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Fine structural features of oyster glycogen: mode of multiple branching

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The fine structural features of oyster glycogen, especially its mode of multiple branching, was investigated by repeated enzymic treatment with β-amylase and pullulanase, followed by the precise analysis of the α-1,4-1inked glucosyl unit-chains by high performance anion exchange chromatography (HPAEC). The purified glycogen (average mol.wt 8.5×10^5, $\overline{CL}$ 11) obtained by DMSO-extraction from fresh oysters (*Crassostrea gigas*) collected in February (a time when the oysters are edible) showed a distribution of α-1,4-D-glucosyl unit-chains, with degrees of polymerization (dp) in the range 2–35 (dp 6, dominant), as measured by HPAEC after complete enzymic debranching. The oyster glycogen was subjected to stepwise degradations with β-amylase and pullulanase, and this procedure was repeated until complete hydrolysis was achieved (extent and degradation of 98% after five treatments). The yield of the limit dextrin formed at each trimming step and quantitative analysis of the unit-chain distributions indicated that the oyster glycogen has a highly branched structure (A:B-chain, 0.7:1), involving five or six times interlinkings of the chains (B-chains). Assuming that B1 chain carrying only A-chains, attaches by α-1,6-bonds to another B-chain (B2 chain), which in turn attaches to a B3-chain, and so on, the molar ratios of the unit-chains (A, B1, B2-) of the dextrins during successive enzymic trimming showed that the ratio of A:B1:B2:B3:B4:B5-chain was 34:25:11:5:5:1, confirming the multiple ramified molecule. In connection with the digestion of oyster glycogen in the mammalian digestive tract, the glycogen was hydrolyzed by salivary and pancreatic α-amylase, and several branched maltosaccharides in the digestion product were fractionated, and their structures determined using HPAEC.

INTRODUCTION

Glycogen, an energy reserve form of glucose, occurs in mammalian cells, shellfish, fungi and bacteria. Early chemical studies established that glycogen consists of linear chains of an average 10–18 of α-1,4-linked D-glucosyl residues, joined by α-1,6-interchain linkages to form a highly branched structure. Based on enzymic studies, a tree-type structure was proposed by Meyer & Fuld (1941). In this type of structure, Peat *et al.* (1956) introduced three types of linear 'basal' chain units, A, B, C: A-chains joined to the other unit chains only by the reducing end; B-chains which were also joined in this way, but also carried A and/or other B chains; and C-chains, if present, only one chain carrying the only reducing terminal in the molecule.

Although the Meyer model represents a basic structural feature of glycogen, there are some modifications, such as the Whelan (1971) model, deduced mostly by other enzymic degradation data. These structural models, however, seem not to represent the complete structure. Thus, the detailed structure of glycogen is still the subject of research interest.

Although our previous study supported a multi-branched, spherical structure for glycogen, based on the gel-filtration data obtained by repeated enzymic degradations by β-amylase and pullulanase (Misaki & Tunoda, 1984; Misaki & Yano, 1985), these conventional gel-filtration chromatographies gave a limited

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resolution of the series of maltosaccharides which were released by debranching enzymes. This disadvantage may be overcome by the use of a high performance anion exchange chromatography (HPAEC) with a pulsed amperometric detector, as developed by Koizumi *et al.* (1988, 1989, 1991) for the precise analysis of the unit-chains of amylopectin. Sandhya Rani *et al.* (1992) also recently applied HPAEC for a structural study of oyster glycogen and confirmed the randomly branched structure containing unit-chains dp 2–30 with dp 6 as the most abundant chains. Our recent study using HPAEC showed that the distributions of α-1,4-unit-chains of glycogens from different biological sources were mostly in the range dp 3–30 (Matsui *et al.*, 1993). These results have prompted us to re-investigate the fine structural features of oyster glycogen, by applying the HPAEC system.

In this paper, we report the detailed structural features of oyster glycogen, by using a novel enzymic degradation, which involves repeating the stepwise enzymic degradation by β-amylase and pullulanase, and precise HPAEC analysis of the changes in the distribution pattern of the unit-chains in the resulting limit dextrin formed at each step of the enzymic degradation. These enzymic degradation studies have enabled the mode of multiple branching of the glycogen molecule, from the newly formed β-limit dextrins to be elucidated.

EXPERIMENTAL

Enzymes

Crystalline, *Pseudomonas* isoamylase (EC 3.2.1.68) and *Klebsiella* pullulanase (EC 3.2.1.41) were donated by Hayashibara Biochemical Laboratories, Okayama. β-Amylase of sweet potato (EC 3.2.1.2) and α-D-glucosidase of bakers' yeast were purchased from Seikagaku Kogyou Co., Tokyo. α-Amylase (human salivary and hog pancreas) and isomaltase of yeast were purchased from Sigma Co.

Preparation of glycogen

Two-year-old fresh oysters (*Crassostrea gigas*) grown in Hiroshima Bay in Japan were harvested in February 1991, the season when the eating quality is highest, homogenized and lyophilized. The lyophilized homogenates were delipidated by two extractions with dichloromethane and methanol (1:1 and 2:1 mixture) and the glycogen was then extracted by stirring with dimethylsulfoxide (DMSO) at 25°C for 48 h. The glycogen was precipitated with three volumes of methanol. After dissolving in water and dialysis, this procedure was repeated three times. The purified glycogen preparation contained 102% of glucose, as measured by the phenol–sulfuric acid method (Dubois *et al.*, 1956). Gas chromatographic analysis showed glucose as the only carbohydrate component.

