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Physicochemical Characterization and Biological Effects of Inulin Enzymatically Synthesized from Sucrose

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We first developed the method to produce inulin from sucrose using an enzyme from *Bacillus* sp. 217C-1. Synthesized inulin consists of a linear polymer having $\beta(2-1)$ linkages of D-fructose with one terminal glucose. The synthesized inulin has similar properties (pH and thermal stability, Maillard reaction, and in vitro fermentation) to plant-derived inulin. The marked difference is the polydispersity of the inulin chain length. Synthesized inulin with a narrow degree range of fructose polymerization shows better solubility in water than plant-derived inulin. Synthesized inulin (5%, w/w) treatment for 12 weeks reduced the elevation in body weight and serum and liver lipids in rats fed high fat- and high sucrose-supplemented diets, and blood glucose in rats fed a standard diet. Synthesized inulin (15%, w/w) significantly suppressed the elevation in blood glucose of human healthy subjects after dextrin loading. These results suggest that daily intake of synthesized inulin modulates carbohydrate and lipid metabolism.

KEYWORDS: Inulin; inulin-producing enzyme (IPE); *Bacillus* sp. 217C-11; dietary supplementation; serum and liver lipids; blood glucose

INTRODUCTION

Inulin is a polysaccharide with $\beta(2-1)$ linkages by which D-fructose is polymerized. Inulin is widely distributed throughout the plant kingdom and exists as a reserve substance in the tubers or tuberous roots of *Asteraceae* plants, such as dahlias, Jerusalem artichokes, and British inula and in chicory roots (*1*). These naturally occurring molecules have fructose polymerization degrees from 2 to >60 (the average degree of fructose polymerization ranges from 32 to 34), and chicory root-derived inulin is commercially available in Europe and North America. In contrast with starch, inulin and oligofructans are soluble and fermentable dietary fiber. Inulin is resistant to hydrolysis by pancreatic amylase and saccharidases (sucrase, maltase, isomaltase, or lactase) in the upper gastrointestinal tract, and it reaches the large intestine unabsorbed and is utilized as a carbohydrate substrate for the growth of indigenous bifidobacteria. Nevertheless, it is noteworthy that potential preventive and therapeutic effects of dietary inulin on blood glucose profiles and serum lipid reduction may be useful in the treatment of subjects with diabetes and cardiovascular disease (*2*−*9*). Inulin-type fructans are either extracted from plants in the form of inulin or synthesized from sucrose through the combined action of microbial enzymes, sucrose:sucrose 1-fructosyltransferase (EC.2.4.1.99) and fructan:fructan 1-fructosyltransferase (EC 2.4.1.100) (*10*, *11*). The purification of plant-derived inulin is extremely difficult because of differing fructose-chain lengths. Although the purity of plant-extracted inulin may be high, a lack of uniformity in the chain lengths is unavoidable. In contrast, both polyfructan and oligofructan are reportedly synthesized enzymatically from sucrose, but the properties are largely different from those of higher-plant inulin. Inulin-type polyfructan is molecularly larger; the molecular weights of polyfructan produced from sucrose by condiospores of *Aspergillus sydowi* are about 10 000 000−20 000 000 (*12*, *13*). Fructan produced from sucrose by fructosyltransferases by *Aspergillus* or *Fuserium* is reportedly an oligosaccharide in which one to four molecules of fructose are bound to sucrose (*14*, *15*). Thus, inulin-type polyfructan with a similar molecular weight to naturally existing inulin could not be produced. In the previous study (*16*), therefore, we screened and found a microorganism that can produce a highly efficient enzyme that converts sucrose into inulin molecules with an approximately similar chain length and structure as plant-origin inulin. Furthermore, in this study, we succeeded in developing methods to produce inulin industrially from sucrose by using one inulin-producing enzyme. The effects of dietary inulin or inulin-type fructans on the metabolism and disease risk are considered to depend on physicochemical properties such as solubility in water, viscosity, and fermentability by microorganisms in the large intestine (*2*−*4*, *7*, *17*). While dietary inulin has been suggested to modulate the levels

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of serum lipids (*5*−*9*), Davidson et al. have reported that the values of serum total cholesterol and LDL cholesterol did not change appreciably during inulin treatment in limited human subjects with hypercholesterolemia (*18*). There is no general agreement that inulin modulates serum cholesterol and triacylglycerol profiles. Hence, the purpose of this study was to examine the physicochemical properties of our synthesized inulin, solubility in water, viscosity, and fermentability by microorganisms, and to assess its usefulness as a dietary supplement compared with plant-derived inulin. Furthermore, the biological effects of inulin-containing foods on serum and liver lipid profiles and serum glucose profiles were compared with the effects of similar foods without inulin using both standard and high-fat diets.

MATERIALS AND METHODS

Chemicals. Raftiline ST (average DP = 10) and Raftiline HP (average DP = 23) were supplied by Orafti (Tienen, Belgium). Raftiline ST is unfractionated, spray-dried inulin isolated from the roots of *Cichorium intybus* L. Raftiline HP is spray-dried inulin, from which the low DP of inulin is removed by fractionation. Inulin from dahlias, Jerusalem artichokes, and chicory, and enzymes from the digestion test (human saliva α-amylase, artificial gastric juice, porcine pancreas α-amylase, and rat intestinal acetone powder) were purchased from Sigma (St. Louis, MO). Xanthan gum was from Dainippon Pharmaceutical (Osaka, Japan). Isomaltooligosaccharide (900P), xylooligosaccharide (95P), lactosucrose (LS-90P), fructooligosaccharide (MeioligoP), nondigestive dextrin (pinefiber), and polydextrose (Litess) were from Showa-Sangyo (Tokyo, Japan), Suntory (Tokyo, Japan), Ensuiko Sugar Refining (Yokohama, Japan), Meiji Seika (Tokyo, Japan), Matsutani Chemical Industries (Itami, Japan), and Danisco Japan (Tokyo, Japan), respectively. 1-Kestose, nystose, and raffinose were from Wako Pure Chemicals (Osaka, Japan).

