HIDEAKI ANDO[1], NAOYOSHI INOUCHI[1], MASAKO ASAOKA[1],
KAZUTOSHI OKUNO[2], HIDETSUGU FUWA[1]

THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON DISTRIBUTION OF SHORT CHAINS OF RICE AMYLOPECTIN

Abstract

The effects of the environmental temperature during the early development of seeds on the characteristics of the endosperm starch were investigated using near- isogenic lines of rice plants grown under temperature controlled conditions. The components of starch and pasting characteristics of starch granules varied by the environmental temperature. The lower temperature increased the amylose content and the ratio of short chain to long chain of amylopectin. Moreover, the starch granules of rice plants grown under the lower temperature were more gelatinized than those grown under the higher temperature. By high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) of *Pseudomonas*-isoamylase-debranched amylopectins we found the amylopectin of Taichung 65 (waxy) grown under the lower temperature (25°C) has decreased amounts of chains with degree of polymerization (DP) of 8 and 9, and slightly increased amounts of chains with DP of 6, 11, 12 and 13 compared with the amylopectin of the same near-isogenic plants grown under the higher temperature (30°C).

Introduction

The amylose content, distribution of α-1,4 chains of amylopectin, and some properties of starch granules in the cereal endosperm are affected by the endosperm mutation and the environmental condition. Environmental effects on properties of endosperm starch are, in general, not as much as effects associated with genetic factors, such as species and varieties.

The effects of the environmental temperature,at the milky stage on properties of endosperm starch in rice have been studied. We showed that the lower temperature (25°C) during the filling period of rice grains increased the amylose content of endosperm starches of non-mutant [1-4] and mutants [4]. In this connection Sano et al. [5] reported that the starch-granule bound *Wx* protein level in the rice endosperm related to the amylose contents was affected by the environmental temperature. Moreover, the

[1] *Department of Food Science and Technology, Fukuyama University, Higashimuracho, Fukuyama 729-02, Japan*
[2] *National Institute for Agricultural Sciences, Tsukuba, Ibaraki 305, Japan*

amylose of rice plants grown at the lower temperature in either the growth chambers or the paddy field gave higher weight-average degree of polymerization (DPw) values than those of plants grown at higher temperature [6]. We also showed that the lower temperature decreased the amount of long B chains of amylopectin and increased that of short B chains, as compared with the higher temperature (30°C) [3, 7]. The pasting characteristics of the starch granules were also affected by the environmental temperature under which the rice plants were grown. Namely, the onset and conclusion temperatures of gelatinization and heats of gelatinization determined by differential scanning calorimetry (DSC) of the endosperm starch from the rice plants grown under the lower temperature were lower than those starch from the plants grown under the higher temperature [1-4, 6, 7].

This paper deals with the determination of structure characteristics by high performance liquid chromatography (HPLC) and high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) of isoamylase-debranched amylopectins of near-isogenic rice plants grown under temperature conto rolled conditions (25°C and 30°C) after heading to elucidate detailed chain-length distributions of endosperm starches of rice plants grown under the different environmental conditions.

Materials and methods

Experimental plants

Near isogenic lines of Taichung 65 for the waxy (*wx*) locus were used. The rice plants having spikelets which flowered within two days after heading were grown in a temperature-controlled growth chamber. The temperature conditions during the grain filling period are shown in Table 1. Heat summation during the grain filling period was 1,000°C. The light intensity for the 12-hr-daylength was about 40,000 lux.

Table 1

Temperature Conditions for Rice Plants (Cultivar; Taichung 65, waxy) during the Grain Filing Period

Group I	Temperature condition	Group II	Temperature condition
I-1	25°C 5 days 30°C 29 days	II-1	30°C 5 days 25°C 34 days
I-2	25°C 10 days 30°C 25 days	II-2	30°C 10 days 25°C 28 days
I-3	25°C 15 days 30°C 21 days	II-3	30°C 15 days 25°C 28 days
I-4	25°C 20 days 30°C 17 days	II-4	30°C 20 days 25°C 22 days
I-5	25°C 40 days	II-5	30°C 34 days

Preparation of rice starch granules

Starch granules were prepared from milled rice by the cold and dilute alkali method of Yamamoto et al. [8, 9].

