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Running title

Screening for Disaccharides with Insulin Mimetic Activity

Title

Screening and Identification of Disaccharides with Insulin Mimetic Activity against L6 Cells

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Abstract

Insulin mimetics are considered as prospecting anti-diabetic agent. Recently, a disaccharide neohesperidose was found to show insulin mimetic activity against L6 cells. Here, several other disaccharides were screened for their insulin mimetic activity and resulted in an identification of three new insulin mimetic disaccharides.

Key words

Insulin mimetic; Disaccharide; Diabetes mellitus; Glucose uptake; L6 Cell

Glucose homeostasis is maintained by multiple hormones with adverse effect. Elevation of blood sugar level is handled together by several hormones, but lowering of the elevated blood sugar level is handled by the peptide hormone insulin alone. Defection in insulin secretion or decrease of its sensitivity easily leads to the impaired control of blood sugar level which status is called diabetes mellitus. In the medical treatment of diabetes mellitus, regulation of blood sugar level is the most important piece, as high level of blood sugar eventually leads to several symptoms like cardiovascular disease, renal failure, blindness and neurological disorders.[1)] Especially, maintenance after dining, the main factor that rapidly elevates blood-sugar, is important. For this purpose, exogenous insulin is currently employed as a medicine to maintain blood-sugar level of the patients.[2, 3)] However, insulin's physical instability due to its peptidic character, restricts the use of insulin only by injection. Development of an alternative agent with a stable character, which can be used orally, should give patients of diabetes mellitus a choice for maintaining their disease.

Natural products have been widely studied as a source of blood sugar controlling agent, and several plants are utilized as an easily applicable supplementary food. Several natural products like flavonoids are reported as insulin mimetic compound, except most of those compounds are known to work as a peroxisomal proliferator activated receptor (PPAR) agonist and does not exactly mimics the activity of insulin.[4,

[5] Other than those PPAR agonists, a complex of flavonoids and an inorganic metal has been reported to resemble the action of insulin.[6-8] Recently, we have explored an organic compound that has insulin mimetic activity.[9] The compound, neohesperidose (**1**), is a disaccharide composed of D-glucose and L-rhamnose and was found as an active principle of insulin mimetic flavonoid glycoside, Kaempferol 3-*O*-neohesperidoside. By treating muscle model cell L6 with neohesperidose (**1**) at concentration of sub-nanomolar range, the stimulated cells rapidly uptake glucose just as it was stimulated by insulin. The simple and stable character of **1** gave an opportunity to utilize it as an insulin alternative, but the maximum uptake of glucose induced by **1** was relatively low compared to insulin. In this study, we presumed that several other disaccharides may exhibit the same activity. And to find a disaccharide with this unique activity, we chose several disaccharides and investigated for their insulin mimetic activity.

Our first choices were disaccharides connected through β1→4 bond as it is one of the most common glycoside bond seen in natural products. Cellobiose (**2**), β-D-glucose-(1→4)-D-mannose (**3**), β-D-mannose-(1→4)-D-glucose (**4**), β-D-mannose-(1→4)-D-mannose (**5**), lactose (β-D-galactose-(1→4)-D-glucose) (**6**) and epilactose (β-D-galactose-(1→4)-D-mannose) (**7**) were tested for their activity [10] but all of these compounds failed to induce insulin mimetic activity (data not shown). We then turned our attention from testing various combinations of β1→4 connected disaccharides to various types of glycoside bond. Di-glucose isomers (**2, 8-15**)[11] with ideally most of the possible structures were selected and tested. Fortunately, three of the tested disaccharides, trehalose (**8**), isomaltose (**12**) and gentiobiose (**15**) showed insulin mimetic activity against L6 cells inducing 130, 136 and 132% increase of 2-deoxyglucose (2-DG) uptake compared to control (Fig. 1). The rest of disaccharides seem to slightly elevate 2-DG uptake, but there were no significant differences to the control. Structure of the active disaccharides varies at this moment. Some restriction to the position of glycoside bond is seen from the result, at least we can point out that

Structures

Fig. 1.

1,4-disaccharide have no activity recognizable from the results of compound **2-7**, **11**. It is fairly difficult to see other structural rules between the three active disaccharides or with the previously found insulin mimetic disaccharide neoheperidose (**1**) except for they are all a disaccharide. However, the results obtained here give an opportunity to find additional active disaccharides. And our first goal to show "Some disaccharides have insulin mimetic activity" has been accomplished.

In conclusion, we have explored three new insulin mimetic disaccharides. Although none of these compounds (**8**, **12**, **15**) induced higher activity compared to neohesperidose (**1**), and the common glycoside bond of these compound seems to have low tolerance under digestive conditions, the result obtained here showed the possibility of several disaccharides to work as an insulin mimetic. Further research about insulin mimetic disaccharides shall give an opportunity to use them as a chemical treatment against diabetes mellitus.

Acknowledgement

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References

1. Coccheri S, *Drugs* , **67**, 997-1026 (2007).

2. Barag SH, *J. Am. Osteopath. Assoc.,* **111**, S13-S15 (2011).

3. Hirsch IB, Bergenstal RM, Parkin CG, Wright E, Buse JB, *Clin. Diabetes*, **23**, 78-86 (2005).

4. Hsu FL, Liu IM, Kuo DH, Chen WC, Su HC, Cheng JT, *J. Nat. Prod.*, **66**, 788-792 (2003).

5. Chacko BK, Chandler RT, D'Alessandro TL, Mundhekar A, Khoo NKH, Botting N, Barnes S, Patel RP, *J. Nutr.*, **137**, 351-356 (2007).

