Carbohydrate Research 344 (2009) 654–659

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Fine structural properties of natural and synthetic glycogens

Hiroki Takata [a,*], Hideki Kajiura [a,b], Takashi Furuyashiki [a], Ryo Kakutani [a], Takashi Kuriki [a]

[a] Biochemical Research Laboratory, Ezaki Glico Co., Ltd, Nishiyodogawa-ku, Osaka 555-8502, Japan
[b] Development Department, Food Material Division, Glico Foods Co., Ltd, 7-16 Kasuga-cho, Takatsuki, Osaka 569-0053, Japan

ARTICLE INFO

Article history:
Received 7 October 2008
Received in revised form 16 December 2008
Accepted 13 January 2009
Available online 19 January 2009

Keywords:
Glycogen
α-Amylase hydrolysis
Structure
Polysaccharide

ABSTRACT

Glycogen, highly branched (1→4)(1→6)-linked α-D-glucan, can be extracted from natural sources such as animal tissues or shellfish (natural source glycogen, NSG). Glycogen can also be synthesized in vitro from glucose-1-phosphate using the cooperative action of α-glucan phosphorylase (GP, EC 2.4.1.1) and branching enzyme (BE, EC 2.4.1.18), or from short-chain amylose by the cooperative action of BE and amylomaltase (AM, EC 2.4.1.25). It has been shown that enzymatically synthesized glycogen (ESG) has structural and physicochemical properties similar to those of NSG. In this study, the fine structures of ESG and NSG were analyzed using isoamylase and α-amylase. Isoamylase completely hydrolyzed the α-1,6 linkages of ESG and NSG. The unit-chain distribution (distribution of degrees of polymerization (DP) of α-1,4 linked chains) of ESG was slightly narrower than that of NSG. α-Amylase treatment revealed that initial profiles of hydrolyses of ESG and NSG were almost the same: both glycogens were digested slowly, compared with starch. The final products from NSG by α-amylase hydrolysis were glucose, maltose, maltotriose, branched oligosaccharides with DP ⩾ 4, and highly branched macrodextrin molecules with molecular weights of up to 10,000. When ESG was digested with excess amounts of α-amylase, much larger macrodextrins (molecular weight > 10^6) were detected. In contrast, oligosaccharides with DP 4-7 could not be detected from ESG. These results suggest that the α-1,6 linkages in ESG molecules are more regularly distributed than those in NSG molecules.

1. Introduction

Glycogen, the major storage polysaccharide in animals and microorganisms, is a construct of highly branched (1→4)(1→6)-linked α-D-glucoses with a high molecular weight (10^6–10^9).[1] Electron microscopy has shown that glycogen consists of spherical particles with diameters of 20–40 nm (β-particles), which often associate into much larger α-particles (~200 nm). The molecular weight of an individual β-particle has been shown to be approximately 10^7.[1] Glycogen is opalescent (milky-white and slightly bluish) in aqueous solution, and gives a reddish-brown color with the addition of iodine. Glycogen was successfully synthesized in vitro several decades ago by Cori and Cori[2] using α-glucan phosphorylase (GP; EC 2.4.1.1), and branching enzyme (BE; EC 2.4.1.18) as catalysts and glucose-1-phosphate (G-1-P) as substrate. Since their study, many researchers have synthesized glycogen and revealed that the enzymatically synthesized glycogen (ESG) has structural and physicochemical properties similar to those of natural source glycogen (NSG). However, no detailed comparison between ESG and NSG has been performed.

Recently, we developed two enzymatic synthesis methods for glycogen. The first method, the SP–GP–BE method, is a modification of Coris' method, in which sucrose is used as a starting material and three enzymes, sucrose phosphorylase (SP; EC 2.4.1.7), GP, and BE, are used as catalysts. In the second method, the IAM–BE–AM method, starch is used as a starting material.[3] First, the branched linkages of starch are hydrolyzed using isoamylase (IAM; EC 3.2.1.68) to produce a mixture of short chain amyloses. Then, the amyloses are assembled into glycogen by the action of BE in the presence of amylomaltase (AM; EC 2.4.1.25). ESG derived by these methods was easily soluble in water and gave an opalescent solution resembling NSG.[3] The structural parameters and iodine-staining properties of ESG and NSG were also similar, as summarized in Table 1, suggesting that the molecular size and shape of ESG closely resemble those of NSG. As a food component, glycogen has long been considered to have health benefits, although there are a few supporting scientific evidences. We have been shown that orally administered ESG has a survival benefit for

Abbreviations: ESG, enzymatically synthesized glycogen; NSG, natural source glycogen; BE, branching enzyme; GP, glucan phosphorylase; SP, sucrose phosphorylase; AM, amylomaltase; IAM, isoamylase; PPA, porcine pancreatic α-amylase; M_r, molecular weight; M_w, weight-average molecular weight; M_n, number-average molecular weight; DP, degree of polymerization; CL, average chain length; ICL, average internal -chain length; ECL, average external-chain length; HPAEC, high-performance anion exchange chromatography; HPLC, high-performance liquid chromatography; HPSEC, high-performance size-exclusion chromatography.

