k_{cat} / K_m
Rice α-glucosidase			
PNP α-Glc	2.62	43.8	16.7
PNP 2D α-Glc (**1**)	6.66	237	35.6
Sugar beet α-glucosidase			
PNP α-Glc	1.04	0.071	0.068
PNP 2D α-Glc (**1**)	5.70	0.64	0.11
Flint corn α-glucosidase			
PNP α-Glc	0.88	2.00	2.27
PNP 2D α-Glc (**1**)	7.38	17.0	2.30
PNP 3D α-Glc (**2**)	9.98	0.44	0.044
A. niger α-glucosidase			
PNP α-Glc	0.59	3.44	5.83
PNP 2D α-Glc (**1**)	6.09	96.9	15.9
PNP 3D α-Glc (**2**)	10.2	4.23	0.41



5: R=CH_2OH **9**: R=$(CH_2)_5OH$
6: R=$(CH_2)_2OH$ **10**: R=Me
7: R=$(CH_2)_3OH$ **11**: R=Ph
8: R=$(CH_2)_4OH$ **12**: R=$C(CH_3)_3$

Fig. 2. Chemical structure of aglycon profiling probes **5**–**12**.



1-deoxynojirimycine castanospermine D-gluconolactone conduritol B epoxide

Fig. 3. Chemical structure of typical α-glucosidase inhibitor.

Table 3. Inhibitory activities of probes **5**–**12** against glycosidases.[12]

Enzyme source	% Inhibition (IC_{50})							
	5	**6**	**7**	**8**	**9**	**10**	**11**	**12**
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
Honey bee I	52.3 (851 μM)	<1.0	<1.0	37.5	<1.0	10.4	4.6	<1.0
Honey bee II	4.4	2.7	3.6	21.4	4.4	<1.0	12.3	<1.0
Honey bee 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 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 31 α-glucosidases, ER glucosidase II: PNP α-Glc, ER glucosidases I: [^{3}H] glucose-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).

pounds, **13**–**24**, shown in Fig. 4, were assessed as inhibitors of glycoside hydrolase family 13 α-glucosidases and family 31 α-glucosidases,[16] and the results are listed in Table 5 (Preparation for publication). Compounds **18** and **24**, with a terminal α-naphthyl group, indicated inhibitions of α-glucosidases from *S. cerevisiae* (IC_{50}=51.7 μM and IC_{50}=74.1 μM) and *B. stearothermophilus* (IC_{50}=60.1 μM and IC_{50}=89.1 μM). We reasoned that the enzymatic liberation of the aglycon from compounds **18** and **24** might be followed by the ejection of a sulfinate anion with the concomitant formation of *p*-benzoquinone and *p*-benzoquinone imine, which would then generate ROS in the enzyme active site, leading to enzyme deactivation.[16,17] Therefore, the effects of compounds **18** and **24** on ROS-mediated DNA breakage were investigated. DNA strand scission in the super coiled pBR322DNA was induced by ROS in the presence of *p*-benzoquinone or *p*-benzoquinone imine, metal ion, and NADH.[17] Compound **24** induced DNA strand breakage condition in the above conditions (data not shown). We suggest that ROS-generated enzyme inhibition might be a new approach for the development of an enzyme inhibitor.

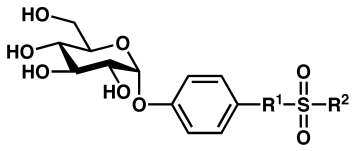
Inhibition of α-glucosidase by catechin derivatives.