Procedure of successive degradations (β-LD and SFD)

The detailed structure of glycogen was investigated mainly by repeated stepwise degradations with β-amylase and pullulanase. Glycogen (800 mg) was dissolved in water (15 ml) by ultrasonication, and after the pH was adjusted to 4.8 with 200 mM acetate buffer (15 ml) the solution was incubated with sweet potato β-amylase (800 units; 1 unit/mg) at 40°C for 48 h. For complete enzymic hydrolysis the dextrin obtained by the first β-amylolysis was precipitated by the addition of methanol (1.5 vol.), collected by centrifugation, and was again incubated with β-amylase. This procedure was repeated until no more maltose was released, as judged by HPAEC. The supernatant solutions of the enzymic digests were combined and maltose was determined by the method of Nelson–Somogyi (Nelson, 1944; Somogyi, 1952). In this manner the β-limit dextrin 1 (β-LD-1) was obtained, and it was purified by methanol precipitation and lyophilized.

The β-LD-1 was dissolved in water by ultrasonication, and after the pH was adjusted to 5.0 with 200 mM acetate buffer, the solution was incubated with pullulanase (1 unit/20 mg) at 40°C for 17 h to remove maltosyl (dp 2) and maltotriosyl stubs (dp 3). The deletion of stubs (dp 2, dp 3) on the β-LD gave a stub-free new dextrin (SFD-1). The SFD-1 was treated with β-amylase and then with pullulanase to give β-limit dextrin 2 (β-LD-2) and stub-free dextrin (SFD-2). In the same way β-LD-3, SFD-3, β-LD-4, SFD-4, β-LD-5, SFD-5 and β-LD-6 were obtained.

Action of debranching enzymes on native glycogen, β-limit dextin and stub-free dextrin

Each sample of native glycogen, β-limit dextrin (β-LD) and stub-free dextrin (SFD) (each 3 mg), was dissolved in water (0.5 ml) by ultrasonication, and after the pH was adjusted to 3.5 with 200 mM acetate buffer (0.5 ml), the solution was incubated with isoamylase (60 units) at 40°C for 24 h. The digest was successively incubated with pullulanase (40 units) at pH 5.0 for a further 24 h to confirm complete hydrolysis of α-1,6-interchain linkages. After heat-inactivation of the enzymes, the reaction mixture was desalted by stirring with an ion-exchange resin (AG501-X8, Bio-Rad), and maltosaccharides released by debranching enzymes were analyzed by HPAEC.

Measurement of average chain length

The average chain length ($\overline{CL}$) of glycogen was measured by methylation analysis using the method of Hakomori (1964).

After complete methylation, as judged by the infrared spectrum, the methylated glycogen was hydrolyzed with 90% formic acid at 100°C for 10 h and then 2 M trifluoro-acetic acid for another 3 h. The partially methylated glucoses in the hydrolysate were converted into the corresponding alditol acetates by reduction with sodium borohydride followed by acetylation (acetic anhydride and pyridine, 1:1, 0.2 ml) at 100°C for 2 h, and were analyzed by GLC, using a capillary column of OV-1 (50 m×0.25 mm i.d., 150–230°C, at a rate of 2°C/min). An average length of repeating unit, which corresponds to $\overline{CL}$, was calculated from the ratio of 2,3,6-tri-*O*-methyl- plus 2,3-di-*O*-methyl-D-glucose to 2,3,4,6-tetra-*O*-methyl-D-glucose.

An average exterior chain length ($\overline{ECL}$ and interior chain length ($\overline{ICL}$) were calculated from $\overline{CL}$ and the β-amylolysis limit, respectively, by the conventional equation (Manners, 1989).

$$\overline{ECL} = \overline{CL} \times (\beta\text{-amylolysis}(\%)/100) + 2^*$$

$$\overline{ICL} = (\overline{CL} - \overline{ECL}) - 1.0$$

*Note: as the oyster glycogen preparation contained A- and B-chains in a ratio 0.7:1.0, the correct value is calculated to 1.9, instead of 2.

Repeated degradations by pullulanase

For the preferential hydrolysis of the outermost branch points with A-chains, the glycogen was dissolved in water by the aid of ultrasonication, and after the pH had been adjusted to 5.0 with 200 M acetate buffer, the solution was incubated with pullulanase (1 unit/5 mg) at 40°C for 17 h. For repeated degradations the first pullulanase-treated glycogen was precipitated by the addition of methanol and was treated again with pullulanase. This procedure was repeated seven times. The maltosaccharides released by each pullulanase treatment were analyzed by HPAEC.

Analysis of maltosaccharides by HPAEC

HPAEC was carried out using a BioLc system, Model 4500i system (Dionex, CA, USA) with a pulsed amperometric detector (Model PAD II). The column used was CarboPac PA-1 (250×4 mm i.d.) with an CarboPac PAGUARD (25×3 mm i.d.). A chromatopac C-R6A digital integrator (Shimadzu, Kyoto) was used to calculate peak areas. Eluent A was 150 mM sodium hydroxide and eluent B was 150 mM sodium hydroxide containing 1 M sodium acetate. These eluent solutions were degassed by bubbling of helium gas before use. The sample and carrier solution were each passed through a 0.2 μm membrane filter, and a sample (25 μl) was injected. All separations were done at room temperature with a flow rate of 1 ml/min.