* Corresponding author. Tel.: +81 (6) 6477 8425; fax: +81 (6) 6477 8362.
E-mail address: takata-hiroki@glico.co.jp (H. Takata).

0008-6215/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2009.01.008

Table 1
Structural parameters of glycogens

ESG/NSG	Sample name or source	Synthetic method or manufacturer	M_w[a] × 10^{-6}	M_n[b] × 10^{-6}	M_w/M_n	α-1,6 Linkage (%)	CL[c]	ECL[d]	ICL[e]	λ_{max} (nm)
ESG	B	IAM-BE-AM	5.0	4.0	1.3	8.6	11.6	7.6	3.0	453
	7M	IAM-BE-AM	7.0	5.6	1.3	10.1	9.9	6.2	2.7	399
	12M	IAM-BE-AM	11.3	3.9	2.9	10.8	9.3	6.3	2.0	396
	A	SP-GP-BE	24.2	19.8	1.2	8.4	11.9	7.5	3.4	464
NSG	Bovine liver	Sigma type IX	2.0	1.7	1.2	8.4	11.9	7.0	3.9	409
	Oyster	Wako	6.0	4.9	1.2	9.8	10.2	6.6	2.7	451
	Mussel	Laboratoirs Serobiologique	5.1	4.5	1.1	9.4	10.6	6.5	3.1	448
	Slipper limpet	Sigma Type VIII	4.8	3.7	1.3	11.1	9.0	5.3	2.7	402
	Rabbit liver	Sigma type III	15.7	12.6	1.2	8.0	12.5	7.7	3.9	468
	Sweet corn	Q.P.	19.8	17.4	1.1	7.5	13.3	8.2	4.1	468

[a] Weight-average molecular weight.
[b] Number-average molecular weight.
[c] Number-average chain length, CL = [Total sugars as glucose]/[non-reducing end residues].
[d] Exterior chain length, ECL = CL × β-Amylolysis (%)/100 + 2.
[e] Interior chain length, ICL = CL–ECL − 1.

tumor-bearing mice.[4] Sasaki and his colleagues also suggested the anti-tumor effects of oyster extracts and sweet corn extracts which contain glycogen.[5,6] For application of glycogen in human health, better understanding of structural properties of glycogen should be important. The aim of this study is to characterize the fine structural properties, such as unit-chain distribution and arrangement of branch linkages, in the ESG and NSG molecules.

2. Results and discussion

2.1. Isoamylase treatment of ESG and NSG

Isoamylase is one of the most powerful tools for structural analysis because the enzyme can completely hydrolyze the α-1,6 linkages of NSG.[7] We tested whether ESG can be completely hydrolyzed by isoamylase. NSG and ESG were treated with isoamylase and then incubated with β-amylase. HPAEC analysis demonstrated that both ESG and NSG were quantitatively converted to glucose, maltose, and a trace amount of maltotriose. β-Amylase is an exo-type α-1,4 hydrolase which liberates maltose molecules from the non-reducing end of glucan. However, the enzyme cannot bypass a branch point. Therefore, the result clearly indicated that ESG consists of α-1,4 and α-1,6 linkages, and that the α-1,6 linkages of ESG can be completely hydrolyzed with isoamylase, as those of NSG can be.

2.2. Unit-chain distribution of ESG and NSG

The structural parameters of glycogens that were used in this study are summarized in Table 1: average chain lengths (CLs), average internal-chain lengths (ICLs), and average external-chain lengths (ECLs). These values are similar to one another and are within the variation of the reported values for glycogens from various sources,[8] but may not reflect differences in fine structure.

In order to compare the structures of glycogens, the unit-chain distributions were analyzed using HPAEC after isoamylase treatment (Fig. 1). The unit-chain distributions of ESGs seemed to be related to the CL values of glycogens as expected: ESG-B and ESG-A, with CL around 12, have relatively large amounts of longer unit chains compared with ESG-7M and ESG-12M. In the case of NSGs, their sources rather than CL values seemed to be related to their unit-chain profiles. NSGs from shellfish (oyster, mussel, and slipper limpet) share similar profiles, and those from animal livers have similar patterns. Additionally, we noticed that NSGs have larger amounts of long unit chains (degree of polymerization (DP): 25–35) than ESGs. In other words, ESGs have narrower unit-chain distributions than NSGs.