The catechin derivatives **25**–**33** shown in Fig. 5 were assessed as inhibitors of family 13 and family 31 α-glucosidases, and the results are listed in Table 6.[18] A comparison of the results against family 13 and family 31 α-glucosidases shows that family 13 α-glucosidases were remarkably inhibited by catechin derivatives compared with family 31 α-glucosidases. The potent inhibition of family 13 α-glucosidases, *S. cerevisiae* and *B. stearothermophilus*, shown by catechin derivative **25** (IC_{50}=1.2 μM and IC_{50}=0.7 μM) and **30** (IC_{50}=0.9 μM and IC_{50}=1.1 μM), are in contrast to the weak activity shown by cate-

Table 4. Kinetic studies of the inhibition of family 13 α-glucosidases.[12]

Probe	*S. cerevisiae*		*B. stearothermophilus*	
	*K*i (mM)	Inhibition type	*K*i (mM)	Inhibition type
8	0.13	Competitive	0.58	Mixed
11	0.50	Competitive	–	–
PNP α-Glc	0.35[a]	–	1.16[a]	–

[a]*K*m value.

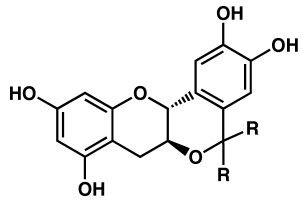


13: R^1=O, R^2=NO_2
14: R^1=O, R^2=Cl
15: R^1=O, R^2=CF_3
16: R^1=O, R^2=CH_3
17: R^1=O, R^2=$C(CH_3)_3$
18: R^1=O, R^2=α-naphthyl
19: R^1=NH, R^2=NO_2
20: R^1=NH, R^2=Cl
21: R^1=NH, R^2=CF_3
22: R^1=NH, R^2=CH_3
23: R^1=NH, R^2=$C(CH_3)_3$
24: R^1=NH, R^2=α-naphthyl

Fig. 4. Chemical structure of ROS-generated compounds **13**–**24**.

Table 5. Inhibitory activity of ROS-generated compounds **13**–**24** against α-glucosidases.

Compound	IC_{50} (μM)		
	Glycoside hydrolase family 13		Glycoside hydrolase family 31
	S. cerevisiae	*B. stearothermophilus*	Rice
13	499	>500	>500
14	437	>500	>500
15	407	>500	>500
16	499	>500	>500
17	391	>500	>500
18	51.7	60.1	>500
19	239	218	>500
20	200	254	>500
21	146	244	>500
22	231	325	>500
23	136	237	>500
24	74.1	89.1	>500



25: R=CH_3
26: R=CH_2CH_3
27: R=$(CH_2)_2CH_3$
28: R=$(CH_2)_3CH_3$
29: R=$(CH_2)_4CH_3$
30: R=$(CH_2)_5CH_3$
31: R=$(CH_2)_6CH_3$
32: R=$(CH_2)_7CH_3$
33: R=$(CH_2)_8CH_3$

Fig. 5. Chemical structure of catechin derivatives **25**–**33**.

chin derivative **26**, which has one methylene group long alkyl side chain compared with **25** (IC_{50}=47.5 μM and IC_{50}=26.8 μM) and catechin derivative **33** which has three methylene groups long alkyl side chain compared with **30** (IC_{50}=64.0 μM and IC_{50}=28.1 μM). From these results, it is thought that the inhibition mechanism of catechin derivative **25** and the inhibition mechanism of catechin derivative **30** are different. The IC_{50} values of typical α-glucosidase inhibitor 1-deoxynojirimycine (see Fig. 3) and catechin derivative **30** against *S. cerevisiae* α-glucosidase

Table 6. Inhibitory activity of catechin derivatives **25**–**33** against α-glucosidases.[18]

Compound	IC_{50} (μM)			
	Glycoside hydrolase family 13		Glycoside hydrolase family 31	
	S. cerevisiae	*B. stearothermophilus*	Rice	*A. niger*
Catechin	>500	>500	>500	>500
25	1.2	0.7	>500	>500
26	47.5	26.8	>500	>500
27	37.5	28.4	>500	>500
28	2.1	14.2	>500	>500
29	5.3	6.8	248	>500
30	0.9	1.1	>500	>500
31	4.9	21.1	>500	>500
32	33.2	13.8	>500	>500
33	64.0	28.1	>500	>500

were 3.3[19] and 0.9 μM, respectively. This result indicated that catechin derivative **30** is about 3.6 times more potent than 1-deoxynojirimycine when their IC_{50} values are compared.