Preparation of Inulin-Producing Enzyme. *Bacillus* sp. 217C-11 was cultivated at 30 °C for 15 h with agitation in culture medium (pH 8.0) containing 1% (w/w) sucrose, 1% (w/w) yeast extracts, 1% (w/w) fish extracts, 1% (w/w) sodium glutamate monohydrate, 0.3% (w/w) K_2HPO_4, and 0.04% (w/w) citric acid. After cultivation, the bacterial cells were removed by centrifugation at 19000*g*, and the supernatant was concentrated by filtration (NTU-3250-C3R) (Nitto Denko, Osaka, Japan) and used as an inulin-producing enzyme (IPE).

Production of Inulin Synthesized Enzymatically from Sucrose by Inulin-Producing Enzyme. A sucrose solution of 40−45% **(**Fuji Nihon Seito, Shizuoka, Japan) was added to IPE and incubated in citrate buffer (pH 6.0−7.0) at 55 °C for 2 days. The reaction was stopped by heat treatment at 85 °C for 30 min. The reaction mixture was decolorized with activated charcoal, filtrated through a reverse osmosis membrane to remove low molecular materials, desalted with ion-exchange resin, and spray-dried to obtain inulin powder. The powder contained more than 98% inulin by HPLC analysis; column, Shinwa Chemical Industries ULTRON PS-80N (300 × 8.0 mm); mobile phase, distilled water; flow rate, 0.5 mL/min; temperature, 50 °C; pump, Hitachi L-6000; detector, Hitachi L-7490 RI detector.

Physicochemical Characterizations of Synthesized Inulin. *Measurement of the Average DP of Inulin.* The average DP of inulin was measured by size exclusion chromatography with Tosoh TSK-Gel G3000PWLX (300 × 7.8 mm) HPLC column under the same conditions described previously (*16*). Furthermore, the average DP of inulin was also defined as the glucose/fructose ratio, after complete hydrolysis with Fructozyme inulinase (Novo Nordisk, Bagsverd, Demmark) at pH 7.0 for 15 h at 37°C. The quantitative analysis of sugars was done by HPLC with Shinwa Chemical Industries ULTRON PS-80N (300 × 8.0 mm) column under the same conditions described above.

Determination of the Polydispersity of Inulin Chain Length. The polydispersity in the chain length of inulin was evaluated using Dionex DX-500 apparatus equipped with an eluent degassing module, Dionex ED-40, pulsed electrochemical detector, GP50 gradient pump and AS50 autosampler. The samples [50 μL, 3% (w/w) solution] passed a CarboPac PA-1 column (4 × 250 mm) and the bound material was eluted by a 150 mM NaOH−150 mM NaOH and 500 mM CH_3COONa gradient solution.

Measurement of the Viscosity of Inulin. The viscosity of the 20% (w/w) solution was determined at 25 °C using a Tokimec DVM-B2 viscometer (Toki Sangyo Co., Tokyo, Japan), which is based on torque measurements when a spindle of various sizes rotates in a container.

pH and Thermal Stability of Inulin and Fructooligosaccharide. The carbohydrates (10%, w/w) were dissolved in citrate buffer of pH 3.0, 4.0, and 5.0 or phosphate buffer of pH 6.0 and 7.0 and heated at each temperature for 15 min, or heated at 70 °C for 60 min, 90 °C for 5 min, or 120 °C for 2 min. The resulting carbohydrates were determined by HPLC as described above.

Maillard Reaction by Inulin. Five milliliters of inulin (20%, w/w) solution and 3 mL of 0.1 M citrate buffer at pH 3.0, 4.0, and 5.0 or 0.1 M phosphate buffer at pH 6.0 and 7.0 were mixed, 2 mL of 2% (w/w) glycine solution was added, and it was heated at 100 °C for 90 min. The absorbance at 420 nm of the resulting solution was then measured.

In Vitro Digestion of Various Carbohydrates by Digestive Enzymes. (a) Carbohydrate (10%, w/w) in 5 mL of 50 mM malate buffer (pH 6.0) containing 1 mM $CaCl_2$ was digested by 1 mL of human saliva α-amylase (40 units/ml) at 37 °C for 30 min. (b) Carbohydrate (1.5%, w/w) was digested in 6 mL of artificial gastric juice solution (pH 2.0) at 37 °C for 100 min. (c) Carbohydrate (1%, w/w) in 5 mL of 50 mM malate buffer (pH 6.6) containing 1 mM $CaCl_2$ was digested by 0.5 mL of porcine pancreas α-amylase (20 units/ml) at 37°C for 6 h. (d) Carbohydrate (1%, w/w) in 5 mL of 50 mM malate buffer (pH 6.6) was digested by 0.5 mL of rat intestinal acetone powder solution at 37 °C for 3 h. (e) Using a kit for the determination of total dietary fiber (Sigma), carbohydrate was treated with α-amylase, protease, and amyloglucosidase according to the manufacturer's instructions.

