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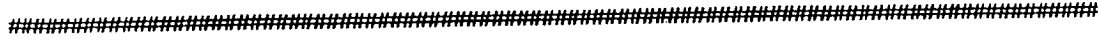
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## Structures of B90 (*sugary*) and W64A (normal) maize starches \*

Yasuhiro Takeda<sup>1</sup> and Jack Preiss

Department of Biochemistry, Michigan State University, East Lansing Michigan 48824 (USA)

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### ABSTRACT

The B90 maize (*sugary*) starch showed an iodine affinity (i.a. = g/100 g) of 8.8, and 30.6% was amylose. The W64A maize (normal) starch and an i.a. of 4.6, and 18.5% was amylose. The starches were fractionated into amylose, amylopectin, and a middle layer (ML)-component by the Schoch method. The amyloses had a number-average dp ( $\overline{dp}_n$ ), 780–830. The B90 amylose had a larger weight-average dp ( $\overline{dp}_w$ , 3360) and a higher number of chains (nc 3.9) than the W64A amylose ( $\overline{dp}_w$ , 2680, nc 2.4) and appeared to contain a large branched molecule. The properties of the amylopectins and ML-components are similar, but the ML-components are larger molecules. These B90 components had higher i.a. (3.1–4.8) than the W64A components (0.8–1.1). The average chain length ( $\overline{cl}$ ) and beta-amylolysis limit of the B90 and W64A components were 20–21 and 59–61%, respectively. However, chromatography on Sephadex G-75SF after debranching with isoamylase indicated that the B90 amylopectin was comprised of larger long B-chains ( $\overline{cl}$  150) and smaller intermediate B-chains ( $\overline{cl}$  40) than the W64A amylopectin ( $\overline{cl}$  100 and 44, respectively). The B90 amylopectin long B-chains appear to be poorly branched and are the reason for its high i.a.

### INTRODUCTION

Accumulating studies on structures of amyloses and amylopectins isolated from starches by the method of Lansky et al.<sup>1</sup> with certain modifications<sup>2</sup> have revealed that the components from different plant origins are unique in their structure. The amyloses differed in number- and weight-average dp, average chain length ( $\overline{cl}$ ), and number of chains, and contained linear and slightly branched molecules at various molar ratios<sup>3–13</sup>. The branched molecule, which is an intermediate molecule between true amylose (linear molecule) and amylopectin<sup>10</sup>, also differed in struc-

*Correspondence to:* Professor Jack Preiss, Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824, USA.

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<sup>1</sup> Present address, Department of Biochemical Science and Technology, Faculty of Agriculture, Kagoshima University, Kagoshima 890, Japan.

ture. The amylopectins are distinct in  $\bar{c}l$  and chain-length distributions<sup>14–18</sup>. The starches from three varieties of sweet potato showed similar structures of amylose and amylopectin<sup>19</sup>, while the starches from several varieties of japonica and indica rice showed different iodine affinities (i.a.), contained a similar amylose content, and differed in chain length distributions of the amylopectins<sup>9,20,21</sup>. Several mutants of maize and normal maize have starches with different i.a. and amylose contents<sup>22–24</sup>. However, their components are not well known<sup>4,8</sup> although the whole starches were investigated by gel-permeation chromatography after debranching with isoamylase<sup>24–27</sup>. The amylose, amylopectin, and an additional fraction (middle layer, ML-component) structures from B90 (a *sugary* mutant of Oh43) and W64A (normal) maize starches are studied and reported herein.