High performance liquid chromatography (HPLC) of isoamylase-debranched amylopectin

Gelatinized amylopectin (2 8 mg) in 3. 5 ml of pure water at 100°C for 6 min was added 100 µl of 1 M acetate buffer (pH 3. 5) and 10 µl of *Pseudomonas* isoamylase (10 µg/10 µl, 590 units/µg protein), and incubated at 45°C for 2.5 h. The reaction mixture was added 200 µl of 0.1 N sodium hydroxide solution, 1.0 ml of 0.5M phosphate buffer (pH 8.5)-0.1 % sodium azide solution and 190 µl of pure water, and filtered through a 0.22 µm filter (Millipore). The filtrate (1.0 ml) was subjected to a HPLC apparatus (LS-8000, Tosoh Co. Ltd., Tokyo, Japan) with 5 columns; TSKgel G3000PWxr, (7.6x300 mm, Tosoh), Asahipak GS-320H (7.6x250 mm, Asahi Kasei Co. Ltd., 'Kanagawa, Japan)x2, TSKgel G2500PWxL, (7.6x300 mm, Tosoh), and TSKgel G-Oligo PW (7.6x300 mm, Tosoh). Detectors were a differential refractometer (RI-8011, Tosoh) and a low-angle laser light scattering photometer (LS-8000, Tosoh) with an interface (IF-8000, Tosoh) and a data processor (software, PC- LALLS vol. 1.03). Chromatograms were eluted with 0.1 M phosphate buffer (pH 8.5)-0.02 % sodium azide solution at 40°C with a flow rate, 0.5 ml/min.

High performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) of isoamylase-debranched amylopectin

Gelatinized amylopectin (5 mg) in 4.69 ml of pure water at 100°C for 6 min was added 1 00 µl of 1 M acetate buffer (pH 3. 5) and 10 µl *Pseudomonas*-isoamylase (10 µg/10 µl, 590 units/µg protein), and incubated at 45°C for 2.5 h. The reaction mixture was added into 200 µl of 0.1 N sodium hydroxide solution and filtered through a 0.22 µm filter (Millipore). The concentration of the filtrate used for analysis was 0.5 mg/ml.

Chain-length distribution was determined by a Dionex model DX-300 system (Dionex Corp., Sunnyvale, CA, USA) and a Model SC-PAD II pulsed amperometric detector consisting of an amperometric flow-through cell with a gold working electrode, a silver-silver reference electrode, and potentiostat according to the method described by Koizumi et al. [10-12] with a minor modification. Briefly, the system was equipped with a Dionex Carbopac PAl column (4x250 mm) in combination with a Carbopac PAl Gurad column (4x15 mm). Repeating sequences of potentials (volts) and durations (ms) on the PAD were as follows: E_1 0.10 (t_1 300), E_2 0.60 (t_2 120), E_3

-0.80 (t_3 300). The sample-injection loop size was 50 µl. Results were recorded on a SC 8020 integrator (Tosoh).

Eluent A was 0.15 M NaOH, and eluent B was 0.15 M NaOH containing 0.5 M sodium acetate. The gradient program was as follows; 40 % of eluent B at 0 min, 50 % at 5 min, 60 % at 20 min, 70 % at 26 min, and 80 % at 40 min. All separations were carried out at ambient temperature with a flow rate of 1 ml/min.

The degree of polymerization (DP) of oligomers was assigned by spiking samples with maltohexaose and standard response curves were prepared by using a mixture of malto-oligosaccharides (Fuji-oligo G67, Nihon Shokuhin Kako Co. Ltd., Fuji, Japan).

The individual peak area obtained from chromatograms of the isoamylase- debranched amylopectin was corrected by dividing the relative detector response. Using the corrected peak area, the exact distributions of chain length (6-17) of amylopectins were obtained [11].

