Some Properties of Starches of Grain Amaranths and Several Millets

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Summary Starch granules were prepared from seeds of Amaranthus hypochondriacus L., A. caudatus L., proso millet, Japanese barnyard millet and foxtail millet. Amylose contents and the distribution of ϕ-1,4 linked chain of amylopectin were determined by gel filtration of isoamylase-debranched starches. Some physical and chemical properties of the starches were also examined by scanning electron microscopy, X-ray diffractometry, photopastegraphy, and differential scanning calorimetry, together with starch-granule susceptibility to amylases. The existence of both normal and waxy types in the same species of a grain amaranth, A. hypochondriacus L., was confirmed. A. caudatus starches were identified to consist of mainly typical amylopectin and 5-7% amylose. The starches have some unique properties, namely, high starch-granule susceptibility values to amylases as well as those of A. hypochondriacus and unique pasting properties.

Key Words properties of starch, grain amaranth starch, millet starch, amylose content, characteristics of amylopectin, Amaranthus hypochondriacus L., Amaranthus caudatus L., proso millet, Japanese barnyard millet, foxtail millet

Both waxy (wx) and glutinous (gl) mutants and their normal or non-glutinous counterparts have been identified in each one of the species of barley (Hordeum vulgare L.), foxtail millet (Setaria italica Beauv.), maize (Zea mays L.), proso millet (Panicum miliaceum L.), rice (Oryza sativa L.) and sorghum (Sorghum bicolor (L.) Moench) among cultivated Gramineae (1). These mutants produce starch granules in the endosperm and the pollen which stain red with iodine and which contain nearly...
100% amylopectin, whereas starch granules of their normal counterparts contain both amylose and amylopectin and stain blue with iodine.

We identified that there are both normal and waxy types in the same species of a grain amaranth, *Amaranthus hypochondriacus* L., which belongs to Amaranthaceae (2). Starch granules are stored in the perisperm of their seeds. The species is considered to be native to Central America (3, 4), has been cultivated as a minor grain crop in Nepal (5) and Mexico (6), and has many synonyms (for example, *A. flavus* L., *A. frumentaceus* Buch.-Hamilt. ex Roxb., *A. anardana* Buch.-Hamilt., *A. hybridius* L. (var.) γ erythrostachys Moq-Tand, *A. leucocarpus* S. Wats, and *A. leucospermus* S. Wats.) (4).

To confirm and extend the previous work (2), starch granules were prepared from seeds of two strains of *A. hypochondriacus* L., three strains of *A. caudatus* L., three strains of proso millet, one strain of Japanese barnyard millet (*Echinochloa utilis* Ohwi et Yabuno) and two strains of glutinous foxtail millet. Amylose contents and the distribution of α-1,4 chains of amylopectin were determined by gel filtration of isoamylase-debranched starches. Some physical and chemical properties of the starches were also examined by scanning electron microscopy (SEM), X-ray diffractometry, photopasteography, and differential scanning calorimetry (DSC), together with evaluation of starch-granule susceptibility to amylases.

**MATERIALS AND METHODS**

*Seeds.* Seeds of *A. hypochondriacus* #76338 and 76345, and *A. caudatus* #76325, 76327 and 76330 were collected in the Langtang area of Nepal in 1975 (2). Seeds of proso millet SGK-110 were collected in Afghanistan in 1978, and proso millet #76373 and 76382 were collected in Nara Prefecture, Japan in 1976, by Sakamoto. These seeds were grown in a glass-house of the Plant Germ-plasm Institute, Kyoto University, in 1976. Seeds of Japanese barnyard millet and two strains of glutinous foxtail millet, one having black seed coat and the other yellow, cultivated at a home farm of Ōtō-son, Nara in 1980 were kindly provided by Mr. and Mrs. Osamu Ebisudani, Shinohara, Ōtō-son, Nara Prefecture, Japan.

*Starch granules.* Starch granules were isolated from the seeds by a modification of the method of MacMasters et al. (7) combined with Schoch’s method (8) as described earlier (2). About 10 g each of the seeds of grain amaranths and proso millet except #76382 (about 2 g of seeds) and about 20 g each of the seeds of the other millets were used for preparation of starch granules. Normal maize starch granules were a commercial product and kindly provided by Dr. Taizo Miwa, Director, Central Research Laboratories, Nihon Shokuhin Kako Co., Ltd., Fuji, Japan.

