Biochemical Response to D-Glucosone in Terms of Motility of the Stomach, Insulin Secretion, and Sweetness

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Summary D-Glucosone (D-arabino-hexos-2-ulose) was prepared by use of an immobilized glucose 2-oxidase-catalase gel, and the biochemical response to D-glucosone by three kinds of glucose-recognizing systems in higher animals was studied. The enhanced motility of rat stomach caused by glucopenia was reduced by carotid arterial injection of D-glucosone like in the case of D-glucose. D-Glucosone (16.7 mM) showed no effect on the secretion of insulin from the pancreatic islets of the rat, and the secretion of insulin caused by D-glucose (16.7 mM) was markedly depressed by the addition of D-glucosone (16.7 mM). The relative sweetness of D-glucosone, compared with that of 5% sucrose as 1.0, was estimated to be 0.4–0.6, which is similar to that of D-glucose. These results suggest that D-glucosone is a useful model compound for D-glucose to investigate the biochemical mechanism of glucose-recognizing systems.

Key Words: D-glucosone, gastric motility, insulin secretion, sweetness, glucose-recognizing systems

D-Glucosone (D-arabino-hexos-2-ulose), a sugar that can exist in acyclic and cyclic pyranose and furanose forms [1], has a very similar structure to D-glucose and 2-deoxy-D-glucose. In matters of the biochemical and physiological actions of D-glucosone, some investigators have reported that D-glucosone has a high affinity for hexokinase [2] and is an inhibitor of glycolysis in yeast and rat brain [3]. Moreover, this sugar acts as a uridylyate trapping agent and causes UTP depression in tumor cells [4]; and the mutagenicity of this sugar also has been reported [5]. In these studies, the D-glucosone used was synthesized chemically from D-glucose by the method of Bayne [6].

Recently, we reported that glucose 2-oxidase (pyranose oxidase; EC 1.1.3.10)

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from *Coriolus versicolor* KY 2912 [7] oxidizes D-glucose to D-glucosone [8]. Thereupon, we have designed a convenient procedure for producing pure D-glucosone using immobilized glucose 2-oxidase, a method like that reported by Liu *et al.*, who used a similar enzyme of different origin [9].

It is well known that many kinds of glucose-recognizing systems exist in higher animals and that these systems play a very important role in the control of the blood glucose level [10]. However, the details of these mechanisms are not well defined. To clarify the molecular action of D-glucose in the above systems, D-glucose anomers [11] and analogues of D-glucose have been used in various experiments.

The present study was designed to investigate the biochemical effect of D-glucosone as an analogue of D-glucose on the following glucose-recognizing systems: suppressive effect of D-glucose on enhanced motility of rat stomach caused by glucopenia [12], glucose-stimulated insulin secretion from rat pancreatic islets, and elicitation of sweet response by human taste buds.

MATERIALS AND METHODS

Reagents. Glucose 2-oxidase purified from *Coriolus versicolor* KY 2912 was kindly supplied by Dr. Y. Yokote of Kyowa Medex Co., Ltd., Tokyo. Catalase (EC 1.11.1.6) from bovine liver and collagenase (EC 3.4.24.3) from *Clostridium histolyticum* were obtained from Sigma Chemical Co., St. Louis, MO. All other reagents were obtained from Nakarai Chemicals, Kyoto.

Preparation of D-glucosone. D-Glucosone was prepared from D-glucose dissolved in water using glucose 2-oxidase immobilized on Affi-Gel 10 (Bio-Rad Laboratories, Richmond, CA). Hydrogen peroxide, a byproduct of the reaction, was eliminated by catalase immobilized on the same gel. Equilibrated D-glucose solution (0.2 M, 20 ml), immobilized glucose 2-oxidase (160 U/g wet gel, 5 g), and immobilized catalase (1,600 U/g wet gel, 5 g) were put into a vessel (outer column of a 50-ml injection syringe) having a cotton filter and a three-way cock. The suspension in the vessel was mixed using an end-over-end mixer for 10 h at room temperature and the supernatant was then removed via the three-way cock. An aqueous solution of pure D-glucosone at the same concentration as that of the initial D-glucose solution was thus obtained, lyophilized and stored at -15°C over desiccant.

