グリコーゲンのin vitroにおける新しい合成方法の開発、および得られたグリコーゲンの性質

<table>
<thead>
<tr>
<th>項目</th>
<th>内容</th>
</tr>
</thead>
<tbody>
<tr>
<td>誌名</td>
<td>Journal of applied glycoscience</td>
</tr>
<tr>
<td>ISSN</td>
<td>13447882</td>
</tr>
<tr>
<td>著者</td>
<td>梶浦, 英樹 高田, 洋樹 空山, 恒久 植山, 玲子 角谷, 亮 古屋, 敷隆 小島, 岩夫 竹田, 良三 栗木, 隆</td>
</tr>
<tr>
<td>巻/号</td>
<td>57巻2号</td>
</tr>
<tr>
<td>掲載ページ</td>
<td>p. 105-111</td>
</tr>
<tr>
<td>発行年月</td>
<td>2010年4月</td>
</tr>
</tbody>
</table>

(Hideki Kajitura, Hiroki Takata, Tsunehisa Akiyama, Reiko Ueyama, Ryo Kakutani, Takashi Furuyashiki, Iwao Kojima, Ryozo Takeda and Takashi Kuriki)

Abstract: A new enzymatic process for glycogen production was developed. In this process, short-chain amylose is used as the substrate for branching enzymes (BE, EC 2.4.1.18). The molecular weight of the enzymatically synthesized glycogen (ESG) depends on the type of BE used and the molecular weight and the concentration of the substrate amylose. Although a plant BE (kidney bean) and Bacillus cereus BE could not synthesize high molecular weight glucan, BEs from 6 other bacterial sources produced ESG. The BE from Aquifex aeolicus was the most suitable for the production of glycogen with a weight-average molecular weight (Mw) of 3000 kDa to 30,000 kDa. Furthermore, the addition of amylomaltase (AM, EC 2.4.1.25) significantly enhanced the efficiency of this process, and the yield of ESG reached approximately 65%. Typical preparations of ESG obtained by this method were subjected to structural analyses. The average chain length, interior chain length, and exterior chain length of the ESGs were 8.2–11.6, 2.0–3.3 and 4.2–7.6, respectively. Transmission electron microscopy showed that the ESG molecules formed spherical particles. Viscometric analyses also supported a spherical nature for the product. Unlike starch, the ESGs were barely degraded by pullulanase. Solutions of ESG were opalescent (milky-white and slightly bluish), and gave a reddish-brown color on the addition of iodine. These analyses revealed that ESG shares similar molecular shapes and solution properties with natural source glycogen (NSG).

Key words: glycogen, branching enzyme, polysaccharide, enzymatic synthesis, α-glucan

Glycogen is the major storage polysaccharide in animals, fungi, yeasts, bacteria, and archaea. Glycogen is a highly branched (1→4)β-glucan with a high molecular weight (10^6−10^9). Based on the results of electron microscopy, it has been shown that glycogen consists of spherical particles with diameters of 20–40 nm (β-particles), and that the β-particles 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. Glycogen is opalescent (milky-white and slightly bluish) in aqueous solution, and gives a reddish-brown color on the addition of iodine. Amylopectin, a major component of starch, is also a highly branched (1→4)α-glucan. However, the degree of branching, namely the number of (1→6)-α-glucosidic linkages, in glycogen is about twice that in amylpectin. A glycogen-type polymer, referred to as phytoglycogen, has also been isolated from several plants. In animal tissues, glycogen is synthesized from UDP-glucose via the cooperative action of glycogenin (EC 2.4.1.186), glycogen synthase (EC 2.4.1.11) and branching enzyme (BE, 1,4-α-glucan:1,4-α-glucan 6-α-d-(1,4-α-glucan)-transf erase, EC 2.4.1.18). Glycogen isolated from shellfish or animal tissues has been used to stimulate the exudation of phagocytic cells into the peritoneal cavities of experimental animals in immunological studies. It has also been reported that glycogen has an antitumor effect, probably through its immunomodulating activity, and that this activity seems to depend on the fine structure of glycogen. Glycogen has long been considered to have health benefits as a food ingredient. As revealed below, we demonstrated that ESG has a stimulating activity on immunocompetent cells such as macrophages. In order to gain information on current public impressions about glycogen, we carried out a questionnaire survey to investigate awareness of glycogen in Japan in April 2009. A total of 2000 men and women (ages teens-60’s) participated in the research. According to the results of the survey, the vast majority (85.6%) knew the word “glycogen”, and most of them seemed to associate the word “glycogen” with impressions of healthiness. The immunomodulating activity might be one of the reasons for the health benefits and impressions of healthiness associated with glycogen. Glycogen has also been used as a raw material in the cosmetic industry and as a carrier to enhance the yield of DNA during precipitation with organic solvent.

