Examination of the Structure of Amylose and Amylopectin by Fluorescent Labeling of the Reducing Terminal

(Received September 15, 2002)

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Abstract: The fluorescent labeling/HPSEC method was developed and the molar-based distribution of amylopectin unit-chains, and molecules of amylose and amylopectin from various sources was examined. The molar-based distribution of A and B chains enabled the estimation of the number of chains per cluster. Cereal amylopectins had a larger number of chains in a cluster than root and tuber amylopectins. A-Crystalline type starches contained amylopectin with a larger number of chains per cluster than B-type crystalline. The molar distribution of C chain suggested the presence of long and short C-chains, which appeared to connect two clusters and were in single cluster, respectively. Amylopectin comprised three molecular species. The large species was major in amount by mole and weight, but the medium and small species were relatively large in amount by mole but very small by weight. The three species appeared to be built up with a similar cluster in structure and differed in the number of clusters. The number of clusters suggested that the large and medium species might be blocklets in granule while the small species immature and/or degraded products of the large species. Amylose comprised several molecular species with different size, and their proportions differed by plant sources. The small amylose species was predominant in cereal amyloses while the large amylose species in root and tuber amylloses. The number-average degree of polymerization of amyloses and the number-average chain length of amylopectins determined by the labeling/HPSEC method were in good agreement with those determined by conventional colorimetric methods.

Key words: amylopectin, amylose, cluster, fluorescent labeling

Starch comprises small, linear and slightly branched molecules of amylose and large, highly branched molecules of amylopectin, and their molecular structures are distinct by plant sources.1,2) For further examination of their structures, we have recently developed a new analytical method3) involving fluorescent labeling of reducing residues of α-glucans. Fluorescent labeling followed by high-performance size-exclusion chromatography (the labeling/HPSEC method) enabled the direct determination of their molar-based distributions, and revealed more detailed molecular structure of amylose and amylopectin than conventional methods for weight-based determination.4,5) The labeling/HPSEC method yielded the molar-based distribution of A, B and C chains of amylopectin,6) and the presence of the multiple species of amylopectin7) and amylose.3) Furthermore, the method enabled the determination of chain length of amylopectin8) and number-average degree of polymerization (DPn) of molecules of amylopectin9) and amylose.10

Determination of number-average chain-length (CLn) and DPn by the labeling/HPSEC method.

The reducing terminal of debranched amylopectin, amylopectin and amylose was labeled with a fluorescent reagent, 2-aminopyridine, and the resulting Schiff’s base was reduced by sodium cyanoborohydride.11 Figure 1 illustrates the analytical system for molar- and weight-based distributions of debranched amylopectin. The system consisted of a HPLC pump, size exclusion columns, two detectors for fluorescence and refractive index, and a computer for data analysis. For analyses of amylose3) and amylopectin7) molecules, a column(s) suitable for their analyses was used.

CLn of amylopectins determined by the labeling/HPSEC method is summarized in Table 1. The values were in good agreement with those determined by the conventional Smith degradation method.8) Procedure for determination of unit chains and main chain of amylopectin.

Figure 2 shows the cluster model of amylopectin proposed by Hizukuri15 and the procedures for determination of unit chains, A and B chains, and the main chain, C chain, of amylopectin. The A chain carries no chain and links to another chain via α-1,6-linkage. The B chain car-
Fig. 1. Analytical system for molar- and weight-based distribution of debranched amylopectin.

Table 1. Number-average chain-length (CLn) of amylopectin.

<table>
<thead>
<tr>
<th>Source</th>
<th>Labeling</th>
<th>Smith degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat, Nohrin-61</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Wheat, Rosella</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Rice, Akitaomachi</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Rice, Nihonbare</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Maize</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>Amylomaize</td>
<td>33</td>
<td>32</td>
</tr>
<tr>
<td>Root and tuber</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweet potato, Joy White</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>Sweet potato, Koganesengan</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>Potato, Eniwa</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>Potato, Benimaru</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>Yam</td>
<td>25</td>
<td>24</td>
</tr>
</tbody>
</table>

Fig. 2. Procedures for determination of unit chains and C chains of amylopectin.