Other method

The procedure for differential scanning calorimetry (DSC) was described earlier [13].

Results and discussion

Effects of environmental temperature on chain-length distribution of amylopectins by HPLC

Rice starch samples under study consisted of nearly 100 % amylopectin as shown by HPCL of iso- amylase-debranched materials. Table 2 shows some of their HPLC characteristics. The amylopectin of group I-1, group II-2, -3, -4 and -5 have decreased

Table 2

HPLC Characteristics of Isoamylase-debranched Materials Obtained from Endosperm Starches of Rice Plants Grown at Different Temperature after Pollination (Cultivar; Taichung 65, waxy)

Group I	BL %	BS + A %	BS + A / BL	Group II	BL %	BS + A %	BS + A / BL
I-1	28.6	71.4	2.5	II-1	26.2	73.8	2.8
I-2	27.1	72.9	2.7	II-2	28.5	71.6	2.5
I-3	26.1	73.9	2.8	II-3	29.1	70.9	2.4
I-4	25.6	74.4	2.9	II-4	29.3	70.7	2.4
I-5	25.8	74.2	2.9	II-5	30.2	69.8	2.3

BL – long chains, BS – short B chains, A – A chains.

amounts of long B chains and increased amounts of short chains as compared with the amylopectin of the group I-2, -3, -4 and -5, and group II-I. Accordingly, the ratio of short chains to long B chains for the amylopectin affected lower temperature (25°C) and higher temperature (30°C) are in a range from 2.7 to 2.9 and from 2.3 to 2.5, respectively.

The development stage, when the chain-length distribution of rice amylopectin was affected by the environmental temperature, was 5-15 days after anthesis (Table 2) and the same as the stage which was the most effective on determination of the amylose content in the endosperm starch in rice seeds [3, 7].

Effects of environmental temperature on short chain-length distribution of amylopectins by HPAEC-PAD

To elucidate detailed distributions of the amylopectin short chain-length, isoamylase-debranched materials of the starch obtained from the near-isogenic waxy rice plants grown under temperatue controlled conditions (25°C and 30°C) by HPAEC-PAD (Figs. 1 and 2).

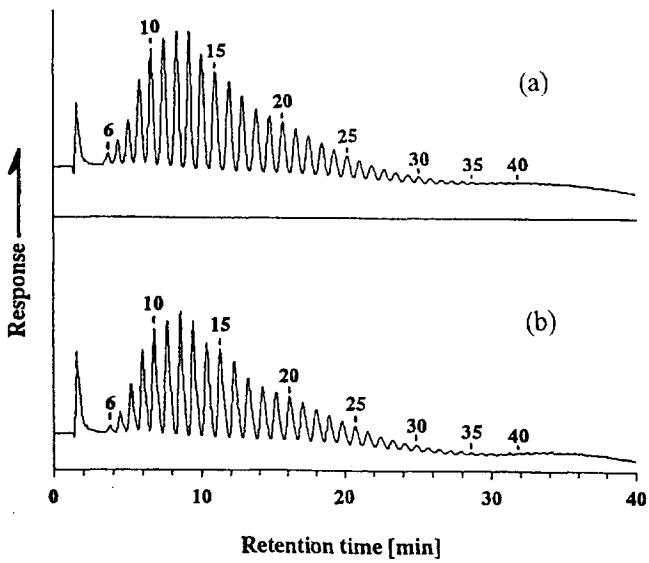

Fig. 1. HPAEC-PAD traces for isoamylase-debranched materials of starches obtained from rice plants grown under different temperature conditions after anthesis.
(a) group I-4; and (b) group II-4.

The amylopectin of Taichung 65 (*wx*) grown at 25°C has decreased amounts of chains with DP of 8 and 9, and slightly increased amounts of chains with DP of 6, 11, 12, 13 compared with the amylopectin of the same near-isogenic plants grown at 30°C.