Anti-viral activity of α-glucosidase inhibitors.

Compounds **1**–**33** were assayed with regard to their ability to inhibit glycoprotein processing at the cellular level. Vesicular stomatitis virus glycoprotein (VSV G) was prepared from VSV-infected and probe-treated baby hamster kidney (BHK) cells.[11] Analyses of the *N*-glycan structure of obtained VSV G using endo H, which is known to have hydrolytic activity against high-mannose type *N*-glycan, failed to confirm that compounds **1**–**24** except for catechin derivatives (**25**–**33**) inhibited processing glycosidases. The catechin derivatives had the possibility of inhibition of processing glycosidases (data not shown). Then, we assayed the anti-virus activities by effects of the catechin derivatives of processing glycosidases on virus glycoprotein synthesis and syncytium formation after newcastle disease virus (NDV) infection, and effects on synthesis and cell surface expression of NDV glycoprotein, hemagglutinin-neuraminidase (HANA) glycoprotein in whole cell lysates were quantified. Moreover, viral infectivity was determined by a plaque assay in BHK cells.[20] In the above assays, catechin derivative **30** showed potent inhibition of the viral infectivity (Table 7, Preparation for publication).

Conclusion and perspectives.

The discovery of glucosidase inhibitors may help us to understand the roles of the oligosaccharides of glycoproteins and glycolipids in cellular functions, and pharmaceutical applications. From this study, it is better to use enzymes of target tissues or organs for the screening of agents for viral diseases, cancer and diabetes. Moreover, in applying glucosidases as inhibitors of glycoprotein processing, inhibitory action of many inhibitors at the cellular levels is not so remarkable, as expected based on their action at the enzyme level. This was speculated to be caused by the difficulty for inhibitors to be able to access the site of action. We think that high throughput

Table 7. Anti-viral activity of catechin derivatives at the cellular level.

Compound	Conc. (μM)	% HAU	SF	% PFU	CPU
Catechin	500	100	+	95	+
	250	100	+	100	+
	125	100	+	NT	+
	63	100	+	NT	+
25	500	0	−	0	+
	250	6	−	14	+
	125	100	+	100	+
	63	100	+	100	+
26	500	0	−	0	−
	250	0	−	0	+
	125	100	+	24	+
	63	100	+	85	+
27	500	0	−	0	−
	250	0	−	0	−
	125	0	−	0	−
	63	100	+	90	+
28	500	0	−	0	−
	250	0	−	0	−
	125	0	−	0	−
	63	100	+	90	+
29	500	0	−	0	−
	250	0	−	0	−
	125	0	−	0	−
	63	100	+	50	−
	31	100	+	100	+
	16	100	+	100	+
30	500	0	−	0	−
	250	0	−	0	−
	125	0	−	0	−
	63	9	+/−	25	−
	31	100	+	95	+
	16	100	+	100	+
33	500	0	−	0	−
	250	0	−	0	−
	125	25	−	50	−
	63	100	+	100	+

screening assays using specific probes and enzymes of target tissues or organs and highly effective design and synthesis of inhibitors *in silico* are necessary for the development of new and potent glucosidase inhibitors.

The authors express sincere thanks to Dr. Seiya Chiba and Dr. Atsuo Kimura for their advice and for some of the α-glucosidases. We thank Ezaki Glico Co., Ltd. for the gift of α-arbutin. This work was performed at the Special Postdoctoral Researchers Program (RIKEN). This research was partially supported by a Ministry of Education, Science, Sports and Culture, Grant-in-Aid for Young Scientists (B), 17790097, 2005.