Molar distribution of C chains of amylopectin.

Much less attention had been paid to C chains due to their very small amount and the lack of appropriate methods to distinguish C chain from A and B chains. The labeling/HPSEC method made it possible to reveal the molar distribution of C chains. The C chains of amylopectin from various plant sources showed asymmetrical, broad distribution (Fig. 3). Other than amylomaize, the distributions were very similar across botanical origins, implying that the biosynthetic process for C chains was similar in different plant sources. The DP values were estimated from a calibration curve obtained from the unit-chain distribution. A peak DP was in the range 38–49 and a shoulder DP 21–27, suggesting that there were two kinds of C chains, long and short C chains. The long C chain was long enough to span two clusters, while the short C chain was involved in a single cluster. The C chain of amylomaize amylopectin was much longer than that of the other plant sources, and showed a single peak with DP 80, being long enough to span two clusters of amylomaize amylopectin, which has long CLn 32 (see Table 1).

Molar-based distributions of unit chains of amylopectin.

The labeling/HPSEC method simultaneously determined the molar- and weight-based distributions of amylopectin unit-chains (Fig. 4). The molar-based distributions were polymodal, similar to the well-known feature for weight-based distribution. However, the relative amount of the long chain fraction (B2 + B3 chains) to the short chain fraction (A + B, chains) was much less in molar basis than in weight basis. The molar-based distributions of root amylopectins showed a peak at DP 6 and a hollow at DP 8. These features were characteristic of root

ries other chains. The C chain is only one chain in an amylopectin molecule and has free reducing terminal residue. The B chain is involved in a cluster together with A chain. The B2 and B3 chains are defined to be the B chain connecting two and three clusters, respectively. The molar-based distribution of unit chains was determined by debranching of amylopectin with isoamylase followed by fluorescent labeling, while the molar distribution of C chains was determined by fluorescent labeling prior to isoamylolysis.
Table 2. Molar distributions of unit chains of amylopectins.

<table>
<thead>
<tr>
<th>Source</th>
<th>Amount in mole (%)</th>
<th>Crystalline type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat (No)</td>
<td>92.8</td>
<td>A</td>
</tr>
<tr>
<td>Wheat (R)</td>
<td>92.5</td>
<td>A</td>
</tr>
<tr>
<td>Rice (A)</td>
<td>91.5</td>
<td>A</td>
</tr>
<tr>
<td>Rice (Ni)</td>
<td>91.0</td>
<td>A</td>
</tr>
<tr>
<td>Maize</td>
<td>90.9</td>
<td>A</td>
</tr>
<tr>
<td>Sweet potato (J)</td>
<td>90.5</td>
<td>A</td>
</tr>
<tr>
<td>Sweet potato (K)</td>
<td>89.9</td>
<td>A</td>
</tr>
<tr>
<td>Potato (E)</td>
<td>86.7</td>
<td>B</td>
</tr>
<tr>
<td>Potato (B)</td>
<td>86.4</td>
<td>B</td>
</tr>
<tr>
<td>Amylo maize</td>
<td>67.8</td>
<td>B</td>
</tr>
</tbody>
</table>

*Number of chains per cluster.

Molar-based distributions and $D_P$ of amylopectin molecule.

Figure 5 shows the molar- and weight-based distributions of unit chains of amylopectins from various plant sources.

- , fluorescent response; ---, RI response; ●, DP. Numbers with arrowheads indicate DP.

Amylopectins, and had been also observed by the HPAEC-PAD method. On the other hand, there was no distinct peak at DP 6 nor hollow at DP 8 for cereal amylopectins, being characteristic of cereal amylopectins and different from root amylopectins. Such difference appeared to be due to the difference in chain transfer action of branching enzyme isoforms. Amylomaize amylopectin had a much larger amount of long chains than the others.