2.3. α-Amylase treatment of ESGs and NSGs

It has been shown that porcine pancreatic α-amylase (PPA) can hydrolyze α-1,4 linkages of NSG, when there are at least two successive α-1,4-linked glucose units between the glucose units holding the α-1,6 linkages.[9,10] The peripheral linear part of the NSG molecule and also long inter-branch regions of NSG are converted into glucose, maltose, and maltotriose.[10] On the one hand, singly, doubly, or triply branched oligosaccharides with DP ⩾ 4 were produced from the branched regions of NSG.[9,10] Furthermore, larger branched molecules with a DP of several tens were detected as the end products from NSG and designated as α-macrodextrin.[8,9] The branch points in the α-macrodextrin region are shown to be separated by only one glucose or two glucose residues.[8,9]

To compare the hydrolysis profiles of ESGs with those of NSGs, the glycogens were treated with PPA. In all cases reducing powers changed similarly: the power increased rapidly and reached 75–90% of the final values (24 h: 34–44% of total sugars) after 0.5 h of the reaction. The reducing powers did not change greatly after 3 h of the reaction (data not shown). The compositions of saccharides in the initial (0.5 h) and end (24-h) products were analyzed by HPLC using an Aminex HPX42A column. In this analysis system, oligosaccharides with DP 1–7 can be separated depending only on their DP values, but not on the number of α-1,6 linkages.[11] However, saccharides with DP ⩾ 8 could not be clearly separated (Fig. 2), and macromolecules eluted together at around 7.5 min. In the initial stages of hydrolysis (0.5 h), the elution profiles of digests of ESG and NSG were similar: the major products were G2, G3, G4, and macromolecules. Slight amounts of G5-7 were detected only in the NSG digests. More noteworthy changes were observed in the oligosaccharide compositions of final products (24-h). In the final products of NSGs, we detected G1–G7 in addition to macromolecules with DP ⩾ 8, in agreement with previous reports.[9,10] From the final products of ESGs, however, we detected only G1, G2, G3, and macromolecules (Fig. 2 and Table 2).

To analyze the macromolecule fractions in α-amylase digests, the reaction mixtures were subjected to HPSEC-MALLS-RI analysis (Fig. 3). Using this method, we can precisely determine the molecular weight (M_r) of glucans with M_r ⩾ 10 k, while glucans with M_r below 10 k are barely detectable because of their weak scattering of light and the interference with refractive index measurement by buffer salts. Starch was rapidly degraded and its M_r was reduced below the lower limit within 10 min under these hydrolysis conditions (Fig. 3A and B). The molecular sizes of ESGs and NSGs were more slowly reduced compared with those of starch (Fig. 3B and D). We again observed remarkable differences between ESG and NSG in the late stages of hydrolysis. Most NSGs, except that from

