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A NEW SYNTHETIC HYPOGLYCAEMIC POLYSACCHARIDE

Kenichi Hatanaka[1]*, Soo-Chang Song[1], Atsushi Maruyama[1],
Akira Kobayashi[2], Hiroyoshi Kuzuhara[3], and Toshihiro Akaike[1]

[1]*Department of Biomolecular Engineering, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 227, Japan*

[2]*KAST (Kanagawa Academy of Science and Technology), Sakado, Takatsu-ku, Kawasaki 213, Japan*

[3]*RIKEN (The Institute of Physical and Chemical Research), Hirosawa, Wako-shi, Saitama 351, Japan*

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SUMMARY: The synthetic (1→6)-α-**D**-glucopyranan with branching and without branching were tested as a new hypoglycaemic drug. (1→6)-α-**D**-glucopyranan having an α-**D**-glucopyranosyl branch at the C-3 position (**1**) showed a remarkable hypoglycaemic activity on *i.p.* injection to mice. The polysaccharide having both α- and β-glucopyranosyl branches (**2**) also lowered the blood sugar (glucose) level in mice. On the other hand, the synthetic linear (1→6)-α-**D**-glucopyranan (**3**) and α-**D**-glucopyranosyl branched polysaccharide (**4**) did not have a hypoglycaemic function, indicating that the branching glucose units are essencial for the biological activity.

Many natural branched polysaccharides participate in a variety of biochemical functions in vivo. It has been noted that both the chemical structures of the main chain and the branch (kind of sugar, type of linkage, distribution of the branch, etc.) are important for the biochemical functions of the branched polysaccharides.

It was reported that polysaccharide of Panaxan A, which was isolated from the roots of *Panax ginseng* C. A. Meyer (Araliaceae), showed physiological activity of lowering the blood glucose level (1). The structure of this polysacharide was analyzed and the three possible structural fragments were also reported (2). Panaxan A is mainly composed of α-1→6 linked **D**-glucopyranose residues having α-**D**-glucopyranosyl branching at C-3 position. However, the definite chemical structure of the polysaccharide, particularly the length of side chains, is not clear yet. In order to clear up the relationship between the chemical structure and the biochemical function, one of the possible polysaccharides, which is monosaccharide-branching glucan, was synthesized by novel reaction route including enzymatic hydrolysis (3).

* To whom correspondence should be addressed.

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In this investigation, we report the hypoglycaemic effect of the monosaccharide-branching glucan in mice and elucidate the importance of the branching glucose units.

MATERIALS AND METHODS

Polysaccharides: (1→6)-α-**D**-glucopyranan with branching and without branching were synthesized by polymerization, glycosylation, and enzymatic hydrolysis as described previously (3, 4). 1,6-Anhydro-glucose monomer has two kinds of protective groups, one is benzyl ether and the other one is benzoyl ester, because hydroxyl group at C-3 will be a branching point after polymerization. The cationic ring-opening polymerization of this monomer was carried out with phosphorus pentafluoride as catalyst at low temperature under high vacuum. The polymerization with 7 - 15% of catalyst gave polymers in high yield. Benzoyl groups of polymers were removed by using sodium methoxide. Glucosylations of polymers with 2,3,4,6-tetra-O-benzyl-β-**D**-glucopyranosyl trichloroacetimidate were carried out. In obtained branched polysaccharide, branch contents were in the range of 48% to 87%. These values were estimated by ^{1}H-NMR spectroscopy. The branches were the mixture of α-glucosyl and β-glucosyl units. Debenzylation of branched polysaccharide was carried out with sodium in liquid ammonia at -78°C. In order to complete the stereospecific branching reaction, unnecessary β-**D**-glucopyranosidic unit was removed from the polysaccharide by enzymatic hydrolysis, using cellulase. The obtained polysaccharide was (1→6)-α-**D**-glucopyranan having α-**D**-glucopyranosyl branch at C-3 position (**1**). On the other hand, the branched polysaccharide before the enzymatic hydrolysis was (1→6)-α-**D**-glucopyranan having α- and β-**D**-glucopyranosyl branches at C-3 position (**2**). In addition, the polysaccha-ride derivative before glycosylation was debenzylated to give a linear (1→6)-α-**D**-glucopyranan (**3**). Mannose-branched polysaccharide (**4**) was synthesized by α-**D**-mannosylation of 2,4-di-*O*-benzyl-(1→6)-α-**D**-glucopyranan with orthoester method and subsequent deprotection.

