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Communication

Synthesis and the Intestinal Glucosidase Inhibitory Activity of 2-Aminoresorcinol Derivatives toward an Investigation of Its Binding Site

Eisuke KATO, Kenichi OIKAWA, Keisuke TAKAHASHI, and Jun KAWABATA[†]

Laboratory of Food Biochemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Kita-ku, Sapporo, Hokkaido 060-8589, Japan

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2-Aminoresorcinol is a potent and selective intestinal glucosidase inhibitor. Unlike the majority of glucosidase inhibitors, it shows an uncompetitive mode of inhibition. In this study, we tested the intestinal glucosidase inhibitory activity of various 2-aminoresorcinol derivatives. We found that structural changes, in amino and two phenolic hydroxyl groups had a negative impact on inhibitory activity, but methylation of the phenolic hydroxyl group was found to maintain its activity and replacement of the aromatic ring with an acyl or alkoxy carbonyl group at the 4th position also retained its activity. This enable us to design a molecular probe for further study of the inhibition mechanism of 2-aminoresorcinol.

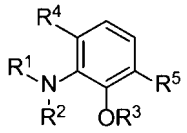
Key words: glucosidase inhibitor; uncompetitive inhibition; diabetes mellitus; structure-activity relationship

The inhibitor of intestinal glucosidase, the maltase-glucoamylase and sucrase-isomaltase complex, is widely studied for the treatment of diabetes mellitus. Inhibition of glucosidases working in the intestine slows the absorption of sugars and prevents rapid elevation of blood sugar level, after diet which has a positive effect on patients with diabetes mellitus. Numbers of small molecule inhibitors have been isolated from nature or designed and synthesized chemically. Currently, two compounds, voglibose and miglitol, are used as anti-diabetic agents.[1,2] These two molecules are competitive inhibitors of glucosidases that mimic a substrate or its transition state and inhibit an active site of the enzyme. Together with these two medicinal compounds, deoxynojirimycin, salacinol, glucoamidine and various sugar mimetics are potent glucosidase inhibitors, with K_i values ranging from low μM to high nM.[3–5] Besides these sugar mimetics, polyphenols also inhibit intestinal glucosidases.[6–8] A majority of plant polyphenols show only mild, unspecific inhibition, but some natural and synthetic polyphenols show inhibitory activity comparable to sugar mimetics and more specificity toward intestinal glucosidase.[9,10] 2-Aminoresorcinol (**1**) is one of these polyphenols. It was designed from baicalein, a natural flavonoid which shows potent and selective inhibition against intestinal sucrase.[11] This small, unique compound is not a sugar mimetic, as seen from its structure. The mode of inhibition of **1** is uncompetitive and differs from sugar mimetics, which usually show a competitive or mixed mode of inhibition.[11] An uncompetitive inhibitor is a compound that binds only to the enzyme-substrate complex. This fact indicates that the binding site of **1** is not a substrate binding pocket. Exploring the binding site of **1** should give an opportunity to enhance the inhibitory potential of **1**, or to develop another potent, selective intestinal glucosidase inhibitor. Since crystallization of intestinal glucosidase is currently only partly successful,[12,13] exploring the amino acid residue placed near the binding site by the molecular probe method should give useful information. In this study, we synthesized various derivatives of **1** and tested their intestinal glucosidase inhibitory activities to design a molecular probe for identification of the binding site.

A molecular probe requires a functional group, a photoaffinity group (benzophenone, diazirine, phenylazide) or a S_N2 type reacting group (iodoacetyl, tosyl), which reacts and creates, a chemical bond between the probe and an amino acid residue of a protein.[14–16] As a first set of derivatives, phenolic hydroxyl and amine group modified derivatives were synthesized and tested for their sucrase and maltase inhibitory activity to determine whether can be utilized to attach a functional group (Experimental procedures; see *Biosci. Biotechnol. Biochem.* Web site). Table 1 shows that amino group modified derivatives **2** and **3** decreased the inhibitory activity, indicating the importance of a free amino group. Dimethylation of the phenolic hydroxyl groups also decreased the IC_{50} value, by 30 fold, for maltase, and no inhibition was seen for sucrase (compound **4**). But monomethylation had almost no effect on the inhibitory activity of **1** (compound **5**). Since the acyl substitute (**6**) showed no activity, the presence of two oxygen atom sandwiching the amino group appears to be the important structure. To examine further the effect of substitution on the phenolic hydroxyl group, propylated (**7**) and heptylated (**8**) derivatives were tested. Unlike methylation, propylation affected the IC_{50} value on the order of 100-fold. Further elongation of the alkyl group slightly elevated its activity, as seen in **8**, but the introduction of a longer alkyl chain was denied, as inhibition of this type of compound probably relies on the hydrophobic interaction of an alkyl groups and the mode of inhibition might change.