To measure the peaks on the chromatograms, detector responses were computed to molar-based quantitites by adopting the data reported by Koizumi *et al.* (1989), (1991). Relative molar responses of >dp 17 were obtained by extroplation from the responses obtained with the lower dps.

Molecular weight (mol. wt) measurement

For estimation of average mol. wt. a glycogen sample (3 mg) dissolved in 100 mM sodium hydroxide (1.0 ml), was loaded onto a column of Toyopearl HW-65S (2.2×100 cm), eluted with 100 mM sodium hydroxide and monitored by the phenol–sulfuric acid method (Dubois *et al.*, 1956).

The mol. wt was also measured by HPLC, using a Shimadzu LC-6A system with RI detector. The column used was a Asahipak GFA-7MF (300×7.6 mm i.d.). Standardized pullulan fractions (Hayashibara Biochemical Lab.) were used for calculation of the mol. wt.

Degradation by α-amylase

Each sample of native glycogen (3 mg) was dissolved in water (0.5 ml) by ultrasonication, and after the pH was adjusted to 6.5 with 5 mM phosphate buffer saline (PBS) (0.5 ml) the solution was incubated with human salivary or hog pancreatic α-amylase (each 60 units) at 37°C for 24 h. After heat-inactivation of the enzymes, the reaction mixture was desalted by stirring with an ion-exchange resin (AG501-X8, Bio-Rad), and the oligosaccharides released by α-amylase were analyzed by HPAEC. Each branched oligosaccharide fraction collected was treated with pullulanase, isomaltase or α-D-glucosidase, and the oligosaccharide structure was deduced from the HPAEC data of the hydrolysis products.

RESULTS AND DISCUSSION

Our preliminary study on the seasonal variation of the glycogen of the fresh oyster showed some changes in the proportions of the exterior chains of α-1,4-linked glucose residues, dependent on the physiological conditions. For instance, the proportions of maltosyl and maltotriosyl unit chains (dp 2 and 3) became significantly higher in summer (spawning season) than in autumn and winter (edible season), although the molecular weights and basic distribution profile of the unit chains, dp from 2 to 35, may not essentially alter throughout a whole year (Matsui *et al.*, 1991). In the present structural investigation glycogen was, therefore, isolated from fresh oysters harvested in February. The glycogen sample was prepared by a mild procedure, involving homogenization of the whole oyster at 5°C, delipidation with dichloromethane and methanol (1:1,

and 2:1 mixture) and extraction with DMSO at 25°C. After dialysis and repeated precipitation the glycogen sample was lyophilized. The purified glycogen, possibly in a natural state, was composed solely of glucose, with a trace amount of protein (< 1.1%). It had a broad apparent molecular weight distribution, ranging from 5.5×10^5 to 20×10^6 (average mol. wt, 8.5×10^5), as measured by gel filtration. Using the same conditions, a commercial oyster glycogen (type II, Sigma Co.) gave mol. wt 4×10^5. The present glycogen preparation had an average length of repeating unit ($\overline{CL}$) 10~11, revealed by methylation 11 analysis. It had a β-amylolysis limit of 30%, which corresponds to an average exterior chain length ($\overline{ECL}$) 5–6, and interior chain length ($\overline{ICL}$) 3~4. These results indicated that the oyster glycogen used in this study has a very highly branched structure with shorter exterior chains, as compared with those present in many samples of mammalian glycogen.

Chain length distribution and A:B-chain ratio

The glycogen sample was completely debranched by the actions of isoamylase and pullulanase according to our previous paper (Akai *et al.*, 1971), and the released oligosaccharides were analyzed by HPAEC under alkaline conditions using a gradient program in the manner reported previously (Matsui *et al.*, 1993).

Figure 1 shows the complete resolution of a series of maltosaccharides by this LC system; individual peaks correspond to their degree of polymerization (dp). This result indicates that the oyster glycogen comprises a homologous series of α-1,4-1inked glucosyl unit-chains with dp ranging from 2 up to 35 (major peak of dp 6), confirming the presence of many short α-1,4-1inked unit chains.

With regard to the quantitative analysis in the present LC system, the response value of any glucooligosaccharide peak on the pulsed amperometric detector (PAD) did not appear to be exactly proportional to the number of glucose residues, but rather related to the numbers of hydroxyl groups in the molecule under alkaline condition (Lee, 1990). Since Koizumi *et al.* (1989, 1991) recently reported the calibration of PAD values for each α-1,4-glucosyl oligosaccharide up to dp 17, we adopted their calibrations for the molar-based estimation of the maltosaccharide peaks up to 17; for those dp higher than 17 the calibration curve could be extrapolated. Thus, the molar-based distributions of the unit chains indicated that the present oyster glycogen has a multiple branched structure, similar to those from other sources as far as the unit-chain distribution is concerned, except for the presence of very short unit chains of dp 2 (maltose) and dp 3 (maltotriose).

In the structural models of glycogen and amylopectin, initially proposed by Meyer (Meyer & Fuld, 1941), a linear A-chain is joined to another unit chain (B-chain) by the α-1,6-linkage at its reducing end. The B-chain carrying other unit chains (A and/or other B-chains) linked to another B-chain, according to the definition of Peat *et al.* (1956).