## EXPERIMENTAL

**Materials.**—The B90 maize starch was a product of Penford Products Co. The B90 maize starch is from a *sugary-2* inbred and was isolated from the kernels by a micro technique approximating the commercial recovery of starch from corn. The starch from the W64A maize was prepared from kernels, of which ears were harvested 22 days after pollination in 1988 and stored at  $-80^{\circ}\text{C}$ . The starch was isolated from the residual precipitate (stored at  $-25^{\circ}\text{C}$ ) obtained from homogenate of kernels when the branching enzyme was prepared<sup>28</sup>. The starch suspension in cold water was squeezed through a four-layered cloth, and the starch was washed with cold water, EtOH, and then ether by centrifugation, and dried over silica gel. The yield of the starch was 15 g (wet) from 100 g (wet) of maize kernels. The fractionation of the starches defatted<sup>2</sup> by repeated dissolution in  $\text{Me}_2\text{SO}$  and precipitation with EtOH was performed under  $\text{N}_2$  by the method of Lansky et al.<sup>1</sup>. Centrifugation of chilled starch dispersion in an aq 1-butanol–3-methyl-1-butanol (6.6% each) gave a middle, loose layer (ML-component) besides precipitate (amylose) and supernatant (amylopectin) layers. The amyloses were purified as previously described<sup>2</sup>. The ML-components were washed four times with aq 10% 1-butanol ( $4^{\circ}\text{C}$ ) by centrifugation, and the supernatants were combined with the amylopectin fraction. The ML-components and amylopectins were precipitated with EtOH washed with EtOH and then ether, and dried over silica gel. The yields of amylose, ML-component, and amylopectin were 0.49, 0.29, and 0.76 g from the B90 starch (2 g) and 0.29, 1.17, and 0.35 g from the W64A starch (2.2 g), respectively. Crystalline *Pseudomonas* isoamylase (EC 3.2.1.68) was obtained from Hayashibara Biochemical Laboratories Inc. (Okayama, Japan).

**Analytical methods.**—The i.a. was determined by a modified<sup>21</sup> amperometric titration<sup>29</sup>. The blue value (BV)<sup>30</sup> and number-average  $dp$  ( $\bar{d}p_n$ )<sup>31</sup> were determined as described. The  $\bar{c}l$  of the amyloses was determined by the rapid Smith-degradation method<sup>31</sup> with minor modifications<sup>32</sup>. The average number of chains per molecule was calculated as  $\bar{d}p_n/\bar{c}l$ . The weight-average  $dp$  ( $\bar{d}p_w$ ) and  $dp$  distribution of the amyloses were determined<sup>33</sup> by gel-permeation HPLC using connected

TABLE I

Properties of the maize amyloses

Amylose	B90	W64A
Iodine affinity (i.a.), g/100 g	21.7	21.3
Blue value (BV)	1.41	1.45
$\lambda_{\max}$ (nm)	648	653
Number-average $\overline{dp}_n$	780	830
Weight-average $\overline{dp}_w$	3360	2680
Apparent $dp_w$ distribution <sup>a</sup>	200–18000	300–9000
$\overline{dp}_w / \overline{dp}_n$	4.3	3.2
Average chain length ( $\overline{cl}$ )	200	340
Number of chains per molecule	3.9	2.4
Beta-amylolysis limit (%)	80	81

<sup>a</sup>  $DP_w$  values of the subfractions (10% by weight) having the lowest and highest molecular weights.

columns (Tosoh, TSKgel G6000PW, G4000PW, and G3000PW) with a differential refractometer (Elma, ERC 7152) and a low-angle laser-light-scattering photometer (Tosoh, LS-8) as detectors. The  $\overline{cl}$  of the amylopectins and ML-components was determined by the rapid Smith-degradation method<sup>34</sup> and debranching with isoamylase<sup>35</sup>. To determine chain-length distributions of the amylopectin and ML-component, the isoamylolyzate<sup>35</sup> (1 mL, ~5 mg) was applied to a column of Sephadex G-75SF (2.4 × 45 cm) at 40°C and eluted with 50 mM NaCl at 29 mL/h (5-mL fractions). The beta-amylolysis limit was determined as described<sup>31</sup>. Carbohydrate and reducing terminal were determined by the phenol-H<sub>2</sub>SO<sub>4</sub> method<sup>36</sup> and the modified Park-Johnson method<sup>31</sup>, respectively.

## RESULTS AND DISCUSSION

*Structure of amylose.*—Table I summarizes the properties of the B90 (sugary) and W64A (normal) amyloses. Both the amyloses were determined to be free of amylopectin, as they gave a single elution profile on Toyopearl HW-75F chromatography<sup>32</sup> (Fig. 1) and gel-permeation HPLC (Fig. 2). Their i.a. and BV were as high as that for pure amyloses<sup>32</sup>. The B90 amylose showed a slightly smaller  $\overline{dp}_n$  (780) than the W64A amylose (830), and their values were between those of amylo maize amyloses<sup>8</sup> (690–740) and the amylose of normal maize of a white dent variety<sup>4</sup> (WD,  $\overline{dp}_n$  990). However, the B90 amylose had the highest  $\overline{dp}_w$  (3360) among the maize amyloses so far examined (W64A 2680, WD 2500 and amylo-maize 1810–1990). The ratio  $\overline{dp}_w / \overline{dp}_n$  (4.3) was also the highest (W64A, 3.2., WD 2.53, and amylo maize 2.9–3.4), indicating that the B90 amylose had a broad distribution of molecular weight ( $dp_w$  200–18000). The B90 and W64A amyloses showed a peak at  $dp$  1710 and 2090, respectively, (Fig. 2). The former had a shoulder ( $dp$  530), while the latter had two shoulders ( $dp$  5370 and 640). The B90 amylose gave a concave slope of  $dp$  plots against retention time and a sharp  $dp$  slope at the side of short retention time, suggesting that it contained a large