Biological Effects of Synthesized Inulin. *In Vitro Fermentation of Inulin and Oligosaccharides.* The organisms (*Bifidobacterium, Bacteroides, Clostridium, Eubacterium, Lactobacillus,* or *Enterococcus,* American Type Culture Collection) were anaerobically cultured in medium (pH 8.0) containing 0.5% (w/w) carbohydrate, 0.5% (w/w) yeast extracts, 0.5% (w/w) peptone, 0.2% (w/w) sodium acetate, 0.05% (w/w) Tween 80, 0.02% (w/w) $MgSO_4 \cdot 7H_2O$, 10 ppm $MnSO_4$ $7H_2O$, 10 ppm $FeSO_4 \cdot 7H_2O$, and 10 ppm NaCl at 37 °C for 24 h. The organism in 10-fold diluted medium (1 vol) was then inoculated in the growth medium containing each of the carbohydrates (99 vol) and anaerobically cultured at 37 °C for 48 h. The growth was determined by turbidity at 660 nm.

Effects of Dietary-Synthesized Inulin on Serum Lipids and Blood Glucose: Animals and Diets. The study was a randomized, double-blind, crossover study. Male Wistar rats (Japan SLC, Hamamatsu, Japan) weighing about 110 g were maintained under a 12-h light-dark cycle with free access to food and water. The animals were acclimatized for one week prior to the experiment. All animals were randomly assigned to standard diet (SD), 5% (w/w) inulin-supplemented standard diet (SD + I), high-fat diet (HF), or 5% (w/w) inulin-supplemented high-fat diet (HF + I) for 4 and 12 weeks. The HF diet consisted of 23.9% (w/w) lipid, 56.8% (w/w) carbohydrate, and 19.3% (w/w) protein (kJ), and the SD diet consisted of 12.9% (w/w) lipid, 60.4% (w/w) carbohydrate, and 26.7% (w/w) protein (kJ) (Oriental Yeast, Tokyo, Japan). Both diets are described in detail in **Table 1**. Three times per week, all rats were weighed, and food intake in grams was monitored. All studies followed protocols approved by the Institutional Animal Care and Life Committee, University of Shizuoka.

Blood and Tissue Sampling. The experiments were run between 9:00 AM and 12:00 AM. Rats were anesthetized with diethyl ether, and then the abdominal cavity was rapidly opened following the median line of the abdomen. Blood was rapidly and simultaneously drawn from the abdominal vena cava (ca. 4 mL) into syringes. Serum samples were separated from blood by centrifugation after standing for 30 min at room temperature. The liver median lobe was excised, frozen in liquid nitrogen immediately after weighing, and used for the determination of triacylglycerol and total cholesterol levels. All plasma and liver samples were stored at −80 °C until analysis.

Blood Glucose Profiles in Normal Human Subjects after Dextrin Load. The study was a randomized, double-blind, crossover study. We studied 12 unrelated, healthy Japanese subjects with a mean age of

Table 1. Composition of the High-Fat and Standard Diets

high-fat diet	g	standard diet (MF chow)	g
casein	19.70	crude protein	23.80
soybean oil	1.0		
lard	10.00	crude fat	5.10
		crude fiber	3.20
mineral mixture	4.00	ash	6.1
vitamin mixture	1.00	nitrogen-free extract	54.0
choline chloride	0.15	humidity	7.8
cholesterol	0.50		
sodium cholate	0.25		
cellulose	3.40		
sucrose	60.00		
total	100		100

43.1 ± 3.1 years (29 to 59) and body mass index of 23−26. None of the subjects had preexisting medical conditions, lipid-altering medications, alcohol or drug abuse, or excessive diet. The conditions and procedures of the study were reviewed, and written informed consent was obtained from each subject. After an overnight fast, blood samples were collected at the baseline from each subject at 9:00 a.m. The subjects then consumed 80 g of dextrin dissolved in hot water and cooled, and blood samples (1.0 mL) were collected into 3.8% (w/w) sodium citrate in a ratio of 9:1 at 0.5, 1, and 2 h after dextrin consumption. One week later, the overnight-fasted subjects consumed 80 g of dextrin and 12 g inulin in the same form, and blood samples were collected at 0, 0.5, 1, and 2 h after consumption and were centrifuged at 2000*g* for 10 min. Samples were stored at −80 °C until assay.

Analytical Procedure. Plasma/serum glucose concentration was determined by the glucose oxidase method with a commercially available kit from Shino Test (Tokyo, Japan). Serum triacylglycerol and nonesterified fatty acid levels were measured enzymatically with kits from Shino Test. Serum total cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured using kits from Wako Pure Chemicals. A piece of liver was homogenized in 20 vol (the SD and SD + I groups) and 100 vol (the HF and HF + I groups) of saline containing 0.1% (w/v) Triton X-100, and the triacylglycerol and total cholesterol concentrations were estimated with the above commercial kits.

Statistical Analysis. Values are expressed as the means ± SE. All data were analyzed using one-way analysis of variance. The difference between the means of the groups was tested for significance using Student's t-test. The level of statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Production of Synthesized Inulin by Inulin-Producing Enzyme and Its Physicochemical Properties. IPE from *Bacillus* sp. 217C-11 was added to a fructose unit by $\beta(2-1)$ linkage to the fructose unit of sucrose to produce GF*n* type inulin and (*n*-1) G, where n represents the number of fructose units (F) linked to each other with one terminal glucose (G). When the reaction reached equilibrium, the composition of inulin and glucose had reached 45% (w/w), respectively, indicating that inulin purification is straightforward. The ratio of glucose/fructose in sucrose-origin inulin was 1:17 after complete digestion with Fructozyme inulinase, while those of Raftiline ST and Raftiline HP, were 1:9 and 1:22, respectively. Although the DP sizes of enzymatically synthesized inulin are reportedly different from those of naturally existing inulin (*12*−*15*), in this study, we succeeded in producing a group of inulin molecules with similar chain length to those of plant-origin inulin (**Table 2** and **Figure 1**). **Figure 2** shows the polydispersity in the chain length of synthesized inulin and plant-origin inulin. The molecular weight of the synthesized inulin was relatively low, and the DP sizes were less than 30, while the DP sizes of plant-origin inulin were dispersed throughout a wider range from 2 to 60. Thus, the physical properties of inulin obtained by IPE were quite different from those of naturally existing plant-origin inulin: (1) The water solubility of synthesized inulin at the temperatures tested was higher than that of chicory root-derived Raftiline HP and Raftiline ST with high polymerized inulin (**Figure 3**). Since the average DP sizes of Raftiline ST are 10−12, but Raftiline ST contains inulin molecules in a wider DP range from 30 to 60, the water solubility is lower than that of synthesized inulin in a narrow DP range from 2 to 30. The water solubility of synthesized inulin was 20% (w/w) at room temperature and 40% (w/w) at 70 °C. (2) The viscosity of synthesized inulin was lower than those of Raftiline HP and Raftiline ST, and was quite different from Xanthan gum with high viscosity (**Figure 4**). (3) The freezing point depression effect of synthesized inulin at concentrations of 10 to 30% was lower than that of sucrose, fructooligosaccharide, and Raftiline ST (**Figure 5**).