*Sources of enzymes.* Crystalline *Pseudomonas* isoamylase [EC 3.2.1.68]: Hayashibara Biochemical Laboratories, Inc., Okayama, Japan. Pancreatin: Type VI-A from hog pancreas, Sigma Co., U.S.A. Crystalline *Rhizopus niveus* glucoamylase [EC 3.4.1.3]: Commercial grade, Seikagaku Kogyo Co., Ltd., Tokyo.

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Japan. Sources of glucose oxidase and peroxidase for glucose determination were reported previously (9).

*Gel filtration of isoamylase-debranched starches.* Procedures for debranching of starches by *Pseudomonas* isoamylase and fractionation of debranched starches on a Sephadex G-75 column were described earlier (10).

*Analytical methods.* Absorption curves of starch-iodine complexes between 500 and 700 nm were recorded by a Hitachi 220-type recording spectrophotometer on mixtures containing 1 mg of starch, 2 mg of iodine and 20 mg of potassium iodide per 25 ml (11). Starch-granule susceptibility to pancreatin was studied by the method reported previously (9). Starch granules were exhaustively degraded by crystalline glucoamylase by the method described earlier (10). Carbohydrate determination by the phenol-sulfuric acid method and glucose determination by the colorimetric glucose oxidase-peroxidase method were reported previously (9, 10).

*Preparation of specimens for SEM, specimen mounting and SEM.* Procedures for specimen preparations, specimen mounting and SEM were described earlier (12–14).

*X-ray diffractometry.* X-ray diffraction diagrams of fully moistured starch granules were recorded by a spectrodiffractometer (JDX-7E, Nihon Denshi Co., Ltd., Tokyo, Japan) by Hizukuri’s method (15), or briefly, by the use of CuKα (Ni filtered), voltage, 35 kV; electric current, 12.5 mA; time constant, 4 sec; scanning speed of goniometer, 1°/min; and chart speed, 10 mm/min.

*Photopastigraphy.* Photopastegrams of starches were recorded by the method of Kainuma et al. (16, 17) using a Hirama photopastegraph, Hirama Rikagaku Kenkyusho, Kawasaki, Japan, on starch suspension (0.10% for maize starch, 0.05 and 0.03% for starches of the grain amaranths, and 0.05% for the other starches at 372 nm).

*Differential scanning calorimetry.* Thermograms were recorded by a thermal analyzer, DSC, catalog No. 8058, Rigaku Denki Co., Ltd., Tokyo, Japan. Starches (a definite dry-weight between 4 and 5 mg) were weighed and two times (w/w) the distilled water was added by a microsyringe in aluminum round-shaped pans (5 × 2.5 mm). Samples were heated in hermetically sealed pans from 30 to 100°C at the rate of 5°C/min. Chart speed, 10 mm/min and instrument sensitivity, ±1 mcal/sec.

**RESULTS**

*Size and shape of starch granules*

The size and shape of starch granules deposited in the perisperm or endosperm of seeds and isolated from the seeds were observed by SEM. Fine starch granules of the grain amaranths were round or polygonal about 1 μm in diameter. The size and shape were very similar to those reported previously (2) and reported in other papers (7, 18, 19). Millet starches were polygonal with about 6–7 μm diameter for proso millet and about 10 μm for Japanese barnyard and foxtail millets. The size and shape were in good agreement with those reported by Kawakami (20).
X-ray diffraction diagrams

X-ray diffraction diagrams obtained from starches of the grain amaranths corresponded to A-type crystalline structure which is typical of starches usually found in grains (Fig. 1b–1f). The diagrams for the millet starches were also of A-type similar to that of the normal maize starch, although the diagrams are omitted.

Absorption spectra of starch-iodine complexes

The iodine-absorption spectra of starches of proso millet SGK-110, A. hypochondriacus #76345-5, Japanese barnyard millet and normal maize revealed absorption maxima for these starches at 584–605 nm showing they are of normal type (Figs. 2a–2d). The waxy-type starches of proso millet #76382, foxtail millet, A. hypochondriacus #76338-2, and proso millet #76373 showed an absorption maximum at 521–529 nm (Fig. 2h–2j). Starches of A. caudatus had an absorption maximum at a longer wavelength (540–544 nm) than the waxy-type starches and the former had curve heights at 680 nm, about twice as high as the latter (Figs. 2e–2g). The results strongly suggest that A. caudatus starches consist of either amylopectin

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Fig. 2. Absorption spectra of starch-iodine complexes. a, proso millet SGK-110; b, *Amaranthus hypochondriacus* #76345-5; c, normal maize; d, Japanese barnyard millet; e, *A. caudatus* #76330-2; f, *A. caudatus* #76327-1; g, *A. caudatus* #76325-3; h, proso millet #76382; i, foxtail millet (yellow and black) and *A. hypochondriacus* #76338-2; and j, proso millet #76373.