Although in the ideal Meyer model, equal numbers of A- and B-chains are present, so that A:B chain ratio should be 1:1, A:B chain ratios of 1:1.2~1:1.5 have been reported for amylopectin, and 0.6:1.0~1.1:1.0 for glycogen (Manners, 1991). Our recent determination of A:B chain ratios, based on the HPAEC data for the proportion of maltose plus maltotriose in β-limit-dextrin, gave values of 0.6:1.0~1.2:1.0 for several glycogen samples (Matsui *et al.*, 1993). Thus, our recent evidence indicates that in most glycogens, there may be more B-chains than A-chains, which is a significant difference from amylopectin.

Enzymic trimming of glycogen by successive degradation with β-amylase and pullulanase

For elucidation of the detailed structural features of the glycogen molecule, especially on the degree of multiple branching in this study, B-chains were sub-divided according to their localizations. A particular B-chain which only carries an A-chain is defined as a B1-chain; this may be linked to another B-chain (B2-chain). The B2-chain carrying B1-chain(s) may also be linked to a B3-chain, which is linked, in turn to B4-chains, and so on, forming a multiple branched, bush-like or spherical structure (see Fig. 5). In such an arrangement of the unit-chains, the possibility that any of B-chains other than B1-chain carries A-chain(s) should not be excluded.

A strategy for the elucidation of the multiple branched structure of oyster glycogen involved enzymic trimming from the outer layer of the molecule by stepwise hydrolysis with β-amylase and pullulanase, and the precise HPAEC analysis of maltosaccharides released by the action of isoamylase and pullulanase on the β-limit dextrin (β-LD), and its stub-free product (SFD) formed at the each step of the trimming reactions. Such an enzymic trimming procedure was repeated until complete hydrolysis of the glycogen was accomplished, according to Fig. 1.

The oyster glycogen was exhaustively digested by β-amylase action (β-amylolysis limit, 30%) to give a β-limit dextrin (β-LD-1, recovery; 72%), which carries stubs of maltose and maltotriose units originating from A-chains. When β-LD-1 was completely debranched by isoamylase and pullulanase, high proportions of maltose (dp 2) and maltotriose (dp 3) were produced in addition to a series of maltosaccharides, as revealed by HPAEC analysis (Fig. 2a).

By careful hydrolysis with pullulanase, maltose and maltotriose (in a molar ratio, 1.0:1.01) together with a trace of maltotetraose were released (Fig. 2b), and the stub-free dextrin 1 (SFD-1, 56.0% of the starting

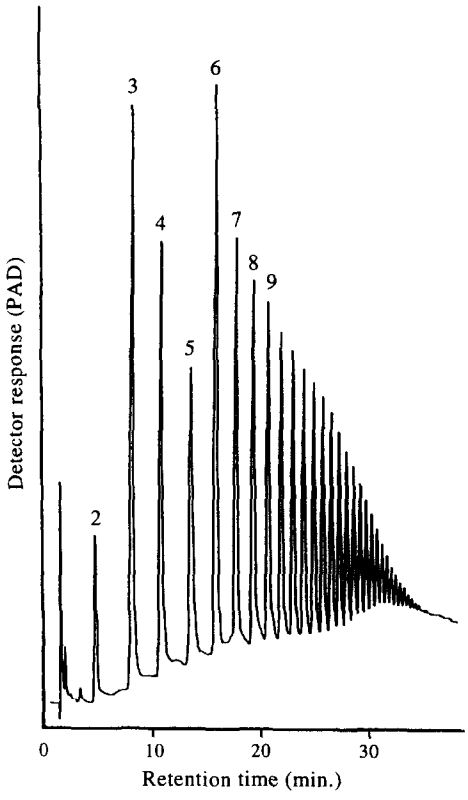


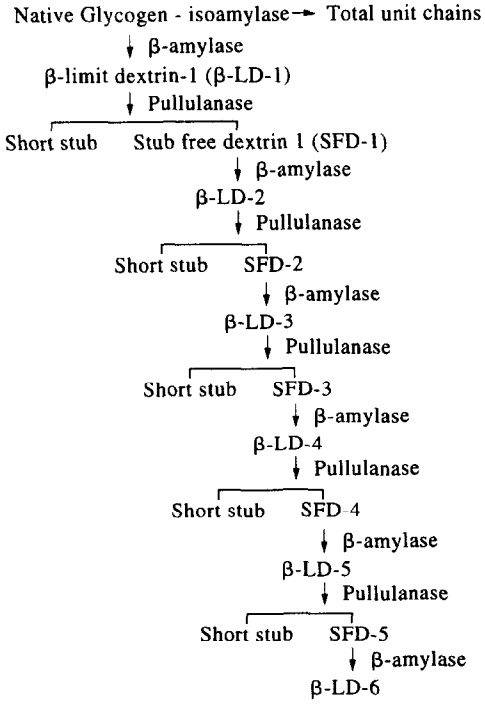
Fig. 1. HPAEC profile of α-(1→4) unit chains of oyster glycogen. The number on each peak indicates its DP. Chromatographic conditions: column, CarboPac PA-1 (250×4 mm i.d.); eluent A, 150 mM sodium hydroxide solution; eluent B, 150 mM sodium hydroxide solution containing 1 M sodium acetate; gradient program, 95% eluent A (5% eluent B) at 0 min. 55% eluent A (45% eluent B) at 40 min; flow rate, 1 ml/min.