High-performance liquid chromatography (HPLC). The liquid chromatograph used in this study was a Triotar-V (Japan Spectroscopic Co., Ltd., Tokyo) equipped with a guard column Shodex Ionpak KS-800 (0.6×5 cm) and a ligand exchange mode column Shodex SUGAR SP-1010 (0.8×30 cm; Showa Denko K.K., Tokyo). Distilled water as the eluant for HPLC was degassed by stirring under reduced pressure. The flow rate was constant at 0.99 ml/min, and the column temperature was maintained at 80°C . The column eluate was monitored by using a Shodex RI SE-51 refractive index detector (Showa Denko K.K.).

Measurement of the effect of D-glucosone on enhanced motility of the stomach. Male Sprague-Dawley rats weighing 250–300 g were bilaterally adrenalectomized about 30 min before the experiment to eliminate the usual variation in blood levels of D-glucose and insulin. The motility of the stomach was evaluated by the changes in intragastric pressure using the balloon method described previously [12]. The initial level of intragastric pressure was adjusted to be 9–11 cm of water. From a cannula placed into the cranial end of the left carotid artery, 50 μ mol (0.5 M, 0.1 ml) of hexose including D-glucosone was injected into the artery 45–90 min after pretreatment with regular insulin (1.0 U/kg i.p.) by which the gastric motility of the rat was increased gradually.

Incubation of rat islets and insulin assay. Islets, isolated from non-fasting Sprague-Dawley rats (250–350 g) by collagenase digestion of the pancreas [13], were incubated at 37°C in a Krebs-Ringer bicarbonate buffer (pH 7.4) containing 2 mg/ml bovine serum albumin and 2.8 mM D-glucose. This basal medium was equilibrated with a mixture of O₂ (95%) and CO₂ (5%) before use. Six to ten islets were placed in each of a series of glass tubes containing 0.1 ml of the above basal medium. The glass tubes were gassed with a mixture of O₂ (95%) and CO₂ (5%) for 2 min and then stoppered tightly. After preincubation for 30 min with shaking in a Dubnoff metabolic shaker (90 strokes/min), the medium was replaced with the basal medium supplemented with D-glucose (total concentration: 0, 5.0, 16.7 mM) and/or D-glucosone (total concentration: 0, 16.7 mM). The glass tubes were gassed again and incubated for 1 h to induce glucose-stimulated insulin release. Insulin was assayed by radioimmunoassay with the use of Sepharose-coupled antibody (Phadeseph insulin RIA, Pharmacia Co., Uppsala) with human insulin used as the standard.

Measurement of relative sweetness of D-glucosone and D-glucose. Thirteen normal subjects in our laboratory, 6 males and 7 females, 22 to 58 years old, were used to evaluate the sweetness of five sugar solutions: 1, 2, 3% sucrose; 5% D-glucose; and 5% D-glucosone. After rinsing their mouths with purified water, the subjects sequentially placed each of these sugar solutions (2 ml) in their mouths for 5 to 10 s to test its sweetness. The relative sweetness of D-glucosone and D-glucose was judged by comparison with the sweetness of the sucrose solutions.

RESULTS

Purity of D-glucosone

Purity of D-glucosone prepared from D-glucose by using the immobilized glucose 2-oxidase and catalase was examined by thin layer chromatography (TLC) [8] and HPLC. No spot or peak except that of D-glucosone was observed.

Effect of D-glucosone on enhanced motility of the stomach

Carotid arterial injection of D-glucosone provoked a reduction in enhanced motility of the stomach caused by insulin, as did D-glucose (Fig. 1). On the con-

Fig. 1. Effects of various monosaccharides injected into the carotid artery on enhanced motility of rat stomach elicited by insulin-induced glucopenia. Arrows indicate the times of administration of saline (100 μ l) and monosaccharide solutions (0.5 M, 100 μ l). (A), Saline; (B), D-fructose; (C), D-galactose; (D), D-glucose; (E), D-glucosone; (F), 2-deoxy-D-glucose; (G), D-mannose.

Table 1. Intensity of maximal response and time required to produce maximal response in terms of intragastric pressure after arterial injection of monosaccharides.