Fig. 3. Elution patterns on a Sephadex G-75 column of isoamylase-debranched starches of several grain amaranths. a, *Amaranthus hypochondriacus* #76345-5; b, *A. hypochondriacus* #76338-2; c, *A. caudatus* #76330-2; d, *A. caudatus* #76327-1; and e, *A. caudatus* #76325-3.
Fig. 4. Elution patterns on a Sephadex G-75 column of several cereal starches debranched by isoamylase. a, proso millet #76382; b, proso millet #76373; c, proso millet SGK-110; d, Japanese barnyard millet; e, foxtail millet, yellow; and f, foxtail millet, black.

and a slight amount of amylose or amylopectin having longer linear chains, as reported for amylopectin of a double-mutant maize having *amylose extender waxy* (*ae wx*) genes (10).

**Elution profiles of isoamylase-debranched starches by gel filtration**

Figures 3 and 4 show elution patterns of debranched starches of *A. hypochondriacus* (Figs. 3a and 3b), *A. caudatus* (Figs. 3c–3e), proso millet (Figs. 4a–4c), Japanese barnyard millet (Fig. 4d), and foxtail millet (Figs. 4e and 4f). The contents of fraction I (Fr. I, amylose) were about 22% for *A. hypochondriacus* #76345-5, 28% for proso millet SGK-110, 26% for Japanese barnyard millet, 0% for *A. hypochondriacus* #76338-2, proso millet #76373 and foxtail millet, and 1% for proso millet #76382, and 5–7% for *A. caudatus* (Table 1). There was no significant difference apparent in the ratios of fraction III (Fr. III) to fraction II (Fr. II) among starches examined. These results indicate that *A. hypochondriacus* #76345-5 starch is of the normal-type, #76338-2 starch is of the waxy-type, *A. caudatus* starches consist of mainly amylopectin and small amounts of amylose, and all of the starches examined have typical amylopectin nearly identical to that of rice and maize starch.

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Table 1. Summary of some properties of isoamylase-debranched components of several cereal starches.a

| Cereal Starch          | Fr. I (%) | Intermediate Fr. (%) | Fr. II (%) | Fr. III (%) | Fr. III/ Fr. II |
|------------------------|-----------|----------------------|------------|-------------|-----------------|
| *Amaranthus hypochondriacus* |           |                      |            |             |                 |
| #76345-5               | 21.8      | 4.7                  | 19.3       | 54.2        | 2.8             |
| #76338-2               | 0         | 3.0                  | 24.3       | 72.7        | 3.0             |
| *A. caudatus*          |           |                      |            |             |                 |
| #76325-3               | 5.0       | 3.4                  | 20.1       | 71.5        | 3.6             |
| #76327-1               | 5.1       | 2.5                  | 20.1       | 72.3        | 3.6             |
| #76330-2               | 6.9       | 2.4                  | 18.9       | 71.8        | 3.8             |
| Proso millet           |           |                      |            |             |                 |
| SGK-110                | 28.4      | 3.7                  | 14.5       | 53.4        | 3.7             |
| #76373                 | 0         | 1.5                  | 22.8       | 75.7        | 3.3             |
| #76382                 | 1.0       | 1.5                  | 20.8       | 76.7        | 3.7             |
| Japanese barnyard millet |       |                      |            |             |                 |
| Yellow                 | 25.6      | 2.3                  | 15.8       | 56.3        | 3.6             |
| Black                  | 0         | 2.3                  | 26.6       | 71.1        | 2.7             |
| Foxtail millet         |           |                      |            |             |                 |
| Yellow                 | 0         | 2.3                  | 26.6       | 71.1        | 2.7             |
| Black                  | 0         | 2.6                  | 20.5       | 76.9        | 3.8             |

a Each fraction (Fr.) was divided according to \( \lambda_{\text{max}} \) of carbohydrate-iodine complexes as follows: Fr. I, \( \lambda_{\text{max}} \geq 620 \text{ nm} \); intermediate Fr., \( 620 > \lambda_{\text{max}} \geq 600 \text{ nm} \); Fr. II, \( 600 > \lambda_{\text{max}} \geq 540 \text{ nm} \); Fr. III, \( 540 \text{ nm} > \lambda_{\text{max}} \).