[†] To whom correspondence should be addressed. Tel/Fax: +81-11-706-2496; E-mail: junk@chem.agr.hokudai.ac.jp

Table 1. IC_{50} Value of 2-Aminoresorcinol (**1**) Derivatives against Intestinal Glucosidase



Compound	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM)	
						Sucrase	Maltase
1	H	H	H	OH	H	4.2	8.5
2	Ac	H	H	OH	H	>1000	>1000
3	Me	Me	H	OH	H	>1000	>1000
4	H	H	Me	OMe	H	>1000	267
5	H	H	Me	OH	H	4.2	13
6	H	H	H	Ac	H	>1000	>1000
7	H	H	Pr	OH	H	429	>1000
8	H	H	Heptyl	OH	H	166	622
9	H	H	Me	OH	$(CH_2)_3OH$	>1000	>1000
10	H	H	H	OMe	$(CH_2)_3OH$	>1000	>1000
11a	H	H	H	OH	Ac	2.4	8.6
12a	H	H	H	OH	CO_2Me	6.0	24.7
13	H	H	H	OH	Me	>1000	511

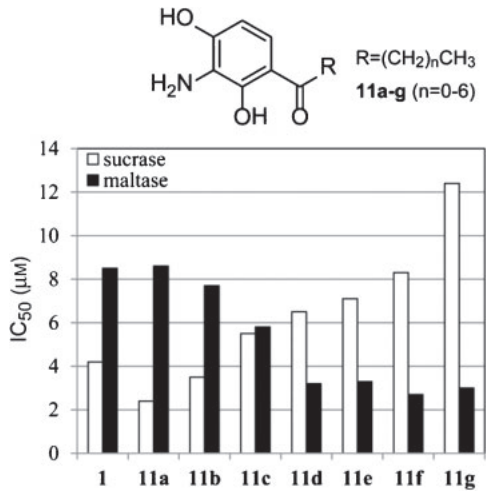


Fig. 1. Effects of Alkyl Chain Length on the IC_{50} Value of Acyl Derivatives **11a–g**.

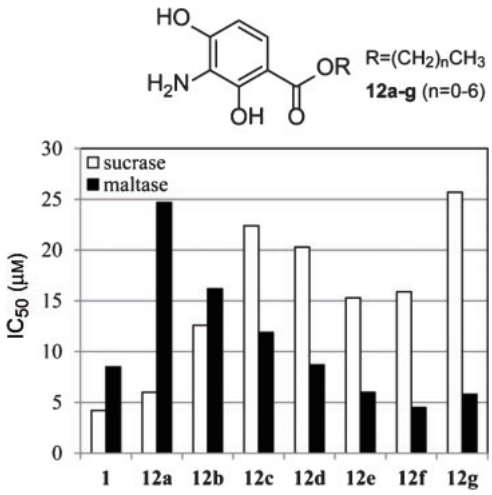
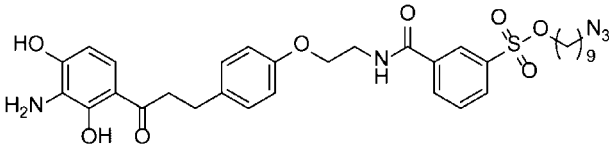


Fig. 2. Effects of Alkyl Chain Length on the IC_{50} Value of Alkoxy Carbonyl Derivatives **12a–g**.