Table 2. Comparison with the Average Degree of Polymerization of Plant-Origin and Synthesized Inulin[a]

	average DP
Sigma's Jerusalem artichoke inulin	29
Sigma's chicory inulin	25
Sigma's dahlia inulin	35
Raftiline HP	23–25
Raftiline ST	10–12
synthesized inulin	16–18

[a] The average degree of polymerization (DP) was determined by the HPLC method with GPC column.

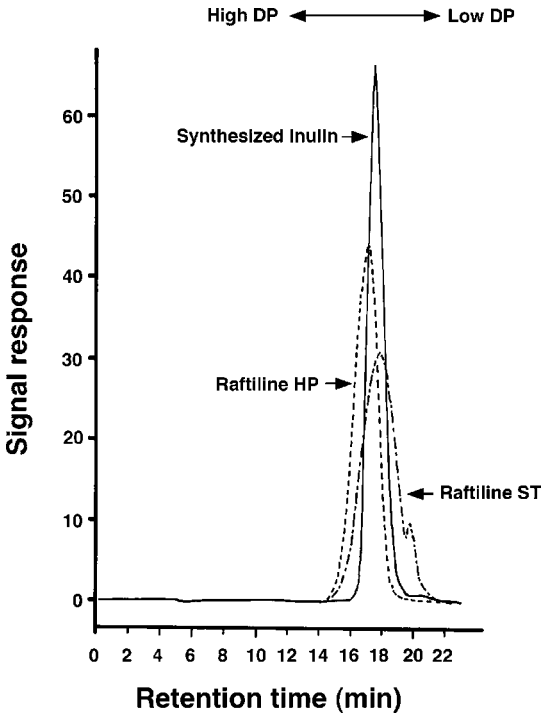

Figure 1. DP-distribution profiles of plant-origin and synthesized inulin.

We also examined the thermal stability of sucrose-origin inulin, plant-origin inulin, and fructooligosaccharide. The residual ratio of synthesized inulin in the after-heating (100 °C) solution at pH 4−7 was more than 96%, while that at pH 3 was drastically decreased to 12% (**Figure 6A**). As shown in **Figure 6B**, the thermal stability order at pH 3 was Raftiline HP > synthesized inulin > Raftiline ST > fructooligosaccharide. These observations indicate that the thermal stability of

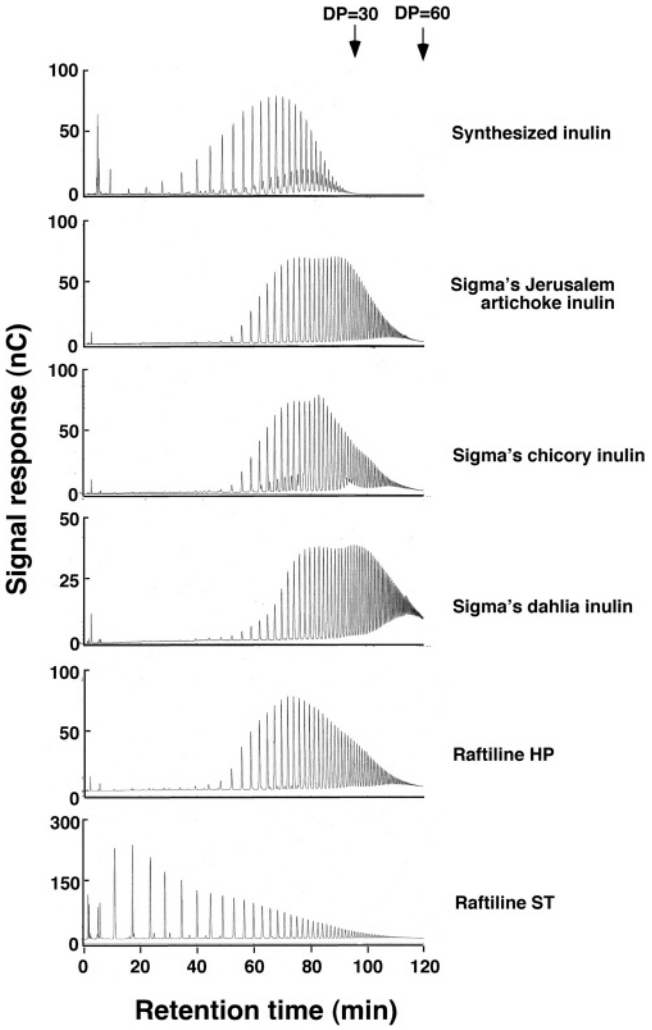

Figure 2. Comparison of chain length polydispersity of plant-origin and synthesized inulin; the broken line indicates a DP of 30 and 60.