Fig. 5. Photopasteographs of several starches. a: 1, *Amaranthus caudatus* #76325-3 (0.03%); 2, *A. caudatus* #76325-3 (0.05%); 3, *A. hypochondriacus* #76345-5 (0.03%); 4, *A. hypochondriacus* #76338-2 (0.05%); and 5, *A. hypochondriacus* #76345-5 (0.05%). b: 1, foxtail millet, yellow (0.05%); 2, proso millet #76382 (0.05%); 3, proso millet #76373 (0.05%); 4, foxtail millet, black (0.05%); 5, Japanese barnyard millet (0.05%); 6, proso millet SGK-110 (0.05%); and 7, normal maize (0.1%). Figures in parentheses were concentrations of the starch suspension used.

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Photopasteigrams of starches

Although the normal-type starches of Japanese barnyard millet, proso millet SGK-110 and maize showed typical two-step gelatinization curves (Figs. 5b 5–7), the waxy-type starches of proso and foxtail millets had one-step curves (Figs. 5b 1–4). Gelatinization profiles of starches of the grain amaranths (Figs. 5a 1–5) are very similar to those reported by Kainuma et al. (17) for fine starch granules such as those of Saponaria vaccaria as described earlier (2). Starches of A. caudatus #76325 exhibited the lowest temperature for initiation of gelatinization (Figs. 5a 1–2).

DSC thermograms of starches

Figure 6 shows the DSC thermograms of the grain amaranths and cereal starches. The gelatinization characteristics of starch in a DSC thermogram can be presented by various temperatures: the onset temperature ($T_o$); the peak temperature(s) ($T_p$); and the conclusion temperature ($T_c$). These temperatures and heat of gelatinization are summarized in Table 2. Linear regression analysis revealed that significant correlation between $T_o$ values and values of temperature for initiation of gelatinization estimated from photopasteigrams ($r=0.781$, $n=10$, $p<0.01$).

Fig. 6. DSC thermograms of several starches. 1, Amaranthus hypochondriacus #76338-2; 2, A. hypochondriacus #76345-5; 3, A. caudatus #76330-2; 4, A. caudatus #76327-1; 5, A. caudatus #76325-3; 6, proso millet #76373; 7, proso millet SGK-110; 8, Japanese barnyard millet; 9, foxtail millet, black; 10, foxtail millet, yellow. $T_o$, onset temperature and $T_c$, conclusion temperature were obtained by extrapolation as shown in thermogram 10. Heat of gelatinization were calculated by measuring the areas surrounded by thermogram curves and base lines.

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Table 2. DSC characteristics of several cereal starches.

| Starch-granule susceptibility to amylases |
|------------------------------------------|
|                                | $T_o$ (°C) | $T_p$ (°C) | $T_e$ (°C) | Heat of gelatinization (cal/g) |
|------------------------------------------|
| Amaranthus hypochondriacus               |
| #76345-5                                | 59         | 67         | 76         | 1.0                              |
| #76338-2                                | 63         | 70         | 78         | 2.5                              |
| Amaranthus caudatus                     |
| #76325-3                                | 53         | 57         | 63         | 1.2                              |
| #76327-1                                | 51         | 56         | 65         | 1.3                              |
| #76330-2                                | 51         | 56         | 64         | 1.6                              |
| Proso millet                            |
| SGK-110                                  | 69         | 74         | 78         | 2.3                              |
| #76373                                  | 62         | 67, 72     | 77         | 3.0                              |
| Japanese barnyard millet                |
| Yellow                                   | 68         | 72         | 80         | 3.7                              |
| Black                                    | 68         | 72         | 78         | 3.9                              |

DISCUSSION

We confirmed that there are both normal- and waxy-types in the same species of Amaranthaceae, *A. hypochondriacus* L., by the investigation of properties of starches isolated from the seeds (Table 1). Starch granules of *A. hypochondriacus* #76338-2 consist of nearly 100% typical amylepectin the same as those of #76343-1 reported previously (2). The widespread distribution of starches staining red with iodine has been recognized for the past century (J). However, their properties have not been fully investigated except those from the cereals such as maize, rice, sorghum and barley. Moreover, the existence of both normal- and waxy-types in the same species of cultivated grain crops which belong to a plant family other than...
Table 3. Susceptibility of starch granules of several cereals to pancreatin.