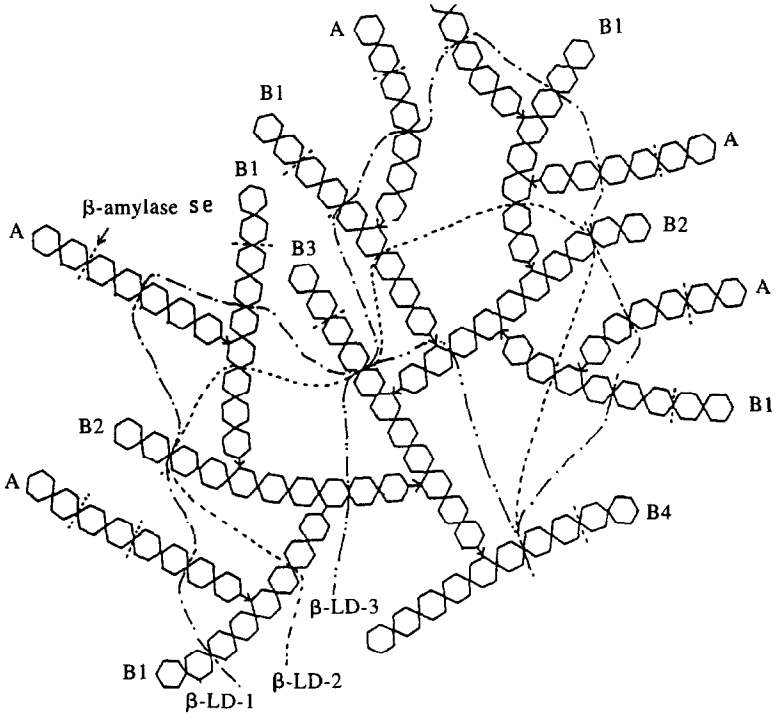
Table 1. Yields and average chain length ($\overline{CL}$) of β-dextrins and stub-free dextrins formed during repeated enzymic trimming of oyster glycogen

Dextrin	Yield (%)	$\overline{CL}$[a]
Native glycogen	100 (800 mg)	11
β-LD-1[b]	72.0	6
SFD-1[c]	56.0	6–7
β-LD-2	—	—
SFD-2	23.8	7–8
β-LD-3	—	5
SFD-3	14.0	8
β-LD-4	—	6
SFD-4	5.1	9
β-LD-5	—	4
SFD-5	1.5	n.d.

[a]Estimated by methylation analysis.
[b]First β-limit dextrin.
[c]Stub-free β-dextrin-1.
n.d.: not determined.

glycogen) was obtained. The fact that equal moles of maltosyl and maltotriosyl stubs were present in β-LD-1 confirms that the glycogen contained equal numbers of A-chains comprising even- and odd-numbered glucose residues. The molar-based calibrations for the HPAEC data of the maltosaccharides released from β-LD-1 (see Fig. 2a) indicated that the molar proportion of (dp 2 + dp 3) in β-LD-1, was 41%, as computed from the molar-based calibrations of HPAEC data of the maltosaccharides indicated that the overall A:B chain ratio of the native glycogen was 0.7:1.0. The newly formed dextrin, SFD-1, free from any stubs,





Scheme 1. Procedure for unit-chain distribution analysis of oyster glycogen. ⬡ (1→4) linked glucose; →, α-(1→6) linkage.

was again subjected to β-amylolysis to give the corresponding β-limit dextrin (β-LD-2), carrying maltose- and maltotriose-stubs, which were derived from B1-chains (see Fig. 3a). These stubs were removed by the action with pullulanase to give the stub-free dextrin, SFD-2 (recovery, 23.8%). In this manner, the molar proportions of (dp 2 + dp 3) to the residual maltosaccharides in β-LD-2 should correspond to the ratio of B1-chains to other B-chains (B2 + B3~). SFD-2 was again treated with β-amylase and then with pullulanase. This procedure yielded a new β-limit dextrin (β-LD-3) and the stub-free dextrin (SFD-3), which was further treated with β-amylase and pullulanase in the same manner Fig. 3(b, c). This enzymic trimming was repeated until the degradation was completed. Yields and average chain lengths of the dextrins obtained by the each step of the enzymic degradations are summarized in Table 1.

The fact that five cycles of such enzymic trimming, resulted in almost complete degradation, leaving a very small residue (1.5%), clearly shows that the α-1,4-linked unit chains in oyster glycogen are most probably five to six times branched by α-1,6-interchain linkages.

At each step of the enzymic degradation, the newly formed β-limit dextrin was completely debranched by isoamylase and pullulanase, and the molar-based proportions of maltosaccharides representing the unit-chain distribution of the individual β-dextrins were computed from HPAEC data, by adopting the calibration reported by Koizumi *et al.* (1989, 1991).