Turning away from the amine and the phenolic hydroxyl group, derivatives with an additional substituent at 4th position was tested next. Alkyl, acyl, and alkoxy carbonyl derivatives **9**, **10**, **11a**, and **12a** were chosen. Monomethylated derivatives **9** and **10** were chosen as alkylated derivatives, as the alkylated derivatives seemed rather unstable, and methylation of phenolic hydroxyl group gave more stability to the 2-aminoresorcinols.[17] Alkylated derivatives **9** and **10** showed only traces of intestinal glucosidase inhibitory activity (Table 1). Acylated derivative **11a** showed inhibition comparable to **1** as previously reported,[11] and alkoxy carbonylated derivative **12a** also showed sucrase inhibition comparable to **1**, but its maltase inhibitory activity was reduced to some degree (Table 1). Compounds **9**/**10** and **11a**/**12a** differed in the methylation of phenolic hydroxide and the presence of a carbonyl group. Since the methylation of phenolic hydroxide has only small effect against glucosidase inhibition, the difference between alkylation and acylation/alkoxy carbonylation is probably due to electronic effect of the substituents on the aromatic ring, the alkyl group has an electron-donating character and the carbonyl group has an electron-withdrawing character, steric effect caused by a rather long alkyl chain is not of concern, as shown by the results below (Fig. 1). To confirm the effect of alkylation, 2-amino-4-methylresorcinol (**13**) was synthesized and tested. Compound **13** showed little inhibitory activity for sucrase as with **9** and **10**. Maltase inhibition of **13** also decreased showing the negative effect of the alkyl substituent and the importance of the carbonyl group in **11** and **12**.

From the above results, acylation or alkoxy carbonylation at the 4th position of the aromatic ring seemed to be the least effecting substitution at the position. Next we examined compounds **11b–g** and **12b–g** to determine the effects of acyl and alkoxy carbonyl chain length in detail. As shown in Figs. 1 and 2, the length of the acyl and the ester alcohol had no negative effect on maltase inhibition, but had some positive effect. The decrease in the IC_{50} value means a favorable effect of hydrophobic interaction for maltase inhibition, and also indicates that

a sterically large functional group can be attached without affecting inhibitory activity. In contrast, the IC_{50} value gradually increased for sucrase inhibition, which means the inhibitory activity decreased as the chain lengthened, but the degree of decrease in inhibition was not large. The results showed a correspondence between attaching functional groups through acylation and alkoxy carbonylation, with a slight advantage for acylation. We synthesized molecular probe **14** with the tosyl group as a reacting group and azide as a pre-tag group.[15,16] An additional aromatic group was inserted in the middle of the linker part to enhance maltase inhibition by hydrophobic interaction. The intestinal glucosidase inhibitory activity of **14** was estimated to be $IC_{50} = 22\,\mu M$ against maltase and $IC_{50} > 100\,\mu M$ (25% at $100\,\mu M$) against sucrase. The inhibitory activity against sucrase decreased and might not be useful but the maltase inhibitory activity fell only 2.6 times as compared to **1** affording an opportunity to use **14** as a probe against maltase.



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In conclusion, we synthesized derivatives of **1** and tested their intestinal glucosidase inhibitory activity. The inhibitory activity of **1** and its derivatives showed a similar tendency as for the inhibition of sucrase and maltase. Uncompetitive inhibition of **1** means that this inhibitor binds to the enzyme-substrate complex. This means it does not inhibit the catalytic site of the enzyme. However, since sucrase and maltase inhibitory activities relate to each other, it is unlikely that **1** binds to a totally different site of sucrase or maltase. Hence the binding site of 2-aminoresorcinol (**1**) must be at a common or a similar region of the two enzymes. The overall results indicate the suitability of attaching functional groups *via* an acyl or alkoxy carbonyl group beside the phenolic hydroxyl group. Hence we designed and synthesized preliminary probe **14**. Since there is no standardized design for the molecular probe method, several variants of this type of molecular probe are being synthesized for further study.

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17) 2-Aminoresorcinol (**1**) changes its color from white to brown in few days at room temperature which causes decrease in its activity. This was not seen in compound **5**.