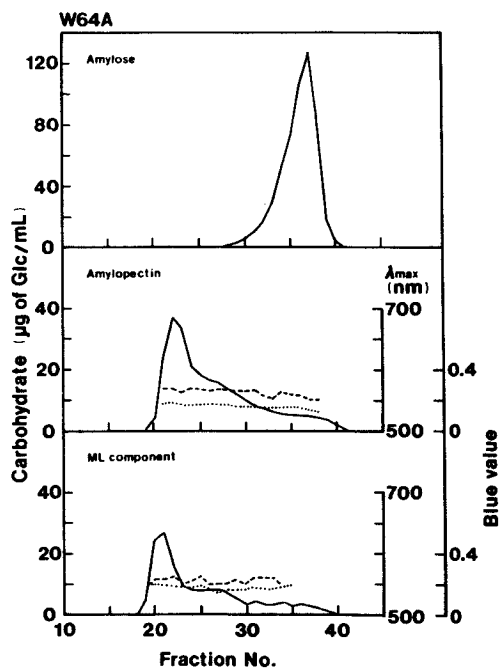
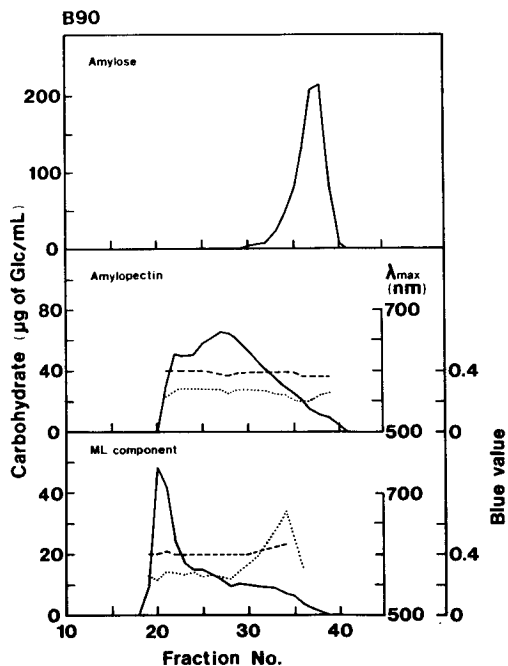


Fig. 1. Gel-permeation chromatograms of amylose, amylopectin, and ML-components from the B90 and W64A maizes on Toyopearl HW-75F: —, carbohydrate; — —,  $\lambda_{\text{max}}$ ; ·····, blue value.

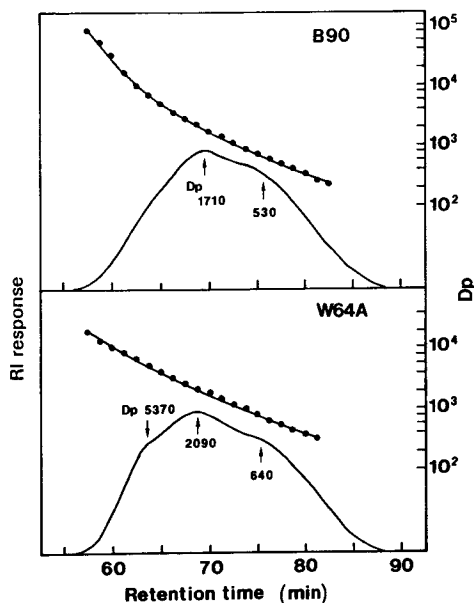


Fig. 2. Gel-permeation HPLC of the B90 and W64A amyloses: —, response of the differential refractometer (RI); ●, dp.

branched molecule. The W64A amylose showed a linear slope. These behaviors of the B90 and W64A amyloses resembled those of amylo maize and WD amyloses, respectively.