The in vitro synthesis of glycogen has been successfully achieved by the combined action of α-glucan phosphorylase (GP, EC 2.4.1.1) and BE using glucose-1-phosphate (G-1-P) as a substrate (GP-BE method). The enzymatically synthesized glycogen (ESG) produced by this method has properties similar to those of the natural.
source glycogen (NSG). Although G-1-P is expensive for industrial applications, it could be obtained from the inexpensive substrate sucrose by using sucrose phosphorylase (EC 2.4.1.7). Furthermore, we have demonstrated that ESG produced by the GP-BE method has immunomodulating activity. However, the yield of glycogen from sucrose was less than 40%, and a more efficient method is required to meet the demand for glycogen for industrial applications.

In the GP-BE method, GP elongates a chain of α-(1→4)-glucan and BE introduces branch points in the growing chains, mimicking the in vivo synthesis of glycogen by glycogen synthase and BE. This raises the question: can we synthesize glycogen simply using amylose as a substrate? The published results to date have all been negative: BE can synthesize branched glucans but their molecular weights are much lower than that of glycogen. For example, Boyer et al. analyzed the action of maize BE isozyme 1 by gel filtration chromatography and observed that the product was eluted more slowly than the substrate. Praznik et al. reported that potato BE on amylose with a weight-average molecular weight ($M_w$) of 67.6 kDa resulted in a branched product with a $M_w$ of 33.5 kDa. Kitamura, using light-scattering analyses, also reported that potato BE brought about a decrease in the molecular weight of the substrate amylose. We have reported that Bacillus cereus BE produced branched glucan with a $M_w$ of 50 kDa from amylose with $M_w$ of 320 kDa. The decreases in the molecular weight of the substrate could be attributable to a cyclization reaction by BE; it has been demonstrated that BE catalyzes a cyclization reaction in addition to a branching reaction in vitro (Fig. 1B). As a result of cyclization, the molecular weight of the substrate is decreased. In contrast, an inter-chain branching reaction results in both larger and smaller molecules if two molecules of the same size are used as a substrate (Fig. 1A). An intra-chain branching reaction should not change the molecular weight (Fig. 1C). However, most studies have used BE from plant sources and relatively long-chain amyloses were used as the substrate. To evaluate the possibility of glycogen synthesis from amylose, we tested the activity of eight types of BE from various sources using relatively short-chain amyloses as the substrates. We found that some BEs from bacterial sources can synthesize glycogen (ESG). We then compared some of the properties of the ESG with those of NSG.

**Fig. 1.** Model of action of BE.

Models of inter-chain branching, intra-chain branching and cyclization of amylose, and glycogen synthesis from amylose are shown. A, α-1,6-glucosidic linkage; $\alpha$-1,4-glucosidic linkage; $\ominus$, glucoseyl residue; $\ominus$, glucosyl residue with reducing end; $\triangledown$, the α-1,4 linkage cleaved by BE; $\blacksquare$, the glucosyl residue used as an acceptor; $n$, number of amylose molecules initially existing in the reaction mixture; $m$, number of synthetic glycogen molecules. Some glucosyl residues connected by α-1,4 linkages are indicated with broken lines.

**Table 1.** Weight-average molecular weight of α-glucans produced by the action of various BEs on amylose.

<table>
<thead>
<tr>
<th>Source of BE</th>
<th>Product from Amylose A</th>
<th>Product from Amylose AS-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus cereus</td>
<td>ND* ND</td>
<td>87 25.5</td>
</tr>
<tr>
<td>Kidney bean</td>
<td>ND ND</td>
<td>185 16.0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>3600 3.9</td>
<td>3900 25.2</td>
</tr>
<tr>
<td>Bacillus caldovelos</td>
<td>5910 4.5</td>
<td>10200 23.7</td>
</tr>
<tr>
<td>Bacillus caldolyticus</td>
<td>36300 1.2</td>
<td>30400 5.3</td>
</tr>
<tr>
<td>Bacillus stearothermophilus</td>
<td>2620 6.7</td>
<td>5760 32.0</td>
</tr>
<tr>
<td>Aquifex aeolicus</td>
<td>9370 10.1</td>
<td>20400 59.0</td>
</tr>
<tr>
<td>Rhodothermus obamensis</td>
<td>10300 31.2</td>
<td>45300 66.7</td>
</tr>
</tbody>
</table>