Monosaccharide (50 μ mol)	Intensity of gastric pressure (% of control)	Time needed to reach maximal response in gastric pressure (s)
D-Glucose	50.6 $\pm$ 1.0 (n=7)	29.3 $\pm$ 2.7 (n=7)
D-Glucosone	52.0 $\pm$ 3.9 (n=8)	34.7 $\pm$ 3.3* (n=8)
D-Fructose	99.7 $\pm$ 0.6 (n=3)	
D-Galactose	99.5 $\pm$ 0.5 (n=3)	
D-Mannose	100.3 $\pm$ 0.6 (n=3)	
2-Deoxy-D-glucose	99.0 $\pm$ 1.0 (n=3)	

Values are means $\pm$ SD. * $p < 0.01$, significantly different compared with D-glucose injection.

trary, saline (0.9% NaCl), D-fructose, D-galactose, 2-deoxy-D-glucose, and D-mannose produced no change in the motility of the stomach. Table 1 shows that the degree of depression of the intensity of gastric pressure was almost the same between D-glucosone and D-glucose. However, the time needed to reach the maximal response to D-glucosone was longer than that to D-glucose ($p < 0.01$).

Inhibition of glucose-stimulated insulin release by D-glucosone

Table 2 shows that the addition of D-glucose at the concentration of 16.7 mM caused stimulation of insulin secretion from rat pancreatic islets. The intensity of the stimulation was about 15 times higher than when 0 or 5.0 mM D-glucose was added. D-Glucosone alone showed no effect on the secretion of insulin. However, the addition of D-glucosone (16.7 mM) markedly depressed the secretion of insulin caused by 16.7 mM D-glucose.

Table 2. Insulin secretion from rat pancreatic islets in the presence of D-glucose and/or D-glucosone.

D-Glucose (mM)	D-Glucosone (mM)	Insulin secretion (μ U/h/islet)
0	0	2.9 ± 0.7
5	0	2.7 ± 0.9
16.7	0	45.7 ± 4.0
0	16.7	3.5 ± 1.1
5	16.7	2.9 ± 1.0
16.7	16.7	7.3 ± 2.1

Values are means $\pm$ SD for three experiments.

Table 3. Comparison of sweetness of 5% D-glucosone solution with sucrose solutions (1–3%) and D-glucose solutions (5%).

(A) Comparison with sucrose

Sweetness	Relative sweetness (5% sucrose = 1.0)	Frequency (n = 13)
less than 1% sucrose	<0.2	0
same as 1% sucrose	0.2	0
between 1% and 2% sucrose	0.2–0.4	0
same as 2% sucrose	0.4	3
between 2% and 3% sucrose	0.4–0.6	9
same as 3% sucrose	0.6	1
more than 3% sucrose	>0.6	0

(B) Comparison with D-glucose (relative sweetness: 0.5–0.6)

Sweetness	Frequency (n = 13)
less than 5% D-glucose	6
same as 5% D-glucose	4
more than 5% D-glucose	3

Relative sweetness of D-glucosone

Nine subjects (69%), 4 males and 5 females, answered that 5% D-glucosone was sweeter than 2% sucrose but not so sweet as 3% sucrose, as shown in Table 3. When the strength of the sweetness of 5% sucrose was regarded as 1.0, relative sweetnesses of 2% and 3% sucrose were supposed to be 0.4 and 0.6, respectively. The relative sweetness of D-glucosone was estimated to be between 0.4 and 0.6.

We confirmed that the relative sweetness of D-glucose (5%) was estimated to be 0.4–0.6 by using the same method. This value agrees well with that (0.5–0.6) reported previously [14]. We also compared the sweetness of D-glucosone with that of D-glucose. Six subjects (46%) answered that 5% D-glucosone was not so sweet as 5% D-glucose, 4 subjects (31%) answered that there was no difference in sweetness between D-glucosone and D-glucose, and 3 subjects (23%) answered that D-glucosone was sweeter than D-glucose (Table 3). The results indicate that D-glucose is slightly sweeter than D-glucosone.

DISCUSSION

In previous investigations on the biological effects of D-glucosone, the D-glucosone used was chemically synthesized from D-glucose by the method of Bayne [6]. We have reported that glucose 2-oxidase from *C. versicolor* KY 2912 can oxidize both α - and β -anomers of D-glucose with the consumption of oxygen and stoichiometric formation of D-glucosone and hydrogen peroxide [8]. So, we immobilized glucose 2-oxidase and catalase on Affi-Gel 10 to prepare pure D-glucosone simply and conveniently. The chromatographic behavior of D-glucosone on both HPLC and TLC indicated that pure D-glucosone can be obtained by this enzymatic method.