The results are listed in Table 2. Since dp 2 (maltose) and dp 3 (maltotriose) in Table 2 have arisen from the new A-chain of the individual β-dextrins, the molar ratio of (dp 2 + 3) to the sum of maltosaccharides higher than dp 4 (dp > 4) must represent the A:B-chain ratio of the dextrin prior to β-amylolysis. Thus the values of the ratio, B1:B2:B3-, could be calculated in a similar

Table 2. Distribution of α-(1→4)unit chains of maltosaccharides on dagradation steps of oyster glycogen[a]

Dextrin	dp2	dp3	dp4	dp5	dp6	dp7	dp8	dp9	dp10	dp11	dp12	dp13	dp14	dp15	dp16	dp17	>dp17
Native glycogen	2.4	10.0	9.1	7.1	11.8	8.6	7.4	7.5	5.8	5.0	4.5	4.1	3.6	2.8	2.4	2.0	5.9
β-LD-1	19.9	21.3	8.2	6.3	4.7	4.4	4.4	4.9	4.4	3.8	3.3	3.0	2.5	2.2	2.0	1.7	3.0
β-LD-2	25.3	26.3	10.0	6.0	6.5	4.8	4.2	3.3	2.8	2.3	1.8	1.5	1.2	1.0	0.8	0.6	1.6
β-LD-3	21.8	23.2	7.9	6.7	4.6	4.4	4.0	3.8	3.7	3.5	3.2	3.0	2.8	2.2	2.0	0.9	2.4
β-LD-4	19.5	21.5	8.5	7.0	7.3	6.0	5.5	4.9	3.9	3.3	2.7	2.3	1.9	1.6	1.3	1.0	1.8
β-LD-5	46.6	21.3	9.8	3.3	2.5	2.0	1.7	1.5	1.2	1.2	0.9	1.6	1.4	1.1	1.1	0.9	1.9
β-LD-6	18.4	21.8	9.2	5.5	6.8	5.6	5.2	4.5	3.9	3.3	3.2	2.8	2.3	2.1	1.6	1.2	2.6

[a]Expressed as molar proportions.

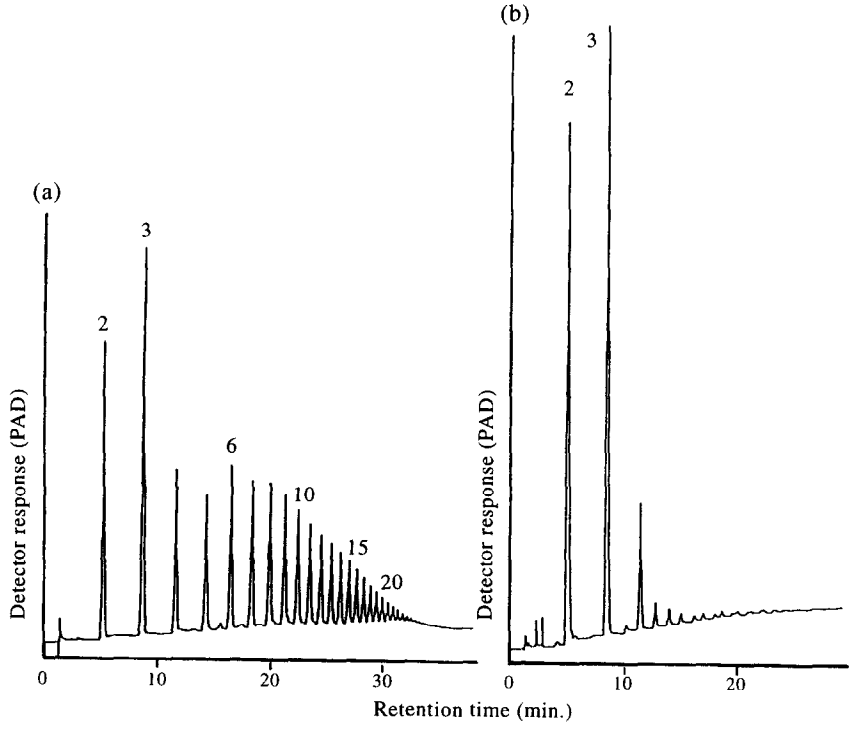


Fig. 2. HPAEC profiles of unit-chain distributions of β-limit dextrin-1(β-LD-1) and stubs: (a) β-LD-1; (b) stubs.