The  $\bar{c}_l$  (200) of the B90 amylose was smaller than that (340) of the W64A amylose, resembling those of amylo maize (215–255) and WD amylose (295), respectively. The B90 amylose had the highest number of chains per molecule (3.9) among W64A (2.4), amylo maize (2.9–3.2) and WD (3.4) amyloses, indicating that the B90 amylose is rich in the branched molecule or highly branched. Both the amyloses had the same beta-amylolysis limit (80 and 81%), similar to that (82%) of the WD amylose and slightly higher than those (75–78%) of amylo maize amyloses.

These results suggested that the normal maize, W64A, and WD amyloses are similar in structure while the B90 amylose differed from amylo maize and normal maize amyloses and contained a large branched molecule. The maize amyloses ( $\bar{d}p_n$  690–990), as well as rice amyloses<sup>6,9,10,21</sup> (920–1110), were small molecules compared with amyloses from root and tuber (2000–4920)<sup>3,5,7,18,19,30,31</sup>.

*Structures of amylopectin and ML-component.*—Table II summarizes the properties of amylopectins and ML-components from B90 and W64A. The ML-component was a middle, loose layer between amylopectin and amylose layers on centrifugation of the chilled starch dispersion. The yields of the ML-component from the starches were 35% for B90 and 16% for W64A. A similar ML-component was obtained from sweet potato starches<sup>19</sup> (10–15% of starch). These B90 components had i.a. 3.1–4.8, about 4-fold higher than those observed for W64A and as



TABLE II

Properties of the amylopectins and ML-components of B90 and W64A

	Amylopectin		ML-Component	
	B90	W64A	B90	W64A
I.a., g/100 g	3.1	0.8	4.8	1.1
Blue value (BV)	0.26	0.15	0.38	0.16
$\lambda_{\max}$ (nm)	610	569	617	570
$\overline{DP}_n$	4800	5100	7900	10000
$\overline{Cl}$				
Smith degradation	20	20	22	21
Isoamylolysis	20	21	21	20
Number of chains per molecule	240	240	370	490
Beta-amylolysis limit (%)	60	59	61	60

high as seen for the amylo maize amylopectins<sup>37</sup> (3.6–4.6), and a higher  $\lambda_{\max}$  and BV than the W64A components. The values of the W64A components were similar to those ( $\lambda_{\max}$  554 nm, BV 0.11) of the WD amylopectin.

Fig. 1 shows gel-permeation chromatograms of the amylopectins and ML-components on Toyopearl HW-75F. Large molecules were preponderant in the components, except that the B90 amylopectin was rich in molecules of medium size. The W64A components gave a lower recovery (51 and 66%) than the B90 components (85 and 95%), possibly due to poor dispersion. The  $\lambda_{\max}$  and BV of the chromatograms were almost constant except for these of the B90 ML-component. Its  $\lambda_{\max}$  and BV increased with the fraction corresponding to amylose. These results indicated that the component except the B90 ML-component were free of amylose. The B90 ML-component appeared to contain an amylose impurity of ~9% calculated from the BV of the ML-component and amylose using an average BV (0.28) of the fractions up to No. 28 (Fig. 1) by the equation given in Table V.

The amylopectins were smaller molecules than the ML-components (Table II) and the WD amylopectin ( $\overline{dp}_n$  7200). The rapid Smith-degradation method and debranching by isoamylase gave the same  $\overline{cl}$  20–22, indicating the complete debranching by isoamylase. The values were a little higher than that (18.6) of waxy maize amylopectin<sup>14</sup>, lower than those of amylo maize amylopectins (29–32), and the same as that for WD (21). The number of chains per molecule was 240 and ~450 for the amylopectins and ML-components, respectively. The beta-amylolysis limit of all the components was 59–60%, which is the same as that for WD.

Fig. 3 shows the chain-length distributions of the B90 and W64A components on Sephadex G-75SF chromatography after isoamylolysis. Complete recovery of carbohydrate was observed. The elution profiles showed three peaks having  $dp > 100$ , 40–44, and 13–15 on a weight basis and a peak having  $dp$  12–13 on a molar basis. The chains were fractionated into three fractions, F1–F3, in order of elution, and their properties are summarized in Table III. The  $\overline{cl}$  of whole fractions agreed with those described above. The B90 amylopectin showed higher F1 and