6. Sakurai H, Shimomura S, Fukuzawa K, Ishizu K, *Biochem. Biophys. Res. Commun.*, **96**, 293–298 (1980).
7. Cazarolli LH, Zanatta L, Jorge AP, Sousa E, Horst H, Woehl VM, Pizzolatti MG, Szpoganicz B, Silva FRMB, *Chem-Biol. Interact.*, **163**, 177–191 (2006).
8. Sakurai H, Adachi Y, *BioMetals*, **18**, 319–323 (2005).
9. Yamasaki K, Hishiki R, Kato E, Kawabata J, *ACS Med. Chem. Lett.*, **2**, 17-21 (2011).
10. Insulin mimetic activity assay was performed as written in reference 9.
11. Commercial products were used without further purification. Compounds **2**, **8**-**12** (>98%) were purchased from Wako Pure Chem. In, Ltd., **13** (>98%) was purchased from Carbosynth Ltd., **14** (>97%) was purchased from Seikagaku Biobusiness Co., **15** (>96%) was purchased from Tokyo Chem. Ind. Co., Ltd.

Figure Legend

Fig. 1. Insulin mimetic activity of di-glucoses.

Differentiated L6 cells were treated with 1 nM of each sample for 4 hours followed by 2-DG treatment for 30 min. Insulin-mimetic activity (i.e., 2-DG uptake) is expressed as a percentage of 2-DG uptake in control cells. Data are means ± SEMs from five independent repeats of the experiment. * denotes a statistically significant difference vs control ($p \leq 0.05$).

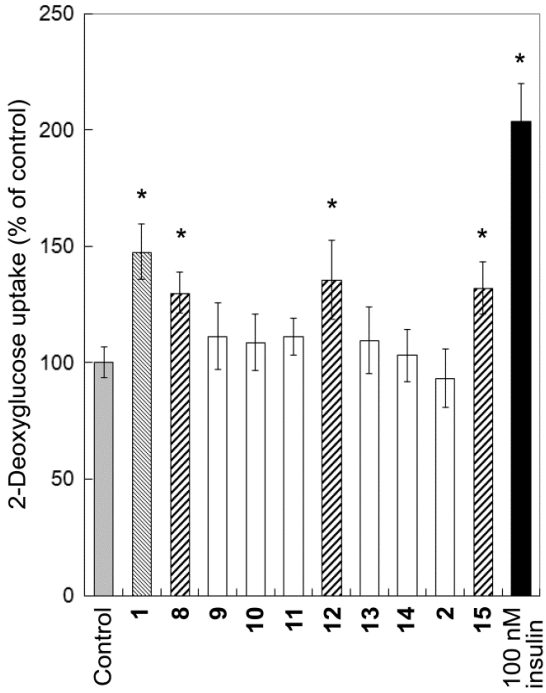

Fig 1.

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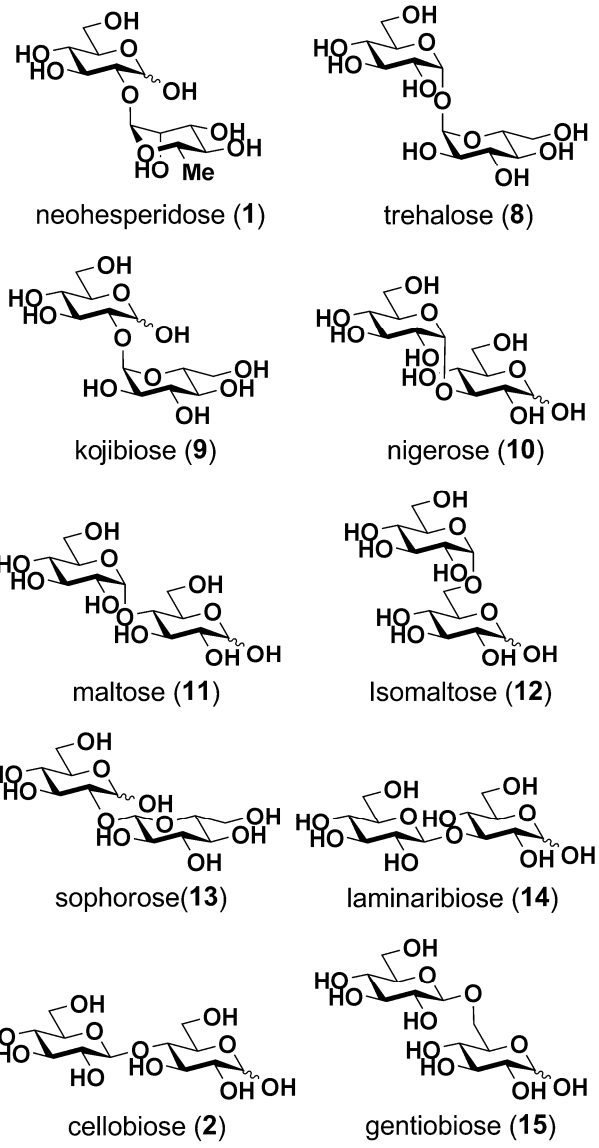
Structures



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