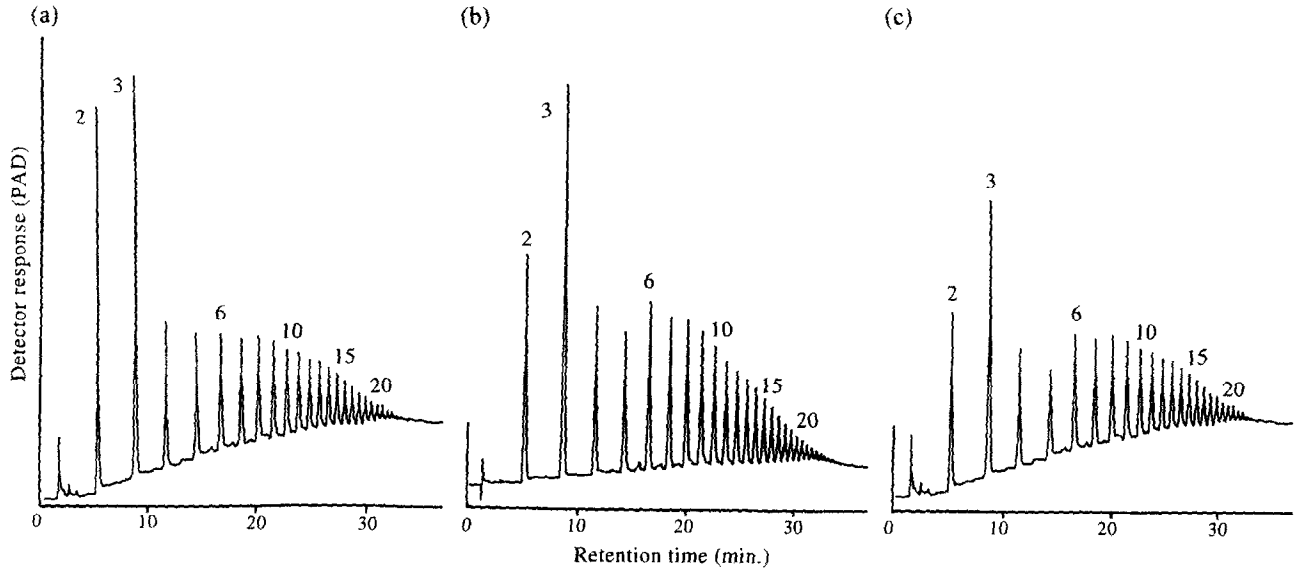


Fig. 3. HPAEC profiles of unit-chain distributions of β-limit dextrins formed by successive degradations: (a) β-LD-2; (b) β-LD-4; (c)β-LD-6.

manner as for the determination of the A:B chain ratio of the original glycogen. For instance, the ratio of moles of (dp2+dp3) to total moles of maltosaccharides (dp≥4) in β-LD-2, 1.1:1.0, corresponds to the ratio of B-1 chains to residual B-chains, including B2 and up to the B5-chain. Similarly, from the molar distributions of the anti-chains in β-LD-3, the ratio of B2-chains, which carry one or more B1-chain(s), to the residual B-chains was estimated as 0.8:1.0. In the same manner as above, the ratio of B3-chains, B2-chain(s), was 0.7:1.0, and the ratio of B4-chains to residual B-chains, 2.1:1.0, as shown in Fig. 4.

Thus, the overall ratio of unit-chains, A:B-chain, 0.7:1.0, comprising A:B1:B2:B3:B4:B5-chains= 34:25:11:5:5:1 as shown in Fig. 4. These chain ratio values in the oyster glycogen would favor a multiple branched spherical molecule. One of possible structural models built up with approximately 100 D-glucose residues is depicted in Fig. 5.

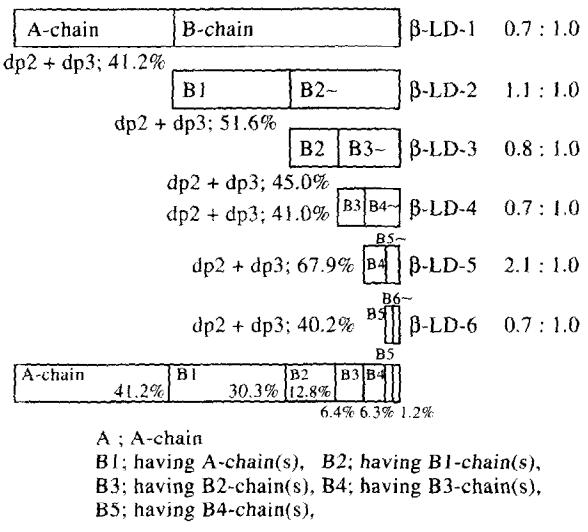


Fig. 4. Comparison of molar proportions of α-(1→4)unit chains in each β-limit dextrin.

Additional information on the multiple branched structure of the oyster glycogen was obtained by controlled, repeated actions with *Klebsiella* pullulanase, which may preferentially delete the exterior A-chains (Yokobayashi *et al.*, 1969; Akai *et al.*, 1971). In the first treatment, some short chain maltosaccharides, dp2–dp5 (dp3, dominant) were released. As the treatment with pullulanase proceeded the distributions of the released maltosaccharides tended to shift to longer, i.e. dp3~dp17, and interestingly even after seven pullulanase treatments, small proportions of longer unit chains were still present, as will be reported elsewhere. These findings suggest that some longer chains are possibly located in the middle or inner tiers of the molecule.

Although deductions of more precise structural features of the oyster glycogen molecule are not warranted, there may be a possibility that the multiple branched high molecular blocks are developed from the inner tiers, so that overall, a huge glycogen molecule would be formed.

Degradation by α-amylase

In connection with the multiple branched structure of oyster glycogen, we were interested in the digestion of oyster glycogen in the animal digestive tract. The oyster glycogen was incubated with salivary α-amylase (human; crystallized) for 24h (α-amylolysis limit, 48.3%). The digestion products, analyzed by HPAEC, were compared with these from amylopectin (α-amylolysis limit, 57.8%).