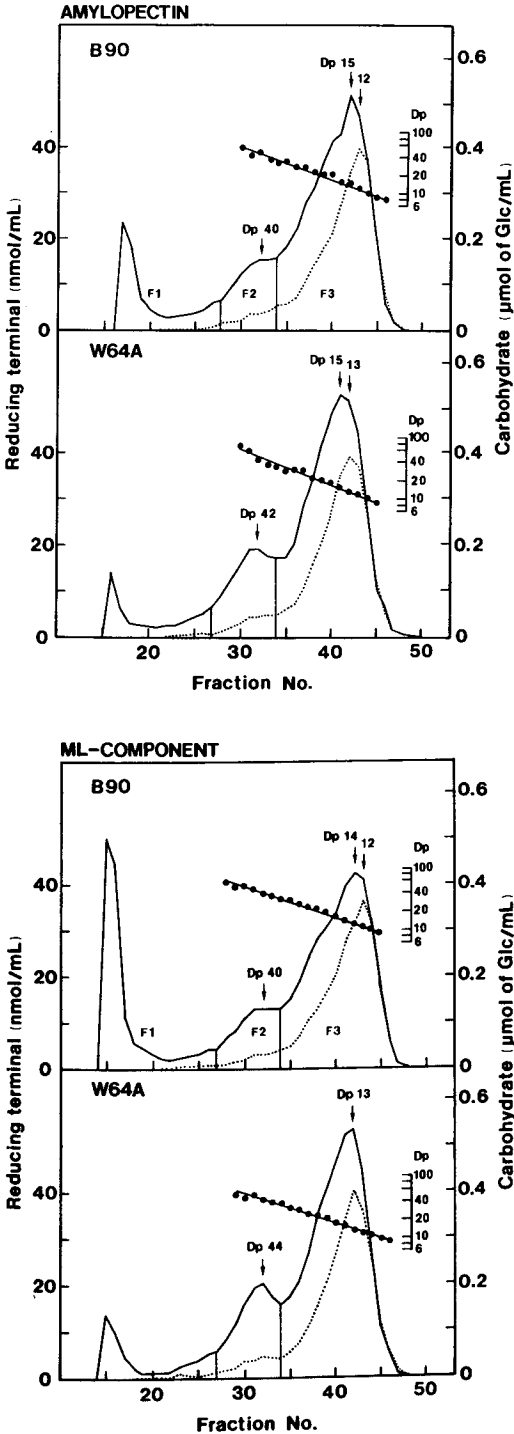


Fig. 3. Gel-permeation chromatograms of debranched amylopectins and ML-components of B90 and W64A on Sephadex G-75SF: —, carbohydrate; ·····, reducing terminal; ●, dp.

TABLE III  
Carbohydrate proportions and  $\bar{D}p_n$  of F1-F3 of the amylopectins and ML-components of B90 and W64A

	Proportion (%) on basis of						$\bar{D}p_n$														
	weight			mole			F1			F2			F3			Whole					
	F1	F2	F3	F3/F2	F1	F2	F3	F3/F2	F1	F2	F3	F3/F2	F1	F2	F3	F3/F2	F1	F2	F3	F3/F2	
Amylopectin																					
B90	16	15	69	4.6	2	8	90	11	150	40	15		150	40	15		150	40	15		20
W64A	10	20	70	3.5	2	9	89	9.9	100	44	16		100	44	16		100	44	16		20
ML-Component																					
B90	26	14	60	4.3	2	8	90	11	240	42	15		240	42	15		240	42	15		22
W64A	10	20	70	3.5	2	9	89	9.9	110	44	16		110	44	16		110	44	16		20

lower F2 weight-proportions than the W64A amylopectin, and no obvious fraction peak in F2. The B90 ML-component gave the highest proportion of F1, due to an amylose impurity. Assuming the impurity as  $\sim 9\%$ , the F1 could be lowered to  $\sim 17\%$ , similar to the B90 amylopectin content. The proportions of F3 were 60–70% and 89–90% on weight and molar bases, respectively, indicating that F3 is the major chain size of all the components. The ratio F3/F2 of each amylopectin was similar to that of each ML-fraction, while the B90 components had a higher ratio than the W64A components. Very similar proportions on a molar basis were observed for all the components, due to the B90 components having larger  $\bar{c}_1$  of F1 and smaller  $\bar{c}_1$  of F2 than the W64A components. In other words, the B90 amylopectin has larger long B-chains (F1) and smaller intermediate B-chains (F2) than the W64A amylopectin. The ML-components are high molecular-weight amylopectins and differ from intermediate fractions of high-amylose maizes previously reported<sup>38</sup>. The reason why the ML-components are a loose layer in the fractionation of starch is unknown. The B90 amylopectin differed from amylo maize amylopectins, which comprised higher and lower amounts of F2 and F3, respectively<sup>24</sup>. The W64A amylopectin resembled the WD amylopectin in chain-length distribution.