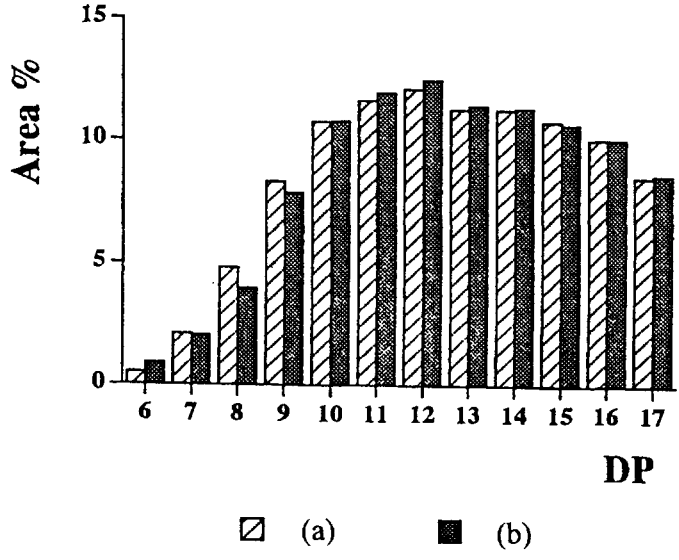

Fig. 2. Chain-length distributions of debranched amylopectins of rice plants grown under different temperature conditions after authesis.
(a) group I-5; and (b) group II-5.

Effects of environmental temperature on DSC characteristics of rice starches

As shown in Table 3, starch granules of the groups I-1, and group II-2, -3, -4 and -5 have higher: onset (T_o) and conclusion (T_c) temperatures and heats of gelatinization (OH) determined by DSC as compared to the groups I-2, -3, -4 and -5, and II-1.

Table 3

DSC Characteristics of Endosperm Starches of Rice Plants (Cultivar; Taichung 65, waxy) Grown at Different Temperature after Pollination

Group I	T_o [°C]	T_p [°C]	T_c [°C]	ΔH [J/g]	Group II	T_o [°C]	T_p [°C]	T_c [°C]	ΔH [J/g]
I-1	64	70	84	14.6	II-1	60	66	80	10.9
I-2	60	–	82	10.0	II-2	64	70	85	12.6
I-3	59	–	81	11.3	II-3	65	71	85	14.2
I-4	61	64, 72	78	11.3	II-4	65	72, 78	85	15.1
I-5	60	66, 73	80	10.0	II-5	65	72	84	14.2

T_o, onset, T_p, peak, and T_c, conclusion temperatures of gelatinization,
ΔH, heat of gelatinization.

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WPŁYW TEMPERATURY OTOCZENIA NA ROZMIESZCZENIE KRÓTKICH ŁAŃCUCHÓW W AMYLOPEKTYNIE RYŻOWEJ

Streszczenie

Na podstawie blisko izogenicznych linii ryżu wzrastającego w kontrolowanej temperaturze zbadano wpływ temperatury otoczenia na charakterystykę skrobi w endospermie ziaren w okresie wczesnego wzrostu. W okresie tym wraz z temperaturą otoczenia zmieniały się składowe skrobi i charakterystyka kleikowania gałeczek skrobiowych. W niższych temperaturach wzrasta zawartość amylozy i stosunek liczby łańcuchów krótkich do długich w amylopektynie. Ponadto gałeczki skrobiowe ryżu wzrastającego w niższej temperaturze kleikowały lepiej niż te wzrastające w temperaturze wyższej. Posługując się wysokosprawną chromatografią anionowymienną z pulsacyjnym detektorem amperometrycznym (HPAEC-PAD) w badaniach amylopektyn, w których odcięto łańcuchy boczne izoamylazą *Pseudomonas* stwierdzono, że amylopektyna woskowego Taichung 65 rosnącego w niższej temperaturze (25°C) zawierała mniej łańcuchów o stopniu polimeryzacji (DP) 8 i 9 i nieco więcej łańcuchów o DP 6, 11, 12 i 13 w porównaniu z amylopektyną z tych samych roślin blisko izogenicznych wzrastających w wyższej temperaturze (30°C).