| Duration | Normal maize | Proso millet | SGK-110 | Japanese barnyard millet | Foxtail millet | Yellow Black |
|----------|--------------|--------------|---------|--------------------------|----------------|--------------|
| 30 min$^a$ | 19.2          | 28.9         | 51.6    | 22.8                     | 48.3           | 45.7         |
| Degradation (%) | 100           | 151          | 119      | 119                      | 119            | 119          |
| Relative susceptibility$^b$ | 100           | 102          | 102      | 102                      | 102            | 102          |
| 1 hr$^a$ | 31.9          | 51.6         | 61.9    | 51.6                     | 51.6           | 51.6         |
| Degradation (%) | 100          | 100          | 100      | 100                      | 100            | 100          |
| Relative susceptibility$^b$ | 100          | 100          | 100      | 100                      | 100            | 100          |
| 2 hr$^a$ | 47.2          | 48.3         | 78.0    | 43.1                     | 84.9           | 76.8         |
| Degradation (%) | 100          | 100          | 100      | 100                      | 100            | 100          |
| Relative susceptibility$^b$ | 100          | 100          | 100      | 100                      | 100            | 100          |

$^a$Duration of enzymatic reaction. $^b$Data were expressed relative to enzyme degradation percentage of commercial normal maize starch, namely % degradation of normal maize starch equals 100.

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Fig. 7. Time courses of hydrolysis of starches by crystalline glucoamylase. 1, *Amaranthus hypochondriacus* #76345-5; 2, *A. hypochondriacus* #76338-2; 3, proso millet #76373; 4, foxtail millet (yellow and black); 5, *A. caudatus* #76325-3; 6, Japanese barnyard millet; 7, proso millet SGK-110. Reaction was achieved at 37°C, and reaction mixture contained 40 mg (on dry weight basis) of starch granules, 3.0 ml of 0.4 M acetate buffer, pH 4.8, and 1.0 ml of crystalline glucoamylase solution (*R. niveus*, 12.5 U/ml). The same glucoamylase solution (0.5 ml) was added at intervals, 6, 24, 48, 72 and 94 hr.

Gramineae is an infrequent case. Amylose content (22%) of the normal-type starch of *A. hypochondriacus* #76345-5 was higher than that (14%) of #76339-2 described earlier (2). The difference in apparent amylose content may involve both cultivation and environmental effects.

We identified that *A. caudatus* starches consist mainly of typical amylopectin and small amounts (5–7%) of amylose (Table 1). Starch granules from maize, sorghum and rice kernels homozygous for wx have been reported to have from 0 to 6% of amylose (22). This apparent amylose content may be due to the measurement technique used, to the effect of non-waxy starch granules from maternal tissue, to differences in the degree of branching, or to the presence of some linear materials as described by Shannon and Garwood (22). A small percentage of amylose usually was present in samples of commercially milled waxy maize starch (2), and this was due to contamination by normal starch, as was readily observed by light microscopy (1). When iodine was added, the presence of a few deep-blue granules among those staining reddish-brown was observed. However, the amylose content of *A. caudatus* starches was not due to contamination. None of the granules stained deep blue or deep brown, but all appeared to be stained reddish-brown and contain small amounts of high molecular-weight linear materials, *i.e.* amylose (Table 1 and Figs. 3c–3e). These results suggest that *A. caudatus* may have a wx perisperm gene similar to the wx⁴ endosperm gene which is found in Argentine waxy maize and reported to deposit starch having 2–5% amylose (23, 24).

Recently, the seeds of *Amaranthus edulis*, which is considered to be of the same
species as *A. caudatus*, have been brought to attention, because very favorable essential amino acid composition of the Australian stock of *A. edulis* seeds was appraised by chemical analysis and in feeding experiments with chick and rats (25, 26). Now, we revealed some unique properties of *A. caudatus* starch, namely, higher starch-granule susceptibility values to amylases as well as those of *A. hypochondriacus* (Fig. 7 and Table 2 in Ref. 2) and pasting properties (Fig. 5 and Table 2). These results may indicate that seeds of the grain amaranths are potentially valuable sources for food, feed and fuel.

Several millets, for example, foxtail, Japanese barnyard and proso millets, and finger millets (*Eleusine coracana* Gaertn.), are grain crops under relict cultivation in Japan. They have been cultivated by diverse and scattered peoples since prehistoric times and were especially important grain crops under barnyard or shifting cultivation. Foxtail and proso millets have been reported to have both glutinous and non-glutinous endosperm and sometimes intermediate ones, while finger and Japanese barnyard millets have just one type, non-glutinous endosperm indicated using the iodine-staining method of the tissue. However, properties of their starches have not been fully investigated in the light of the present knowledge of starch chemistry and biochemistry.

We identified that amylose contents of proso millet starches are 28% of the normal, 0% of the waxy and 1% of the intermediate type and all of the starches have typical amylopectin (Fig. 4 and Table 1). Thus, cereals, especially the so-called intermediate types of cereal grains by the iodine-staining method may have to be re-examined for properties of their starches.

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