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N-結合型糖鎖プロセッシング酵素を分子標的とした創薬

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現在，新 H5N1 型インフルエンザや SARS など続々と出現する新興ウイルス感染症や鳥インフルエンザのヒトへの伝播等，新興ウイルス感染症は人類の脅威となっている．しかし，ウイルス感染症に対する有効な薬剤の開発は，細菌感染症の抗生物質に比べ遅れている．そこで，ウイルス共通の感染機序に基づいた薬剤の開発が重要と考え，外被を有する多くのウイルスの感染・増殖には複合型の *N*-結合型糖鎖が関与している知見を基にして，小胞体 *N*-結合型糖鎖プロセシング酵素を標的酵素とした分子標的薬の開発を目指して研究を行っている．分子標的薬の開発には，標的酵素である糖鎖プロセシング酵素の基質特異性の解明が必要であると考えた．そこで，合成プローブを用いて *N*-結合型糖鎖プロセシングの第 1 段階を担うプロセシンググルコシダーゼ I (EC 3.2.1.106)と第 2 段階を担うプロセシンググルコシダーゼ II (EC 3.2.1.84)のグリコンおよびアグリコン特異性を調べ，α-グルコシダーゼ (EC 3.2.1.20, GH13 and GH31) のそれと比較した．その結果，グルコシダーゼ I のグリコン特性は GH13 α-グルコシダーゼと，グルコシダーゼ II のグリコン特性は GH31 α-グルコシダーゼと同様であった．またグルコシダーゼ I とグルコシダーゼ II のアグリコン認識は同様であり，GH13 および GH31 α-グルコシダーゼとは異なっていた．そこで，プロセシンググルコシダーゼ I および II を標的として，酵素阻害剤候補化合物の設計と合成を行った．これら候補化合物の *in vitro* 酵素阻害活性と細胞レベルでのウイルス外被糖タンパク質の合成・成熟・転送阻害およびプラーク法による感染性ウイルス数の測定を行った．その結果，*in vitro* においてヘプチトール誘導体，スルフォニル誘導体の一部に IC_{50} 約 50 μM の阻害活性を，カテキン誘導体の一部に IC_{50} 0.9 μM の強力な阻害活性を見いだした．さらに，細胞レベルではカテキン誘導体の一部にプロセシンググルコシダーゼ阻害を作用点とするとみられる比較的強い抗ウイルス活性を見いだした．今後，ウイルス外被糖タンパク質の糖鎖構造解析等により詳細な作用機序の解明を行う予定である．

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〔質　問〕　食総研　徳安

1）安全性の高いカテキン骨格をリード化合物として，カテキン骨格を含む阻害剤の設計と合成を行っていますが，その阻害剤の「安全性が高い」という理由はなにか．

2）ウイルスに対してカテキン誘導体の効果があったが，α-グルコシダーゼに対して作用した結果なのか．

〔答〕

1）誘導体合成前のカテキンの安全性が高いからといって，カテキン骨格を有する誘導体の安全性が高いということはできません．しかし，安全性の高い骨格を創薬リード化合物として用いることは，毒性を回避するという目的において理にかなっていると考えています．また，本誘導体はカテキン骨格をほぼ維持しているので，毒性発現の可能性を低く抑えられるのではないかと考えております．

2）α-グルコシダーゼに対する阻害効果なのかどうか，直接の証拠はありませんが，*in vitro* での強い阻害活性およびウイルスを感染させた培養細胞の形態から α-グルコシダーゼ阻害を作用機序とする抗ウイルス作用であると考えております．今後，ウイルス粒子を回収し，そのウイルス外被糖タンパク質の糖鎖解析を行うことにより，作用点を解明したいと考えております．

〔質　問〕　食総研　北岡

1）グルコシダーゼ阻害剤のリード化合物として，数ある安全性の高い物質の中から，カテキンを選択した理由はなにか．

2）グルコシダーゼ以外の糖質加水分解酵素の阻害剤になっている可能性はあるのでしょうか．

〔答〕

1）安全性の高い物質は他にもたくさんありますが，カテキンには弱いながらも血糖上昇抑制作用が報告されており，腸管グルコシダーゼ阻害が示唆されておりますので，リード化合物として選択いたしました．

2）グルコシダーゼ阻害以外の阻害活性があることは否定できません．現時点では，一部の α-マンノシダーゼに対する阻害活性がないことだけ確認しております．