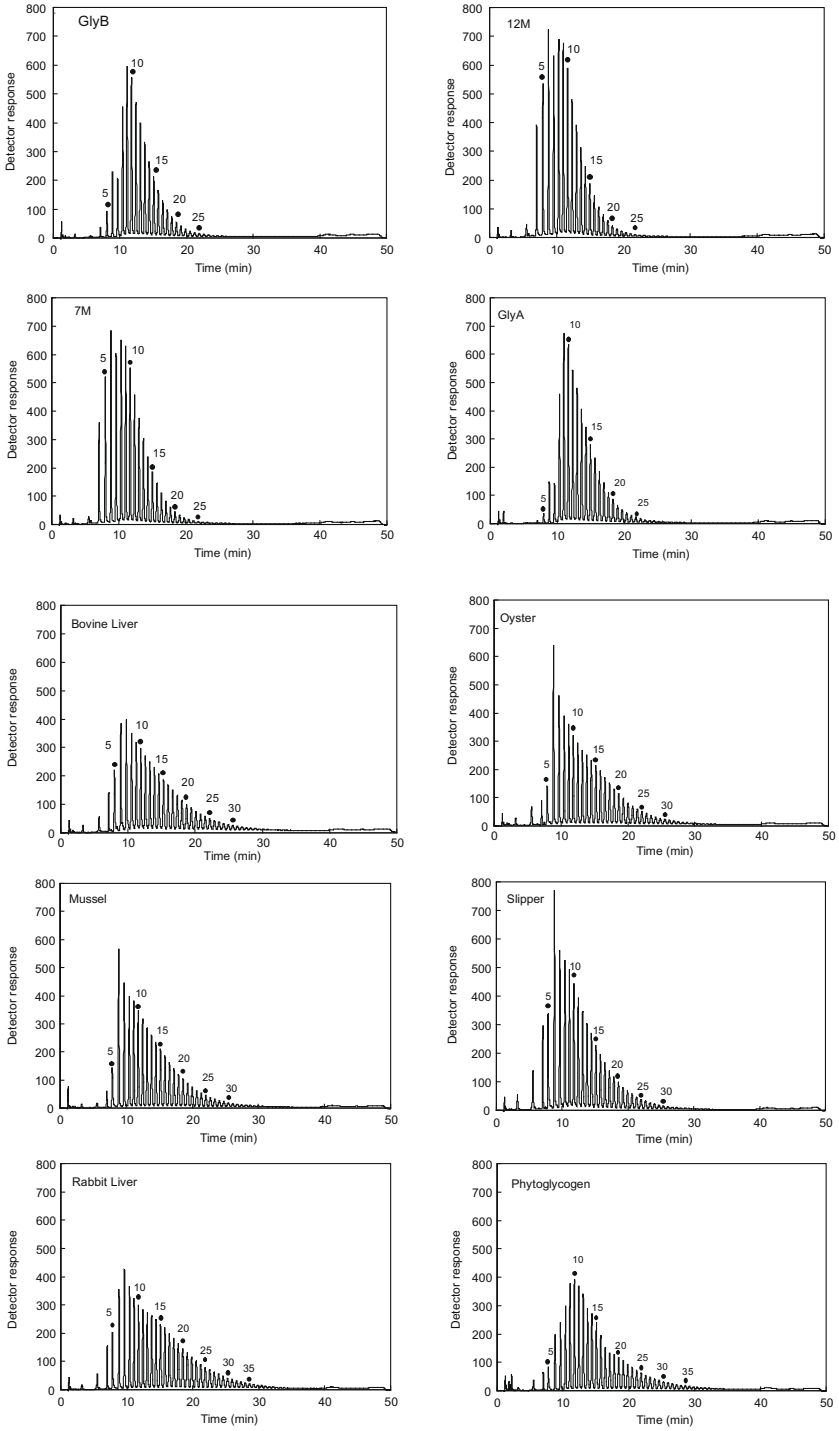

Figure 1. Chain-length distributions of glycogen. Glycogens were subjected to HPAEC analyses after debranching with isoamylase. Chain lengths (DPs) are indicated above the peaks.

the slipper limpet, were degraded to glucans with M_r <10 k after 1 to 6 h reaction. In contrast, large molecules with 10–30% of the original size remained from all ESGs and slipper limpet NSG even after 24 h of hydrolysis. These large molecules were not contaminants such as proteins, because the molecules were susceptible to isoamylase treatment (data not shown). The proportion of large molecules from ESG was around 40%, agreeing with the quantity of macromolecules determined by Aminex HPX42A column (Table 2). Therefore, most macromolecules from ESG detected by the Aminex column were likely to have $M_r \geqslant 10$ k. On the other hand, the proportion of large molecules from NSG was less than 20%, indicating that most macromolecules from NSG were relatively small ($M_r < 10$ k).

It has been shown that the α-macrodextrin regions with M_r up to several thousands (DP; ∼several tens) are randomly dispersed throughout the NSG molecule and that such regions are connected to one another via less-dense regions that are susceptible to α-amylase (Fig. 4A).[8,9] In living cells, NSG molecules are subjected to successive synthesis-degradation cycles corresponding to the glucose/energy demand in cells, and an increased branching