It has been accepted that changes in the blood glucose level alter gastric motility by a glucose-recognizing system located within the brain [12]. Because the depression of D-glucose has been shown to activate cells in the hindbrain and hypothalamus which in turn stimulate vagal motoneurons to produce an increase in gastric motility, the administration of D-glucose from the carotid artery into the brain causes the suppression of the enhanced motility of the stomach evoked by insulin-hypoglycemia (Fig. 1 and Table 1). This glucose-recognizing system also showed a clear response to D-glucosone introduced into the brain at the same molar concentration as used for D-glucose (Fig. 1). Because the other monosaccharides, including D-fructose, D-galactose, 2-deoxy-D-glucose, and D-mannose, produced no appreciable change in the motility, the response seems to be specific to D-glucose and D-glucosone.

The glucose-recognizing system in the brain has an anomeric preference for the β -form of D-glucose [12]. D-Glucosone appears to have several anomeric configurations [1]. However, it is not possible to state which type of D-glucosone is more effective, since we have not yet succeeded in separating D-glucosone anomers.

The molecular mechanism of glucose-stimulated insulin secretion remains unclear. Recently, a multiplicity of evidence has suggested that the metabolism of D-glucose following its phosphorylation by pancreatic islet glucokinase (ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1), which is one of the isozymes of hexokinase, is intimately involved in triggering insulin secretion. This recognition mechanism for D-glucose in pancreatic islets showed no response to D-glucosone as shown in Table 2. However, D-glucosone drastically inhibited the secretion of insulin caused by a high level (16.7 mM) of D-glucose. It is well known that 2-deoxy-D-glucose inhibits the glucose-induced secretion of insulin from rat pancreatic islets. This phenomenon is explained by the inhibition of glucose-6-phosphate isomerase (D-glucose 6-phosphate ketol-isomerase, EC 5.3.1.9) by 2-deoxy-D-glucose 6-phosphate (2-deoxy-D-*arabino*-hexose 6-phosphate), a product of the glucokinase reaction, which causes depression of glycolysis in the pancreatic islets. We have demonstrated that glucokinase of rat liver, as well as hexokinase, can phosphorylate

D-glucosone. However, the phosphorylated D-glucosone (D-glucosone 6-phosphate), as well as 2-deoxy-D-glucose 6-phosphate, cannot be metabolized by the coupling system of glucose-6-phosphate isomerase and 6-phosphofructokinase (EC 2.7.1.11) from rabbit muscle (unpublished results). In the pancreatic islets, D-glucosone may also inhibit the insulin secretion by a similar mechanism as 2-deoxy-D-glucose.

The different biological responses to D-glucosone described above appear to be attributable to differences in the glucose-recognizing mechanisms between the system present in the brain and that in pancreatic islets. Previously, we reported that the glucose-recognizing system of the insulin secretion shows the α -anomeric preference, whereas β -D-glucose is more effective than α -D-glucose in the suppression of enhanced motility of the stomach [11, 12].

Regarding the molecular features of sweet-tasting compounds, the AH and B systems and the third hydrophobic component (X) established by Shallenberger and Acree [15] may be important for the sensation of sweet taste. In the case of D-glucose, the hydroxyl group on C-4 has been assigned as the AH and the oxygen atom of the hydroxyl group on C-3 has been ascribed to the B. The X function has been assigned to C-6. As the configuration of hydroxyl groups of D-glucosone is similar to that of D-glucose, it can be assumed that D-glucosone is a sweet-tasting compound. In fact, we found that D-glucosone tastes sweet and the sweetness of D-glucosone is almost the same as that of D-glucose.

It has been reported that D-glucosone is metabolized to UDP-glucosone in cultures of TA3 mammary tumor cells [4]. More definitive experiments about the metabolic pathway of D-glucosone will be needed to clarify the mechanism of the biological action of D-glucosone as an analogue of D-glucose. One such study on the metabolism of D-glucosone in rat hepatocytes is now in progress in our laboratory. Details of the study will be reported elsewhere in the future.

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