The amylopectin with a higher i.a. contained higher weight proportion of F1 as observed for the rice amylopectins<sup>21</sup>. The waxy maize amylopectin having a low i.a. almost lacks<sup>15,16,24</sup> F1, and the B90 amylopectin having a high i.a. have high amounts of F1. The normal maize amylopectins from WD and W64A are intermediate in F1 content. The long B-chains of the B90 amylopectin appear to be poorly branched, and thus they complex with iodine. This was supported by lower  $\lambda_{\max}$  and BV of the beta-limit dextrins compared with their parent components and the higher values of the B90 dextrins than those of the W64A dextrins (Table IV).

The normal maize of W64A contained the multiple forms of branching enzymes, BE I, IIa, and IIb<sup>28</sup>. On amylose branching, BE I rapidly reduced long chains with gradual increase of intermediate and short chains, whereas both BE IIa and IIb gradually decreased the long chains and rapidly produced the short chains<sup>39</sup>. The activity balance of these different types of the enzymes may determine the structure of maize amylopectins. The B90 amylopectin is comprised of larger long B-chains and shorter intermediate B-chains than is the W64A amylopectin. This

TABLE IV

The blue value and  $\lambda_{\max}^a$  of beta-limit dextrins from the amylopectins and ML-components

Origin of beta-limit dextrin	Amylopectin		ML-Component	
	B90	W64	B90	W64A
Blue value (BV)	0.19	0.12	0.25	0.11
$\lambda_{\max}$ (nm)	558	528	579	529

<sup>a</sup> Calculated from the blue value and  $\lambda_{\max}$  of the isoamylolyzates.

TABLE V

Properties of the B90 and W64A starches<sup>a</sup>

Starch	B90	W64A
I.a., g/100 g	8.8	4.6
Blue value (BV)	0.63	0.37
$\lambda_{\max}$ (nm)	632	614
Amylose content <sup>b</sup> (%) from		
I.a.	30.6 (44) <sup>c</sup>	18.5 (23) <sup>c</sup>
Blue value (BV)	32.2	16.9

<sup>a</sup> Defatted by repeated dissolution in Me<sub>2</sub>SO by heating and precipitation with ethanol. <sup>b</sup> Calculated by the equation ( $X = \text{i.a. or BV}$ ):  $[(X_{\text{starch}} - X_{\text{amylopectin}})/(X_{\text{amylose}} - X_{\text{amylopectin}})] \times 100$ . <sup>c</sup> Calculated by the equation:  $(X_{\text{starch}}/X_{\text{amylose}}) \times 100$ , where  $X_{\text{amylose}}$  was assumed to be 20.

might indicate that the B90 maize contains a lower ratio of the activities (BE I)/(BE IIa + BE IIb) than the W64A maize.

*Properties and amylose content of starch.*—The B90 starch showed a higher i.a., BV, and  $\lambda_{\max}$  than the W64A starch (Table V). The W64A starch had a slightly lower i.a. than that of the WD starch<sup>4</sup> (5.18 g/100 g). The apparent amylose contents of the B90 and W64A starches were calculated as 44 and 23%, respectively, on the basis of assumed amylose i.a. (20 g/100 g), without consideration of the amylopectin i.a. The value for B90 was a little higher than the published value. Apparent contents for *sugary-2* maize [40%, (variety, not indicated)<sup>40</sup> and 43.3% (Oh43)<sup>41</sup>] and that for W64A was lower than those observed for the same variety<sup>42,43</sup> (25.4 and 29.1%). The reasons for these discrepancies are unknown but may be due to the published values being determined by a colorimetric method using different standards<sup>44,45</sup>. The actual amylose contents were calculated by considering the i.a. or BV of the respective amylose and amylopectin, and the contents from the i.a. were found to be 30.6 and 18.5% for B90 and W64A, respectively, similar to the contents calculated from the BV. The B90 starch amylose content is between the amylo maize<sup>8</sup> (36–59%) and normal (W64A and WD<sup>4</sup> 20%) starches. The higher apparent content is due to a higher amount of long chains binding with iodine in the amylopectins.

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