A reaction mixture (1 mL) containing 0.02 g of Amylose A or Amylose AS-10, 50 mM buffer (optimum pH) and 200 U of various BEs was incubated at optimum temperature for each enzyme for 24 h. *Not detected by HPSEC-MALLS-RI

The salts in the reaction buffer. However, if glucan with a high molecular weight (>1000 kDa) is produced, its peak is easily detected. Table 1 summarizes the results. In the reaction mixtures including Bacillus cereus BE and kidney bean BE, high molecular weight glucans could not be detected. However, glucans with $M_w$ more than 1000 kDa were detected in the mixtures including other BEs. R. obamensis BE gave glucans with an extremely high molecular weight, whereas A. aeolicus BE produced glucan that was similar in size to the β-particles of NSG. BE from A. aeolicus was selected for further analyses.

The products were easily dissolved in water giving
opalescent (milky-white and slightly bluish) solutions. The solutions gave a reddish-brown color with iodine. These properties were similar to those of NSG. Therefore, the high-molecular-weight glucan produced by this method (Fig. 1D) can be considered to be enzymatically synthesized glycogen (ESG). Figure 2 shows the time-course of the A. aeolicus BE reaction with Amylose A. After a 24 h reaction, the Mw of ESG increased to 6100 kDa. During the reaction, there were no changes in reducing power (data not shown).

From a practical perspective, Amylose A is preferable to AS-10, since amylose of this size can be easily obtained by treating starch with isoamylase, although the yield of ESG from Amylose A was lower than that from AS-10. However, during studies on the reaction conditions, we found that the addition of amyloylmalate from Thermus aquaticus (AM, EC 2.4.1.25) to the reaction mixture significantly improved the yield of ESG.

The yield reached 64% under optimal conditions.

**Effect of substrate concentration on product size.**

We debranched dextrin (DE 8–9.5) by isoamylase treatment, and used it at various concentrations as the substrate for BEs in the synthesis of ESGs. At a higher substrate concentration, the Mw values of ESGs were lower (Fig. 3). Thus, we can control the Mw of ESGs. Our hypothesis regarding the mechanism of this phenomenon is as follows. In the branching reaction, a singly-branched molecule produced in the first stage of the reaction is used as a recipient for subsequent reactions. Thus, very few branched molecules are produced in the initial stage of the reaction and are used as a “nucleus” or “core” for the growth of a macromolecule. The number of cores is inversely proportional to the molecular size of ESGs. Thus, the Mw values of ESGs should be low under a high concentration of substrate, because many cores are produced.

**Properties and structures of ESG.**

We synthesized ESGs (A-I) under various reaction conditions (Table 2), and compared the properties of the synthesized glycogens with those of NSGs (Table 3). The structures of glycogens from various origins have been compared mainly in terms of three parameters: the average chain length (CL), the exterior chain length (ECL), and the interior chain length (ICL). The structures of NSGs depend on the source and extraction method, and therefore they show structural heterogeneity (Table 3). The CLs of ESGs (A-I) tended to be slightly shorter than those of NSGs, whereas ECLs and ICLs were within the variations of the values for NSGs (Table 3). On the other hand, the unit-chain distributions of ESGs and NSGs as analyzed by high-performance anion exchange chromatography (HPAEC) after isoamylase treatment were slightly different from each other. Typical profiles are shown in Fig. 4. 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.

![Fig. 2. Action of A. aeolicus BE on short-chain amylose.](image)

A reaction mixture (40 mL) containing 0.8 g of short-chain amylose, 50 mM potassium phosphate buffer (pH 7.5) and 8000 U of A. aeolicus BE was incubated at 75°C. At the indicated time points, 150 μL of the mixture was removed, 37.5 μL of 5N NaOH was added to stop the reaction, and the sample was subjected to HPLC-MALLS-RI analysis. This analysis cannot determine low-molecular-weight (<10 kDa) substances. Mw of ESG; Δ, yield of ESG.