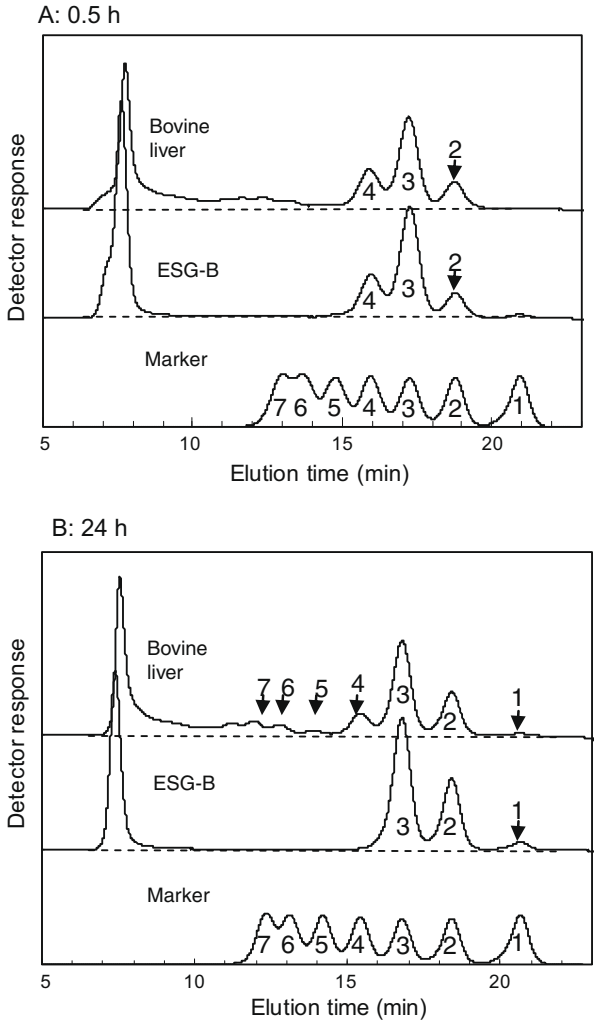

Figure 2. Oligoaccharide compositions of α-amylase hydrolyzates of NSG and ESG. A 20-μL portion of the hydrolyzates was loaded onto the Aminex HPX42A column. The HPLC chromatograms of 0.5-h (A) and 24-h (B) digests of ESG-B and NSG from bovine liver are shown as typical examples for ESG and NSG, respectively. Marker is a mixture of glucose (1), maltose (2), maltotriose (3), maltotetraose (4), maltopentaose (5), maltohexaose (6), and maltoheptaose (7). Concentration of each marker saccharide was 0.7 mg/mL.

density can result from the random placement of new branches on existing structures during the synthesis cycle and from the relative stability of the densely branched regions against degradative enzymes.[9] Our results indicated that ESG has a huge α-macrodextrin component in the molecule (Fig 4B). On the other hand, α-amylase treatment of ESG did not produce branched oligosaccharides (G4–G7). These results suggested that all α-1,6 linkages of ESG were present in the huge α-macrodextrin region and that there are no long spans between α-1,6 linkages. Because of such structural properties, α-amylase seemed to trim off only the peripheral parts of the ESG molecule. We have already reported the isolation of partially degraded molecules from ESG-B using human salivary α-amylase.[12] Structural parameters confirmed that almost all α-1,6 linkages in ESG remained in the partially degraded molecules (Table 3).

Hydrolysis profiles of NSG indicated that α-1,6 linkages in NSG were more irregularly positioned than those in ESG: the span between α-1,6 linkages is only 0-2 in some parts, while in another parts the spans are wide enough to permit attack by α-amylase. ESG is synthesized under much simpler circumstances than NSG, in which no trimming reactions occur during the synthetic process. This simplicity can result in a regular internal structure without long spans between α-1,6 linkages. The narrower unit-chain distribution (Fig. 1) should also reflect this simplicity. Slipper limpet NSG also yielded a huge α-macrodextrin after hydrolysis. Even in the natural environment, conditions in which the synthetic reaction is greatly favored over any trimming reaction may result in NSG molecules having a huge α-macrodextrin region. Since slipper limpet NSG gave branched oligosaccharides (G4-7), the NSG may be a mixture of NSG- (Fig. 4A) and ESG-type molecules (Fig. 4B).

2.4. Dietary fiber contents of ESG and NSG

The results shown above imply that orally ingested ESG and NSG may be differently degraded from each other as they pass through the gastrointestinal system. To test this possibility, we used the Resistant Starch Assay Kit (Megazyme). This kit is designed to evaluate the dietary fiber content of starchy food after treatment with a mixture of pancreatic α-amylase and glucoamylase to detect 50% ethanol insoluble material as 'dietary fiber'. Considerable amounts of fiber were detected from all ESG samples (16.9% from ESG-A, B, and 12M, and 22.1% from ESG-7M), whereas none (<0.2%) was derived from NSGs. The α-macrodextrin of slipper limpet NSG may be more sensitive to glucoamylase than that of ESG.

All results shown in this paper strongly suggested that glycogen, especially ESG, is digested from its external chains, and that macromolecule fractions of glycogen molecules probably reach the intestinal tract after oral ingestion. Recently, we demonstrated that glycogen with M_w 5000–6500 k has a stimulating activity on immunocompetent cells such as macrophages,[12,13] and that the activity is strictly related to its molecular weight. Furthermore, orally administered ESG-A has a survival benefit for tumor-bearing mice.[4] We hypothesize that the macromolecule fraction after partial hydrolysis of ESG that reached the intestinal tract stimulated immunocompetent cells resulting in the health benefits. Investiga-

Table 2
Oligosaccharide compositions of 24-h α-amylase hydrolyzates of ESGs and NSGs

ESG/NSG	Sample name or source	Relative composition of saccharides (%)							
		⩾G8	G7	G6	G5	G4	G3	G2	G1
ESG	B	34.6	N.D.[a]	N.D.	N.D.	N.D.	41.6	21.5	2.4
	7M	38.2	N.D.	N.D.	N.D.	N.D.	40.7	19.7	1.4
	12M	41.8	N.D.	N.D.	N.D.	N.D.	37.3	19.4	1.5
	A	32.0	N.D.	N.D.	N.D.	N.D.	43.3	22.1	2.5
NSG	Bovine Liver	44.3	3.7	2.8	1.5	7.0	28.0	12.4	0.4
	Oyster	41.4	2.6	4.3	2.6	1.8	13.3	28.0	6.0
	Mussel	35.5	3.6	4.2	1.8	0.5	28.1	22.7	3.5
	Slipper limpet	43.0	2.6	2.8	0.9	0.2	29.1	18.5	2.9
	Rabbit liver	34.8	4.5	3.7	1.4	0.5	32.6	20.5	1.9
	Sweet corn	33.4	4.2	3.4	1.4	0.5	31.3	23.2	2.5

[a] N.D.: Not detected.