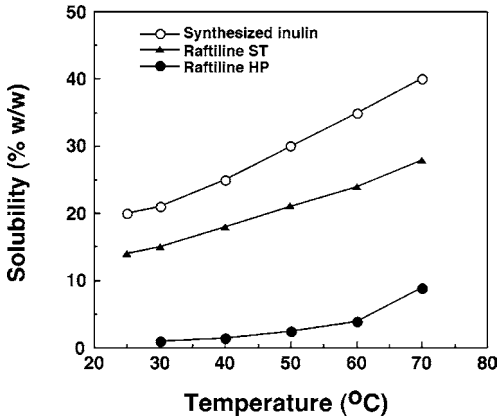

Figure 3. Differences of solubility between plant-origin and synthesized inulin.

synthesized inulin and plant-origin inulin is likely to depend on the chain length of fructan. To investigate whether the synthesized inulin product contained carbohydrates having a reducing end, we compared the Maillard reaction by the synthesized inulin with those by plant-origin inulin, sucrose and glucose. The Maillard reaction by the synthesized inulin was detected in rather low levels compared with that by glucose, and the reactivity of the synthesized inulin was almost the same as those of Raftiline ST and sucrose (**Figure 7**).

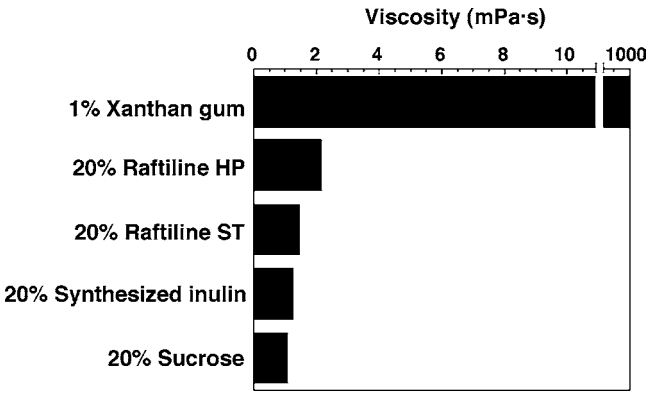

Figure 4. Comparison of viscocity of xanthan gum, plant-origin inulin, synthesized inulin, and sucrose.

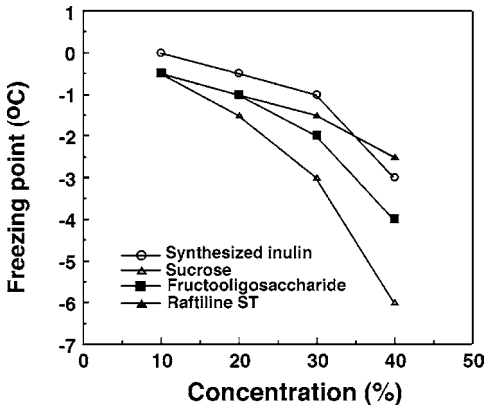

Figure 5. Freezing-point depression effect of various fructans.

Next, since plant-origin inulin is reportedly resistant to digestion in the upper gastrointestinal tract, we examined the digestion of synthesized inulin by various digestive enzymes compared with 1-kestose and nystose, which are constituents of fructooligosaccharide. While 1-kestose and nystose, fructans with a short chain length, were slightly hydrolyzed by artificial gastric juice (7.9 and 5.1% of the original, respectively), synthesized inulin and Raftiline HP were resistant to digestion by human saliva α-amylase, artificial gastric juice, porcine pancreas α-amylase, and rat intestinal acetone powder (**Table 3**). Also after treatment with combined digestive enzymes (i.e., α-amylase, protease, and amyloglucosidase) using a kit for the determination of total dietary fiber, synthesized inulin and Raftiline HP were not hydrolyzed by these enzymes. Accordingly, these observations suggest that synthesized inulin reaches the large intestine essentially intact in the same manner as plant-origin inulin, and functions as dietary fiber.

Biological Effects of Synthesized Inulin. *Effect of Various Carbohydrates on the in Vitro Growth of Intestinal Bacteria.* The growth of intestinal bacteria on culture medium containing various carbohydrates such as synthesized inulin, plant-origin inulin, and oligosaccharides was compared. **Table 4** shows that *Bifidobacterium adolescens* grew better on synthesized inulin and Raftiline ST than on Raftiline HP. *Enterococcus faecalis* and *Clostridium butyricum* grew on synthesized inulin, but harmful bacteria such as *Lactobacillus fermentum*, *Bacteroides fragilis*, and *Eubacterium aerofaciens* did not. While beneficial bacteria grew better on fructooligosaccharide than on synthesized inulin, synthesized inulin was shown to suppress more strongly the growth of harmful bacteria than fructooligosaccharide. There was no significant difference in the availability of synthesized inulin and chicory root-derived inulin by intestinal bacteria.