![Fig. 3. Effect of the substrate concentration on ESG size.](image)

A reaction mixture (1 mL) containing (0.02–0.16 g) of dextrin (DE 8–9.5) and 5000 U/g substrate of Pseudomonas amylodera-mosa isomylase was incubated at 40°C for 4 h. To this mixture were added 5000 U/g substrate of A. aeolicus BE and 0.08 mL of 0.5 M potassium phosphate buffer (pH 7.5), and the sample was incubated at 65°C for 44 h.

<table>
<thead>
<tr>
<th>Product name</th>
<th>Substrate</th>
<th>Substrate concentration (%)</th>
<th>Amount of amylomalase (U/g substrate)</th>
<th>Reaction time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESG A</td>
<td>Dextrin</td>
<td>10</td>
<td>146</td>
<td>58</td>
</tr>
<tr>
<td>ESG B</td>
<td>Dextrin</td>
<td>4</td>
<td>100</td>
<td>47</td>
</tr>
<tr>
<td>ESG C</td>
<td>Dextrin</td>
<td>8.5</td>
<td>200</td>
<td>64</td>
</tr>
<tr>
<td>ESG D</td>
<td>Dextrin</td>
<td>8</td>
<td>400</td>
<td>62</td>
</tr>
<tr>
<td>ESG E</td>
<td>Starch</td>
<td>1</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>ESG F</td>
<td>Dextrin</td>
<td>8</td>
<td>200</td>
<td>48</td>
</tr>
<tr>
<td>ESG G</td>
<td>Dextrin</td>
<td>8</td>
<td>200</td>
<td>48</td>
</tr>
<tr>
<td>ESG H</td>
<td>Dextrin</td>
<td>8</td>
<td>200</td>
<td>48</td>
</tr>
<tr>
<td>ESG I</td>
<td>Dextrin</td>
<td>8</td>
<td>200</td>
<td>66</td>
</tr>
</tbody>
</table>

A reaction mixture (1 mL) containing (0.01–0.1 g) of substrate and 5000 U/g substrate of Pseudomonas amylodermosa isomylase was incubated at 40°C for 4 h, and 5000 U/g substrate of A. aeolicus BE, the indicated amount of amyloylase, and 0.08 mL of 0.5 M potassium phosphate buffer (pH 7.5) were then added. The mixture was incubated at 65°C for the indicated number of hours.

**Transmission electron microscopy (TEM).**

TEM images (Fig. 5) revealed that both ESGs and NSGs have a slightly distorted circular shape, with diameters of 20–40 nm. The shapes and sizes were in good
agreement with those of β-particles previously reported by Hata et al. In the images of NSGs, a few large and irregular shaped particles with diameters of around 80–100 nm were found. These large particles may be aggregates of smaller particles, since it is known that the β-particles sometimes associate to form larger α-particles (60–200 nm) with a rosette-like appearance. In the images of ESGs we did not find any aggregated forms (α-particles) of glycogen.

**Viscosity.**

The intrinsic viscosities of ESGs and NSGs were very low (around 8 mL/g), and showed no molecular weight dependence (Fig. 6). In contrast, Kitamura et al. demonstrated that the intrinsic viscosities of amylose and amylopectin increase with their molecular weights. The intrinsic viscosity result suggested that ESG molecules behave as hard spheres in solution, resembling the behavior of NSG. NSG from sweet corn showed a higher intrinsic viscosity than the other glycogens. This high value may be due to the effect of slight structural differences such as slightly longer CL than other glycogens, or to a slight contamination with amylose and/or amylopectin. We also tested the viscosity of glycogen at higher concentrations. At concentrations of ≤20%, aqueous solutions of ESG showed low viscosity (<70 mPas) (Fig. 7). However, in the higher concentration range (>20%), sharp increases in viscosity were observed. The viscosities of ESGs showed no molecular weight dependence. The solutions of NSGs showed similar relationships between viscosity and concentration.

**Susceptibility of ESG to pullulanase and α-amylase.**

Pullulanase (EC 3.2.1.41) hydrolizes α-1,6 linkages of α-1,4/1,6 glucans such as pullulan, starch, and glycogen. However, it has been shown that the enzyme only partially hydrolyzes glycogen, trimming its outermost branches. ESG synthesized by the GP-BE method was shown to be resistant to pullulanase. Therefore, we tested the pullulanase digestibility of the ESG (Fig. 8).  