The molar-based elution profile was divided into the long ($B_2+B_3$) and the short ($A+B_1$) chain fractions, and the proportion in mole was calculated for each fraction (Table 2). The amount of the short chain fraction was in the range of 93–68% by mole, and that of the long chain fraction was 7–32%. The short chain was involved in a single cluster, and the long chain was the chain that connected clusters. Therefore, the ratio of the short chain fraction to the long chain fraction, ($A+B_1$)/($B_2+B_3$), implied the number of chains per cluster. The number of chains varied by the plant origin and was in the range of 2–13. Cereal amylopectins had a larger number of chains per cluster than root amylopectins. Starches of A-type crystalline tended to have amylopectin with a larger number of chains per cluster than starches of B-type crystalline. Such information obtained by the labeling/HPSEC method would be valuable for modeling a cluster structure.

**Structure of three molecular species of amylopectin.**

Waxy rice amylopectin was labeled and fractionated into large, medium and small species (see Fig. 5), and their molar-based distribution showed three molecular species differing in size: large, medium and small species. The large species was predominant, and the medium and small species had relatively large amounts by mole, but very small amounts by weight. The $D_P$ of the large, medium and small species was 22,000, 7200 and 1200, respectively, and whole amylopectin had a $D_P$ of 12,900. Amylopectin from other plant sources, normal maize, rice (waxy, japonica and indica), sweet potato and potato, also had three molecular species, and the size and proportion of these species differed by the plant sources.

The number of clusters for molecular species from cereal, root and tuber amylopectins was calculated by the equation: $(D_P) / [CL x (number of chains per cluster)]$. The large species comprised 60–120 clusters, while the medium and small species comprised 20–40 and 4–15 clusters, respectively. The number of clusters of the species differed by the plant origin. Gallant et al. suggested the presence of blocklets organizing starch granule observed under scanning and transmission electron microscopes. The blocklets were 20–500 nm in diameter, which worked out to be 2–50 clusters in length arranged in tandem, because a cluster was 10 nm long. The number of clusters suggested that the large and medium species was a blocklet while the small species immature blocklet and/
Molar- and weight-based distributions of waxy rice amylopectin.

Symbols, see Fig. 4. Single column packed with Toyopearl HW-40S, HW-50S and HW-755 (1:3:2 by volume) was used.

Fig. 6. Molar- and weight-based distributions of amyloses from various plant sources.

Symbols, see Fig. 4. Column, TSKgel G6000PW, G4000PW and G3000PW connected in series.

or degraded product of large and small blocklets.

**Molar-based distribution of amylose molecule.**

The distribution of amylose molecules on a molar basis was more characteristic by plant origin than on a weight basis (Fig. 6). Amylose comprised several molecular species with different sizes, and the size and proportion of these species differed by plant origin. Potato amylose was composed of at least three species, large (peak DP, 10,200), medium (4520) and small (2230) species. Sweet potato, barley and wheat amyloses had two molecular species, relatively large species (2210-3090) and small species (500-730). Rice and maize amyloses had mostly single, small species (500-730). It was not known how these species were synthesized in the starch granule. But the presence of these amylose species in starch granule was very interesting.

The DP of amyloses determined by the labeling/HPSEC method are listed in Table 3. In general, root and tuber amyloses were larger than cereal amyloses, and the values were in good agreement with the values determined by a conventional colorimetric method.13)

**Conclusion.**

The labeling/HPSEC method gives molar- and weight-based distributions simultaneously and provides new information on the molecular structure of amylose and amylopectin. Amylopectin comprised three molecular species with different differences, and all species appeared to be built up with a cluster having similar structure, and each species composed of different number of clusters. There appeared to be two kinds of C chains differing size. The long C chain appeared to connect two clusters while the short C chain resided in single cluster. Amylose consisted of several molecular species with different size. The size and proportion were different by the plant origin.

The authors thank Messrs. S. Shibahara (Nihon Shokuhin Kako Co., Ltd., Japan), M. Tagawa (Nihon Starch Co., Ltd., Japan) and K. Iwata (Advantec Toyo Kaisha Ltd., Japan) for their collaboration with this study.

**REFERENCES**