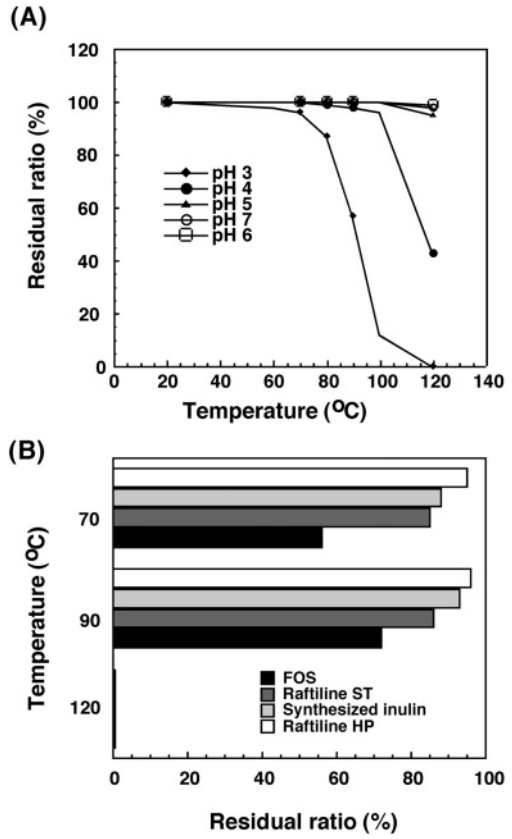

Figure 6. Comparison of thermal stability of synthesized inulin (**A**, **B**), Raftiline HP, Raftiline ST, and fructooligosaccharide (FOS) (**B**) under different pH conditions. Residual ratios (%) were calculated after the HPLC analysis of the carbohydrates treated for 15 min under various pH conditions at various temperatures (**A**), and for 60 min at 70 °C and pH 3, for 5 min at 90 °C and pH 3, or for 2 min at 120 °C and pH 3 (**B**) and nontreated carbohydrates as described in Materials and Methods.

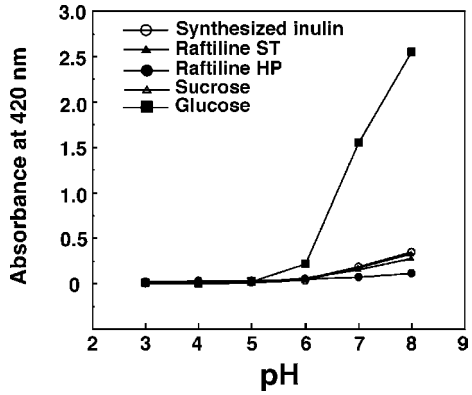

Figure 7. Comparison of Maillard reaction by various carbohydrates.

Effects of Dietary-Synthesized Inulin on Serum Lipids, Liver Lipids, and Blood Glucose in Rats Fed a Standard Diet or High-Fat Diet. We examined the effect of synthesized inulin (5%) consumption on the body weight of rats fed a standard diet mainly supplemented with naturally existing starch as carbohydrate or in rats fed a diet supplemented with high fat and high sucrose instead of starch. While inulin consumption for 4 and 12 weeks in the SD group did not affect the rat body weights, synthesized inulin consumption in the HF group reduced the body weight (**Table 5**). In addition, serum triacylglycerol levels decreased significantly with synthesized inulin consumption in the HF group, while a trend toward reduced serum triacylglycerol levels was observed in the SD group after inulin consumption for 4 and 12 weeks (**Figure 8A**). There was no significant change in serum total cholesterol levels (**Figure 8B**). We next analyzed the liver lipid content: No significant change in triacylglycerol and total cholesterol levels of the liver in 4-weeks-fed rats was detected. These observations indicate that triacylglycerols did not accumulate in the liver due to blockage in the lipoprotein secretion. After 12 weeks of

Table 3. Comparison of in Vitro Enzymatic Digestion toward Various Carbohydrates

	hydrolysis rate (%)			
carbohydrates	α-amylase from human saliva	artificial gastric juice	α-amylase from porcine pancreas	intestinal acetone powder from rat
synthesized inulin	0	0	0	0
1-kestose	0	7.9	0	0
nystose	0	5.1	0	0
raffinose	0	0	0	0
Raftiline HP	0	0	0	0

Table 4. Comparison with Availability of Various Carbohydrates by Intestinal Bacteria[a]

bacteria	IM	XO	LS	RF	FO	ND	PD	ST	HP	SI
Bifidobacterium infantis ATCC 15697	++	−	++	++	+	±	−	+	+	+
Bifidobacterium adolescens ATCC 15703	+++	+	+++	++	+++	±	+	++	+	++
Bifidobacteriurn breve ATCC 15700	++	−	+++	+	+	−	−	±	−	−
Lactobacillus fermentum ATCC 14931	++	−	+	+	−	−	−	±	−	−
Enterococcus faecalis ATCC 19433	+++	+	+	+	+	++	++	+	±	+
Clostridium butyricum ATCC 19398	+++	+	+	+++	+++	+	+	+	−	+
Bacteroides fragilis ATCC 25285	+	±	+	+	+	±	+	±	−	±
Eubacterium aerofaciens ATCC 25986	+	±	+	−	+	±	−	−	±	±

[a] A portion of bacterial culture fluid was inoculated in the medium containing 0.5% various carbohydrates. Judgment of bacterial growth: −, 0.1 > OD at 660 nm; ±, 0.1 ≤ OD at 660 nm < 0.2; +, 0.2 < OD at 660 nm < 0.4; ++, 0.4 < OD at 660 nm < 0.6; +++, 0.6 < OD at 660 nm. IM, isomaltooligosaccharide; XO, xylooligosaccharide; LS, lactosucrose; RF, raffinose; FO, fructooligosaccharide; ND, nondigestible dextrin; PD, polydextrose; ST, raftiline ST; HP, raftiline HP; SI, synthesized inulin.