The HPAEC profile of maltosaccharides produced by the α-amylase action is shown in Fig. 6. The hog

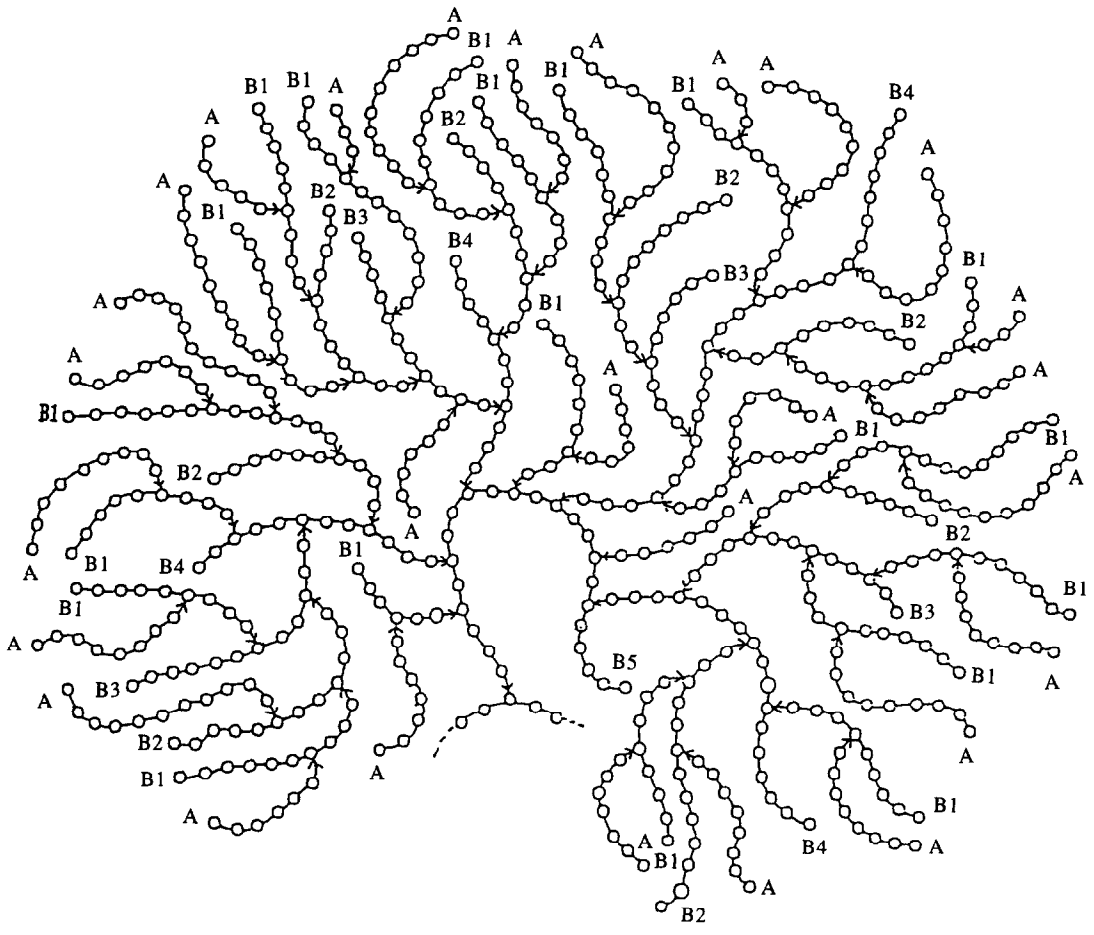


Fig. 5. A hypothetical molecular model for oyster glycogen. ○, glucose; -, α-(1→4)linkage; →, α-(1→6)linkage.

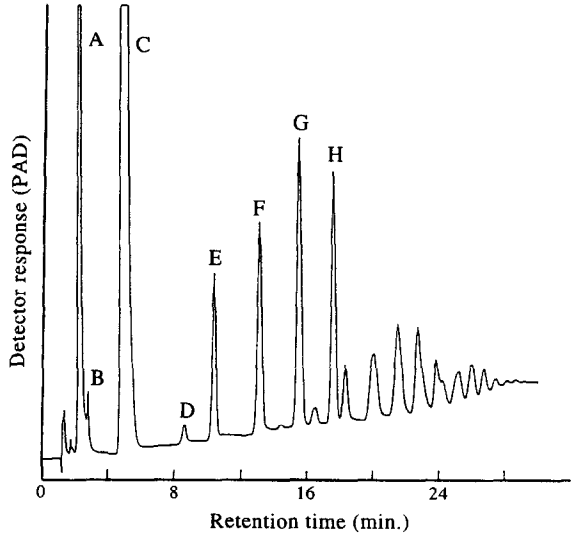


Fig. 6. HPAEC profile of the degradation products after salivary α-amylase action. A: glucose; B: isomaltose; C: maltose; D: maltotriose; E: 6^6-α-glucosyl-maltotriose (B_4); F: 6^6-α-glucosyl-maltotetraose ('fast' B_5) and 6^6-α-maltosyl-maltotriose ('slow' B_5); G: 6^6-α-maltosyl-maltotetraose ('2 on 4' B_6) and 6^3-α-maltotriosyl-maltotriose ('pullulan' B_6); H: 6^6-α-maltotriosyl-maltotetraose ('3 on 4' B_7).

pancreatic amylase action also gave similar maltosaccharides. Each branched oligosaccharide collected from the column (see Fig. 6) was characterized by identification of the hydrolysis products after action with α-D-glucosidase, isomaltase or pullulanase. In this manner, the following branched oligosaccharides were identified: 6^3-α-glucosyl-maltotriose, and -maltotetraose, 6^3-α-maltosyl-maltotriose and -maltotetraose, 6^3-α-maltotriosyl-maltotriose and -maltotetraose, in addition to several unidentified doubly branched maltosaccharides, as previously reported by Kainuma & French (1970). It is noted that branched oligosaccharides, especially those having double branches were present in glycogen, but not in amylopectin, supporting the presence of a higher degree of multiple branching in glycogen compared to amylopectin.

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