Starch (from corn) was easily degradoid into small fragments by pullulanase. However, NSG from oyster and the ESG were barely degraded. This result indicates that the ESG was similar to NSG, but was unlike amylopectin. Furthermore, we tested the digestibility of ESG by excess amounts of α-amylase. The final products of α-amylase hydrolysis of NSG were glucose, maltose, maltotriose, branched oligosaccharides with DP ≥4, and highly branched macrodextrins with molecular weights of up to 10 kDa. In the final products of α-amylase hydrolysis of ESGs, we detected much larger macrodextrins (molecular weight >1000 kDa). In contrast to NSG, oligosaccharides with DP 4–7 could not be detected in the

![Fig. 4. Chain-length distributions of ESGs and NSGs.](image)

ESGs and NSGs were subjected to HPAEC analyses after debranching with isomylase. Chain lengths (DPs) are indicated above the peaks.

**Table 3. Properties and structural parameters of various glycogens.**

<table>
<thead>
<tr>
<th>Sample name or source of native glycogen</th>
<th>$M_n$ (kDa)</th>
<th>$M_w$ (kDa)</th>
<th>$M_n/M_w$</th>
<th>β-Amyloylisis (%)</th>
<th>CL*</th>
<th>ECL*</th>
<th>ICL**</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESG A</td>
<td>2720</td>
<td>2170</td>
<td>1.3</td>
<td>35.2</td>
<td>9.3</td>
<td>5.3</td>
<td>3.0</td>
</tr>
<tr>
<td>ESG B</td>
<td>4960</td>
<td>3990</td>
<td>1.2</td>
<td>47.9</td>
<td>11.6</td>
<td>7.6</td>
<td>3.0</td>
</tr>
<tr>
<td>ESG C</td>
<td>7080</td>
<td>5610</td>
<td>1.3</td>
<td>45.9</td>
<td>9.3</td>
<td>6.3</td>
<td>2.0</td>
</tr>
<tr>
<td>ESG D</td>
<td>7940</td>
<td>6250</td>
<td>1.3</td>
<td>35.0</td>
<td>8.5</td>
<td>5.0</td>
<td>2.5</td>
</tr>
<tr>
<td>ESG E</td>
<td>9720</td>
<td>7760</td>
<td>1.3</td>
<td>44.8</td>
<td>9.1</td>
<td>6.1</td>
<td>2.0</td>
</tr>
<tr>
<td>ESG F</td>
<td>11,800</td>
<td>9670</td>
<td>1.2</td>
<td>26.9</td>
<td>8.2</td>
<td>4.2</td>
<td>3.0</td>
</tr>
<tr>
<td>ESG G</td>
<td>13,900</td>
<td>11,600</td>
<td>1.2</td>
<td>32.5</td>
<td>8.7</td>
<td>4.8</td>
<td>2.8</td>
</tr>
<tr>
<td>ESG H</td>
<td>15,000</td>
<td>12,700</td>
<td>1.2</td>
<td>35.0</td>
<td>8.6</td>
<td>5.0</td>
<td>2.6</td>
</tr>
<tr>
<td>ESG I</td>
<td>28,700</td>
<td>25,400</td>
<td>1.1</td>
<td>29.3</td>
<td>8.9</td>
<td>4.6</td>
<td>3.3</td>
</tr>
<tr>
<td>Synthetic glycogen†</td>
<td>24,200</td>
<td>19,800</td>
<td>1.2</td>
<td>46.1</td>
<td>11.9</td>
<td>7.5</td>
<td>3.4</td>
</tr>
</tbody>
</table>

*Produced by the GP-BE method. †Number-average chain length, CL = [Total sugars as glucose] / [non-reducing end residues]. †Exterior chain length, ECL = CL x β-amylolysis (%) / 100 + 2. †Interior chain length, ICL = CL-ECL-1.
A New Method for Glycogen Synthesis, and its Structure and Properties

ESG hydrolysates. These results suggest that the α-1,6 linkages in ESG molecules are more regularly distributed than those in NSG molecules. ESG is synthesized in much simpler circumstances than is NSG, in which no trimming reactions occur during the synthetic process.

"Function of ESG."