Table 5. Initial and Final Body Weight, Food Intake, and Energy Intake in Standard-, Inulin-Supplemented Standard-, High Fat-, and Inulin-Supplemented High Fat-Fed Rats for 12 Weeks[a]

	SD	SD + I	HF	HF + I
n	12	12	12	12
initial body weight (g) 5-wk-aged rats	111.9 ± 2.2	112.9 ± 1.1	110.6 ± 1.1	112.3 ± 3.0
final body weight (g)				
4 wk-fed rats	242.0 ± 4.8	233.9 ± 4.2	229.7 ± 4.7	207.2 ± 1.0***
12 wk-fed rats	329.1 ± 9.6	322.9 ± 7.1	325.9 ± 6.1	293.5 ± 4.3***
food intake (g/day)	15.3 ± 0.4	15.2 + 0.3	13.9 ± 0.3	13.2 ± 0.3
energy intake (kJ/day)	225.1 ± 5.2	215.7 ± 4.4	240.3 ± 4.9	223.3 ± 5.2*

[a] Values are means ± SE; *n*, number of animals; SD, standard diet; SD + I, inulin-supplemented standard diet; HF, high fat diet; HF + I, inulin-supplemented high fat diet. Food and energy intakes represent the daily mean over the 12-wk protocol. * $p < 0.05$, *** $p < 0.001$ for synthesized inulin-supplemented HF-fed animals versus HF-fed animals.

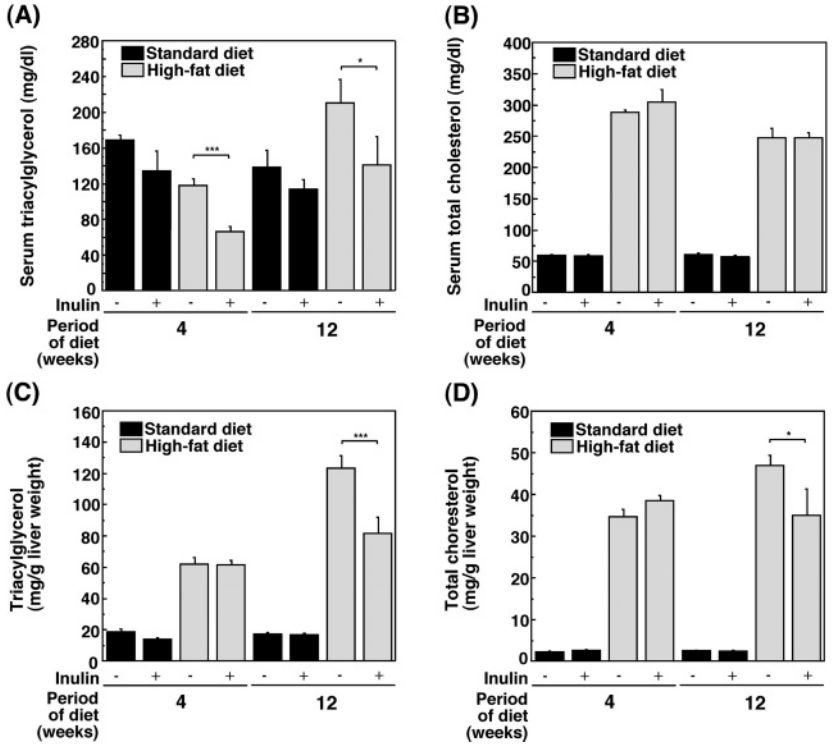

Figure 8. Response of serum and liver lipids to dietary supplementation with synthesized inulin for 4 and 12 weeks. Male rats were maintained on regular MF chow (standard diet, solid bars) or a high-fat diet (gray bars) with or without synthesized inulin for 4 and 12 weeks after which the concentrations of serum (**A**, **B**) and liver (**C**, **D**) triacylglcerols (**A**, **C**) and total cholesterol (**B**, **D**) were determined. Values are the means ± SE of six determinations. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for synthesized inulin-supplemented HF-fed versus HF-fed animals.

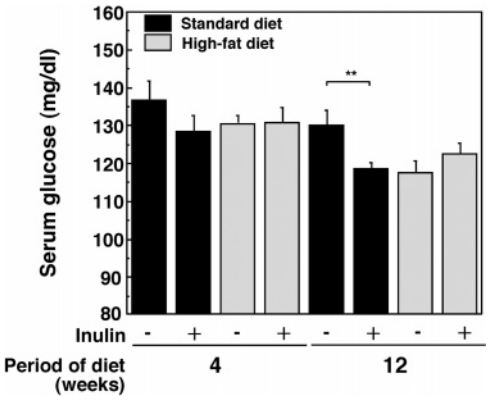

Figure 9. Response of serum glucose to dietary supplementation with synthesized inulin for 4 and 12 weeks. Male rats were maintained on regular MF chow (standard diet, solid bars) or high-fat diet (gray bars) with or without synthesized inulin for 4 and 12 weeks after which the concentrations of serum glucose were determined. Values are the means ± SE of six determnations. * $p < 0.05$ for inulin-supplemented diet versus the counterpart.

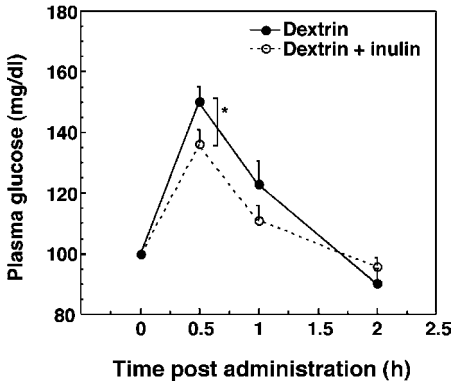

Figure 10. Plasma glucose profiles in human subjects after dextrin load. Blood glucose levels of each subject before dextrin load was presented as 100, while the plasma glucose levels measured were 94.2 ± 1.9 mg/dL on day 0 and 96.6 ± 1.9 mg/dl on day 7. Values are the means ± SE of 12 subjects. * $p < 0.05$ for inulin-supplemented subjects compared to the controls.

treatment with synthesized inulin in the HF group, the accumulation of triacylglycerols and total cholesterol in the liver was suppressed by inulin consumption. These results suggest that inulin consumption suppressed the further development of fatty liver (**Figure 8C**,**D**). Liver lipids in rats fed a diet supplemented with high fat and high sucrose were markedly high, but serum AST and ALT levels were unaffected by the high-fat diet and also synthesized inulin consumption (data not shown).