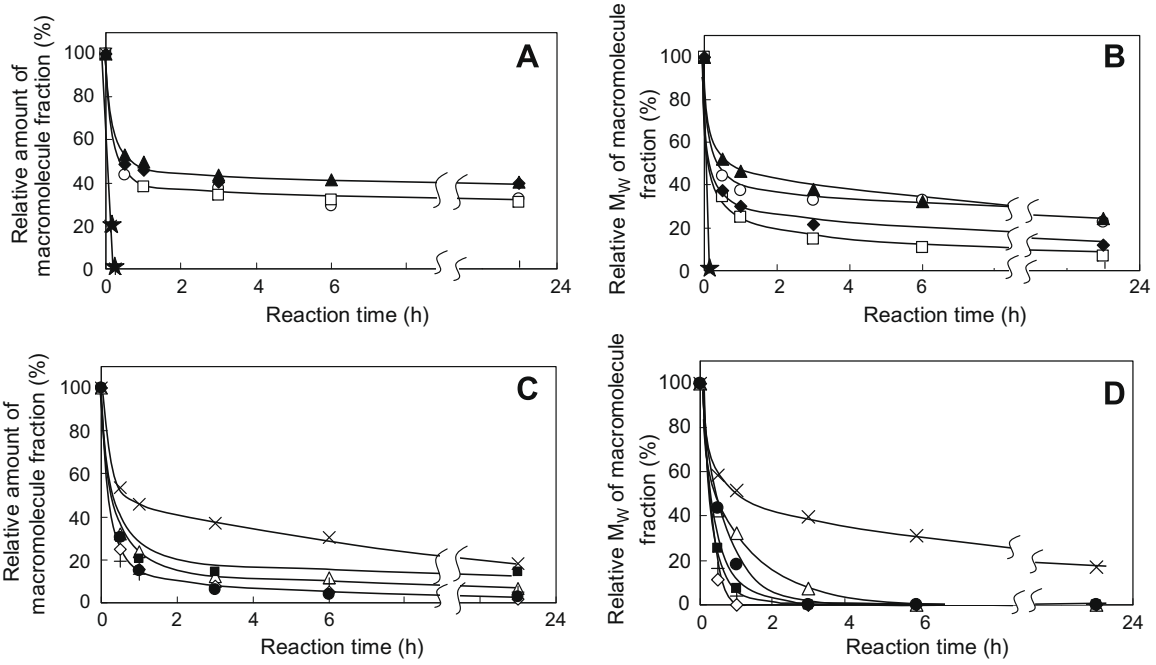

Figure 3. Changes in amount (A, C) and relative M_w (B, D) of macromolecule fraction after α-amylase treatments of ESG (A, B) and NSG (C, D). A 30-μL portion of the hydrolyzates was subjected to HPSEC-MALLS-RI. Fraction eluted until 10 min in this system was taken as macromolecule to determine amounts and M_w. Symbols in A and B: ○, ESG-B; ▲, ESG-7M; ♦, ESG-12M; □, ESG-A; ★ amylopectin from waxy starch. Symbols in C and D: ■, NSG from bovine liver; △, NSG from oyster; +, NSG from mussel; ×, NSG from slipper limpet; ●, NSG from rabbit liver; ◇, NSG from sweet corn.

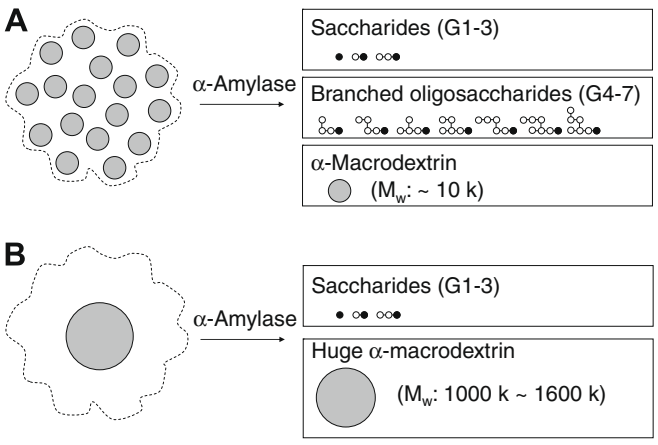

Figure 4. Models of molecular structures of glycogens and the α-amylase-hydrolysis profiles of glycogens. (A) Model of the molecular structure of NSG, based on the study by Brammer et al. [9] The gray circles indicate α-macrodextrin regions and the dotted line indicates the periphery of the glycogen molecule. The remaining part of the molecule indicates the less branched region, which is susceptible to α-amylase treatment. Three types of products were observed in the α-amylase digests: saccharide (glucose, maltose, and maltotriose), branched oligosaccharides (G4–G7), and α-macrodextrin. Open and closed circles indicate glucosyl units and reducing-end glucosyl units, respectively. Horizontal and vertical lines in the illustration of oligosaccharides indicate α-1,4 and α-1,6 linkages, respectively. The structures of branched oligosaccharides are presumed from the oligosaccharide structures derived from amylopectin.[10] (B) Models of the molecular structure of ESG and its hydrolysis profile with α-amylase.

tions in vivo are now in progress to gain a further understanding of the effect of orally administered glycogen.