Hypoglycaemic Activity: Male mice (Std:ddy, 8 weeks old) were used in groups of five. The mice was given drinking water freely and was not feeded for two hours just before the injection and the measurement of the blood glucose level. The synthetic polysaccharides (**1**, **2**, **3**) were dissolved in physiological saline and injected *i.p.* (10, 30 mg/kg) to the mice. As control experiment, the saline solution without polysaccharide was also injected to the mice. After 5, 10, and 24 hours from the administration, the blood was drawn from the tail. The glucose level of the drawn blood was determined by a glucose level analyzer (Glucoboy, Eiken Chemical Co. Ltd., Tokyo: glucose oxydase method). In addition, the same experiment was carried out for the hyperglycaemic mice (BALB/c, male, 14 weeks old) which were pretreated with streptozotocin (250 mg/kg, *i.p.*) 12 days before the polysaccharide injection.

RESULTS AND DISCUSSION

The results in the normal mice are summarized in Table 1. α-**D**-glucose-branched polysaccharide (**1**) showed a remarkable hypoglycaemic activity at a dosage of 10 and 30 mg/kg. After 5 and 10 hours from the administration of **1**, the blood glucose level was in the range of 65 to 81% of the value before administration. And after 24 hours after administration, the blood glucose level was restored to normal value. The hypoglycaemic activity existed in α,β-**D**-glucose-branched polysaccharide (**2**) as well, indicating that β-**D**-glucose branch at least did not inhibit the biological functions.

On the other hand, since the linear (1→6)-α-**D**-glucopyranan (**3**) and mannose-branched polysaccharide (**4**) were tested on the different day, the blood glucose level was normalized using the value of control experiment. The normalized values were plotted in

Table 1
Effect of Synthetic Polysaccharides on Blood Glucose Level in Normal Mice[a]

Polymer	$10^{-4}\overline{M}_n$	Ds[b]	Dose (mg/kg, *i.p.*)	Relative glucose level[c] 0	5	10	24
Control	—	—	—	100	91±3	91±7	97±4
1	1.8	0.38	10	100	65±1	81±4	99±4
			30	100	77±5	69±7	99±5
2	1.9	0.52	10	100	68±6	80±6	103±7
			30	100	68±6	89±4	100±3
Control	—	—	—	100	94±3	81±8	107±12
3	1.3	no	10	100	108±9	88±8	114±13
			30	100	108±15	85±17	101±14
4	1.6	0.64	10	100	104±4	92±5	113±8
			30	100	97±19	86±6	106±8

[a] n=5.

[b] Number of branching unit per main chain glucose unit.

[c] Relative blood glucose level at 0, 5, 10, 24 h after administration (value at 0 h = 100).

Figure 1. As shown in Figure 1, linear glucan and the mannose-branched polysaccharide did not exhibit hypoglycaemic action. From these data, it was indicated that α-glucosyl branch was essencial to the hypoglycaemic activity.

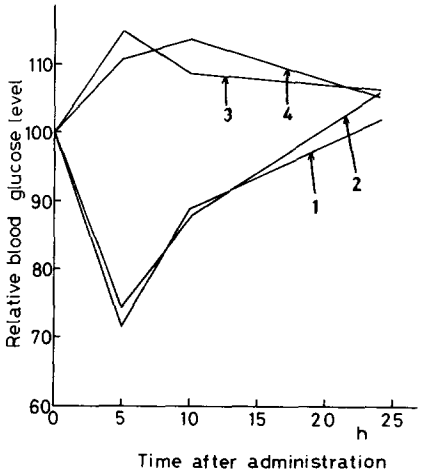

Figure 1. Effect of synthetic polysaccharide on blood glucose level in normal mice (dose = 10 mg/kg). Relative values against the control experiment were plotted.

I.p. administration of **1** to the hyperglycaemic mice pretreated with streptozotocin did not reduce the blood glucose level, suggesting that β-cells in islets of Langerhans, which are destructed by streptozotocin, can participate in this hypoglycaemic activity of the branched polysaccharides. In the experiment using natural polysaccharides, panaxans A and B, it was reported that the natural branched polysaccharides were quite effective drugs for alloxaneinduced hyperglycaemic mice (1). The difference between alloxan-treated mice and streptozotocin-treated mice can be given to explain by the extent of hyperglycaemia, i.e. the extent of destruction of β-cells. β-cells were completely destructed with streptozotocin, since the blood glucose level was more than 500 mg/kg, whereas the alloxan-treated mice had the blood glucose level of 250-450 mg/kg (1). In the preliminary experiment, the incubation of isolated β-cell in the presence of 2 secreted insulin. The mechanism of secretion of insulin by synthetic branched polysaccharide is now in progress.

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