In the SD group, blood glucose levels were significantly reduced when rats consumed 5% synthesized inulin for 12 weeks, while a trend toward reduced levels was observed after 4-weeks inulin consumption (the levels averaged 137 mg/dL in the control diet and 128 mg/dL in the inulin diet) (**Figure 9**). In contrast, no significant changes in blood glucose levels by 5% synthesized inulin consumption were observed when rats were fed a high sucrose-supplemented diet in the HF group (**Figure 9**).

Blood Glucose Profiles in Normal Human Subjects after Dextrin Load. Furthermore, we examined the effect of synthesized inulin on blood glucose profiles in healthy human subjects after dextrin loading on day 0 and day 7. Since the fasting blood glucose levels on day 0 and day 7 were slightly changed as shown in the **Figure 10** legend, the values of fasting blood glucose were calculated as 100. The increased blood glucose level of the subjects 30 min after oral dextrin loading when it

reached the maximum level was significantly suppressed by synthesized inulin administration (**Figure 10**). During the test periods, no gastrointestinal symptoms and signs such as diarrhea were observed in any subjects.

GENERAL DISCUSSION

In this study, we succeeded in producing inulin enzymatically synthesized from sucrose by IPE on the industrial scale. The structure of synthesized inulin is a linear polymer linked by $\beta(2-1)$ glycoside bridges of D-fructose with one terminal glucose similar to that of plant-origin inulin such as Raftiline ST and Raftiline HP. Thus, the synthesized inulin has a similar pH and thermal stability and Maillard reaction to plant-origin inulin (**Figures 6** and **7**). The marked difference between the synthesized inulin and plant-origin inulin was polydispersity (**Table 2** and **Figures 1** and **2**). The synthesized inulin has a narrower range of polydispersity, with a DP lower than 30, compared with Raftiline ST, Raftiline HP, and other plant-origin inulin in which the DP ranged between 2 and 60. Since synthesized inulin does not contain highly polymerized fractions more than 30 of DP, the water solubility was higher and the viscosity was lower (**Figures 3** and **4**). Furthermore, the freezing point depression effect of synthesized inulin at concentrations of less than 30%, of which the concentration is utilized as a dietary supplement, was smaller than plant-origin inulin (**Figure 5**). These observations indicate that synthesized inulin is superior to plant-origin inulin as a dietary fiber.

Synthesized inulin not only had in vitro intestinal bifidogenesis effects but also led to modulate serum and liver lipid profiles and serum glucose profiles as a dietary supplement (**Table 4** and **Figures 8−10**). The relatively high amounts of plant-origin inulin such as chicory root-derived inulin and oligofructose in the supplemented diet (10 to 20%, w/w) have been reported to lower serum lipids (*5*−*7*). This study demonstrates that the supplementation of rats with quite a small amount of synthesized inulin (5%, w/w) for 12 weeks reduced serum triacylglycerols and liver triacylglycerols and total cholesterol, accompanied by suppressing the elevation of body weights in rats fed a high-fat diet (**Table 5** and **Figure 8**). It has not yet been explained how synthesized inulin prevents the further development of fatty liver. One possible explanation is that synthesized inulin may inhibit the biosynthesis of triacylglycerols and cholesterol in the liver, which utilizes metabolites from the consumption of high fat and high sucrose in the HF diet. In contrast, it is likely that because of the absorption of high cholesterol in the HF diet from intestinal mucosa, serum total cholesterol levels may not be reduced even in an inulin-supplemented diet. The common disorder of lipid metabolism in subjects suffering from hyperlipidemia is considered to be related to the increased production of VLDL. The effect of lowering serum lipids by oligofructose has been reported to result from the reduction of VLDL production (*19*). Accordingly, the data in this study suggest a novel approach for the therapeutic reduction of serum and liver lipid levels. We found decreased blood glucose in rats fed the standard diet by a small amount of synthesized inulin (5%, w/w), but not in rats fed a high fat and extremely high sucrose diet (**Figure 9**). In addition, as reported by Alles et al. (*20*), there was no significant effect of inulin consumption on blood glucose in Type 2 diabetes. This may be why rats fed HF and HF + I diets showed no significant difference in serum glucose levels. Williams has pointed out that convincing lipid-lowering effects of the fructooligosaccharide inulin have been demonstrated in animals, yet attempts to reproduce similar effects in humans have generated conflicting results (*5*). Furthermore, Williams has stated that future attempts to demonstrate lipid-lowering effects of inulin should consider the nature of the background diet as a determinant of response because the pathway of hepatic fatty acid synthesis, which is the major site of action for the triacylglycerol-lowering effects of inulin, is relatively inactive in humans unless a high carbohydrate diet is fed (*5*). In fact, there was no change in serum levels of triacylglycerol and glucose of rats fed a high-fat but not a high-carbohydrate diet containing 23% (w/w) protein, 19.8% (w/w) lard, 19.8% (w/w) corn oil, 24.5% (w/w), sucrose and 5% (w/w) cellulose for 4 weeks, and synthesized inulin (5%, w/w) did not affect the serum levels of triacylglycerol and glucose in rats fed the high-fat diet for 4 weeks (our unpublished data). This study indicates that synthesized inulin administration may be useful in lowering body weight, serum and liver lipid profiles, and blood glucose profiles, and improving colonic health, while it is dependent on diet conditions such as the amount of inulin, sugar, and fat.

ABBREVIATIONS USED

IPE, inulin-producing enzyme; DP, degree of polymerization; AST, aspartate aminotransferase; ALT, alanin aminotransferase.

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