3. Experimental

3.1. Glycogens

NSG from oysters was purchased from Wako Pure Chemical (Osaka, Japan), and NSGs from bovine liver, rabbit liver, and slipper limpet were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. NSG from mussels was a product of Laboratoires Serobiologique, France. Phytoglycogen of sweet corn was obtained from the Q.P. Corporation, Tokyo, Japan. ESG-A synthesized by the SP–GP–BE method was described previously.[4,12,13] By changing reaction conditions in the IAM–BE–AM method, molecular sizes can be controlled.[3] ESG-B[12,13] was synthesized by this method from 4% (w/v) of partially hydrolyzed starch with dextrose equivalent 3.[3] ESG-7M was synthesized from 10% (w/v) partially hydrolyzed starch (dextrose equivalent, 9). ESG-12M was also synthesized from the same substrate, but a small amount of ESG-7M was added in the reaction mixture as a primer molecule. By addition of the primer, a larger size of ESG can be obtained although molecular weight distribution is broadened. The structural parameters of NSGs and ESGs that are used are summarized in Table 1. Number-average chain length (CL), exterior chain length (ECL), and interior chain length were calculated by the following equations:

$$\mathrm{CL} = [\text{Total sugars as glucose}]/[\text{non-reducing end residues}];$$

$$\mathrm{ECL} = \mathrm{CL} \times \beta\text{-amylolysis}(\%)/100 + 2;$$

$$\mathrm{ICL} = \mathrm{CL}—\mathrm{ECL} - 1.$$

Total sugars were determined by the phenol-sulfuric acid method.[14] Non-reducing end residues were quantified as glycerol by using a commercially available kit (Behringer Mannheim, Tokyo, Japan) after rapid Smith-degradation.[15] β-Amylolysis limit was determined as described by Takeda et al.[16] The absorption spectrum of iodine-glycogen complex was determined by the method of Krisman.[17]

3.2. Enzymes

Isoamylase from *Pseudomonas amyloderamosa* (crystalline form) was purchased from Hayashibara Biochemical Laboratories (Okayama, Japan). Isoamylase treatment of glycogen was carried out as described by Rani et al.[18] β-Amylase from sweet potato (Type I-B) and α-amylase from porcine pancreas (Type I-A) were

Table 3
Structural parameters of partially degraded ESG

Sample name	$M_w \times 10^{-6}$	$M_n \times 10^{-6}$	M_w/M_n	α-1,6 Linkage (%)		CL	ECL	ICL
				%	No/molecule[a]			
ESG-B	5.0	4.0	1.3	8.6	2120	11.6	7.6	3.0
α1-ESG-B	3.1	2.4	1.3	16.4	2430	6.1	3.1	2.0
α2-ESG-B	1.9	1.5	1.3	23.3	2140	4.3	2.2	1.1

[a] The number of α-1,6 linkages in a molecule, No/molecule = $M_n/162 \times$ α-1,6 linkage (%).

obtained from Sigma. α-Amylase treatment of glycogen was carried out as follows. The reaction mixture (6 mL) consisting of 5 mg/mL glycogen in 10 mM sodium phosphate buffer (pH 7.0) and α-amylase (300 U/g of substrate) was incubated at 37 °C. At intervals, 1 mL of the mixture was removed and boiled for 5 min to stop reaction.

3.3. High-performance liquid chromatography (HPLC) analysis

Three types of HPLC were used as follows. Glycogen was completely debranched with isoamylase, and the unit-chain distributions were analyzed using high-performance anion exchange chromatography (HPAEC) as described previously.[19]

The compositions of oligosaccharides after α-amylase treatment of glycogens were analyzed by HPLC using an Aminex HPX42A column (Bio-Rad Laboratories, CA, USA). A 20 μl sample of each digest was injected into the column and elution was monitored with a differential refractive index detector. Elution was carried out at 75 °C with distilled water at a flow rate of 0.5 mL/min.