Glycogen is exclusively known as the energy and carbon reserve in animal cells and microorganisms. However, that glycogen has an immunological activity has long been suggested, although strong scientific evidence...
Fig. 8. Susceptibility of α-glucan to pullulanase.

ESG-D (●), NSG from oysters (○) and gelatinized starch (from corn) (▲) were treated with pullulanase. Mₙ values of hydrolyzates were analyzed by HPSEC-MALLS-R.

Fig. 9. Relationship between molecular weight and macrophage-stimulating activity of ESG.

RAW264.7 was cultured with ESGs or hypopolysaccharide (LPS) in the presence of interferon-γ. After cultivation for 48 h, Nitric oxide concentration in the culture supernatant was determined. Open and slanted bars indicate the results without and with polymyxin B treatment, respectively.

CONCLUSION

We have described a new method (IAM-BE-AM method) for producing glycogen from short-chain amylose by using BE. This method has two advantages compared with the GP-BE method, for which sucrose is used as a starting material. First, the yield of glycogen is higher. The yield of glycogen by the GP-BE method was less than 40%, whereas that by the IAM-BE-AM method reached 65% under the best conditions. Second, the molecular weight of glycogen is controllable within the range of 3000 kDa to 30,000 kDa. The structures and properties of the ESGs were quite similar to those of NSGs (Figs. 4-8 and Table 3). Despite these similarities, there were two differences (the unit-chain distribution (Fig. 4) and final products by α-amylase hydrolysis) between the ESG and NSG. Furthermore, we have demonstrated that ESG has immunomodulating activity. We hypothesize that the macromolecule fraction after partial hydrolysis of ESG that reaches the intestinal tract stimulates immunocompetent cells resulting in the health benefits. Investigations in vivo are now in progress to gain a further understanding of the effect of orally administered glycogen.

We deeply appreciate Dr. S. Kitamura of Osaka Prefecture University for his kind help in performing structural analyses and testing the solution properties of ESG. We also thank Drs. H. Matsui and H. Ito of Hokkaido University for providing the plasmid used to produce the BE of Phaeolus vulgaris L. We also thank Drs. N. Ohno and Y. Adachi of Tokyo University of Pharmacy and Life Science for their advice and valuable discussions during the course of the immunological studies. This work was supported in part by a grant from 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

4) Y. Takaya: Biological activities of natural resources around us are now in the limelight. Yakugaku Zasshi, 120, 1075–1089 (2000).
9) D.S. Tolmasky and C.R. Krisman: The degree of branching in
グリコーゲンのin vitroにおける新しい合成方法の開発、および得られたグリコーゲンの性質

[質問] 秋田県立大 中村

[回答] グリコーゲン合成用の新しい合成方法を開発した。この方法では、プレンチングエンザイム（BE, EC 2.4.1.18）を基質として短鎖アミロースを使用する。得られた酵素合成グリコーゲン（酵素由来グリコーゲン, ESG）の分子量は、BEの種類、基質であるアミロースの分子量、基質濃度に依存した。植物由来BEとBacillus cereus由来BEはこの方法で高分子グリコーゲンを合成することができなかったが、他の6種類の細菌由来BEはESGを合成することができた。特にAquifex aeolicus由来BEが分子量30,000Daから30,000kDaの大きなESGを合成するのに最も適していた。さらにアミロマルターゼ（AM, EC 2.4.1.25）を反応系に加えることにより収率が大幅に上昇し、最大約65%まで達した。この方法で得られたESGの構造を分析したところ、平均単位鎖長は8.2-11.6。内部平均鎖長は2.0-3.3。外部平均鎖長は4.2-7.6であった。透過型電子顕微鏡による観察および固有粘度の分析によって、ESG分子が球形であることが示された。また、ESGは、澱粉と違ってプルラナーゼなどで容易に分解されなかった。ESGの水溶液の色は、オパール色（煮ひがしの乳白色）であり、ヨウ素によって赤茶色に呈色した。これらの分析結果からESGと植物由来グリコーゲン（自然由来グリコーゲン, NSG）がほぼ同じ分子形状、溶液性をもつことがわかった。

＊＊＊＊＊

[質問] 秋田県立大 中村

[回答] グリコーゲン合成用の少ないBacillus cereus由来BEやkidney bean由来BEは、なぜ機能をもたないのでしょうか。

秋田県立大 中村