Macromolecule fractions after α-amylase treatment of glycogens were analyzed by high-performance size-exclusion chromatography (HPSEC) equipped with a multi-angle laser light scattering detector (MALLS; DAWN HELEOS, Wyatt Technology Corp., CA, USA) and a differential refractive index detector. Columns were Shodex OH-pak SB-806M HQ (6.0 × 300 mm, Shoko Co., Ltd., Tokyo, Japan) with a precolumn (OH-pak SB-G). Elution was carried out at 40 °C with 0.1 M $NaNO_3$ at a flow rate of 1.0 mL/min. Weight-average M_r and quantities of macromolecule fractions eluted from 6.5 min to 10 min were calculated using the software ASTRA (Wyatt Technology).

3.4. Other procedures

The reducing power of glucan was determined with the dinitrosalicylic (DNS) acid method as follows. Sample solution (0.3 mL) was mixed with DNS reagent (0.6 mL) and heated in a boiling water bath for 5 min. The mixture was cooled with tap water and diluted with 2.7 mL of distilled water. Absorbance at 535 nm was measured to determine the reducing power using glucose as a standard. The DNS reagent was prepared by mixing 0.5 g of 3,5-dinitrosalicylic acid dissolved in 20 mL of 2 N NaOH and 30 g of sodium potassium tartrate dissolved in 50 mL of distilled water and by adjusting the volume to 100 mL with distilled water. Dietary fiber content of glycogen was tested with the Resistant Starch assay kit of Megazyme International Ireland Ltd (Wicklow, Ireland) according to manufacturer's instructions. Unless otherwise specified, all chemicals were obtained from Wako Pure Chemicals (Osaka, Japan).

Acknowledgment

This work was supported in part by a grant under the "Program for New Technology Development to Activate Agriculture, Forestry, Fisheries and Food Industry by Cooperating Industry, Academia, and the Government" from the Ministry of Agriculture, Forestry and Fisheries, Japan.

References

1. Geddes, R. *Biosci. Rep.* **1986**, *6*, 415–428.
2. Cori, G. T.; Cori, C. F. *J. Biol. Chem.* **1943**, *151*, 57–63.
3. Kajiura, H.; Kakutani, R.; Akiyama, T.; Takata, H.; Kuriki, T. *Biocatal. Biotransform.* **2008**, *26*, 133–140.
4. Ryoyama, K.; Kidachi, Y.; Yamaguchi, H.; Kajiura, H.; Takata, H. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 2332–2340.
5. Kumaki, N.; Satoh, T.; Sutoh, C.; Itoh, Y.; Chiba, K.; Norigami, A.; Sakuta, K.; Lu, C.; Wakatsuki, Y.; Nagamori, Y.; Sasaki, J. *BCG BRM Ryoho Kenkyukai Kaishi* **2005**, *29*, 17–21.
6. Sasaki, J.; Soga, H.; Shinohara, C.; Itoh, Y.; Sasahara, R.; Yoshida, K. *BCG BRM Ryoho Kenkyukai Kaishi* **2003**, *27*, 27–31.
7. Harada, T.; Misaki, A.; Akai, H.; Yokobayashi, K.; Sugimoto, K. *Biochim. Biophys. Acta* **1972**, *268*, 497–505.
8. Manners, D. J. *Carbohydr. Polym.* **1991**, *16*, 37–82.
9. Brammer, G. L.; Rougvie, M. A.; French, D. *Carbohydr. Res.* **1972**, *24*, 343–354.
10. Sakano, Y.. In The Amylase Research Society of Japan, Ed.; Handbook of amylase and related enzymes. Their sources, isolation methods, properties and applications; Pergamon Press: Oxford, 1988; pp 22–26.
11. Kuriki, T.; Yanase, M.; Takata, H.; Imanaka, T.; Okada, S. *J. Ferment. Bioeng.* **1993**, *76*, 184–190.
12. Kakutani, R.; Adachi, Y.; Kajiura, H.; Takata, H.; Kuriki, T.; Ohno, N. *Carbohydr. Res.* **2007**, *342*, 2371–2379.
13. Kakutani, R.; Adachi, Y.; Kajiura, H.; Takata, H.; Ohno, N.; Kuriki, T. *Biocatal. Biotransform.* **2008**, *26*, 152–160.
14. Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Robers, P. A.; Smith, F. *Anal. Chem.* **1956**, *28*, 350–356.
15. Hizukuri, S.; Osaki, S. *Carbohydr. Res.* **1978**, *63*, 261–264.
16. Takeda, C.; Takeda, Y.; Hizukuri, S. *Cereal Chem.* **1983**, *60*, 212–216.
17. Krisman, C. R. *Anal. Biochem.* **1962**, *4*, 17–23.
18. Rani, M. R. S.; Shibanuma, K.; Hizukuri, S. *Carbohydr. Res.* **1992**, *227*, 183–194.
19. Takata, H.; Takaha, T.; Okada, S.; Hizukuri, S.; Takagi, M.; Imanaka, T. *Carbohydr. Res.* **1996**, *295*, 91–101.