YOSHINORI UTSUMI*, D. V. GLOVER**, NAOYOSHI INOUCHI*, HIDETSUGU FUWA*

STRUCTURE AND THERMAL PROPERTIES OF STARCHES OF ENDOSPERMS POSSESSING DIFFERENT ALLELES AT THE AMYLOSE-EXTENDER (*ae*) LOCI IN MAIZE

Summary

TSK gel permeation chromatography of non-granular starches, amylopectin chain-length distributions measured by HPAEC-PAD, and DSC characteristics of starches of maize endosperms possessing different alleles at the amylose-extender (ae) loci were studied. GPC of non-granular starches through Toyopearl columns showed that elution profiles for 5 ae mutants, Oh43 inbred line ae (standard ae), ae-RWB-2 and ae-RWB-3, and W23xL317 hybrid line ae-PP and ae-Bol 561, were similar to a commercial ae starch, Hylon VII and different from Hylon ae starch and normal maize starches. The elution profile for W23xL317 ae-emll was similar to Hylon and different from Hylon and normal maize starches. HPAEC-PAD of isoamylase-debranched starches showed that the 5 ae mutants were uniquely ae type similar to Hylon VII and different from Hylon V. W23xL137 ae-emll had the amylopectin chain-length distribution similar to Hylon . Gelatinization temperatures (Tp) of the ae starches measured by a Setaram Micro DSC III were high compared with the normal counterpart starches except for Oh43 ae-RWB-1 starch. Oh43 ae-RWB-1 starch had structure and thermal characteristics similar to the normal maize starch.

Introduction

Recently, there has been increasing interest in modifying the starch composition and content in plants, producing novel starches, from the standpoints of fundamental and applied researches [1-4]. For the purpose evaluation of genetic resources for the novel starch is very important as well as creation of new mutants producing the starch by biotechnology and genetic engineering.

Several maize endosperm genes are known to have mutant alleles at the some loci, for example, the amylose-extender (ae), waxy (wx), and sugary-1 (su-1) genes. A

*Department of Applied Biological Science, Fukuyama University, Fukuyama 729-0292, Japan
**Department of Agronomy, Purdue University, West Lafayette, IN 47907-1150 USA

potential exists for kernels homozygous for any one of these alleles to have a starch with unique structure and physicochemcial properties. We reported that starches of six ae mutants, Oh43 inbred line ae (standard ae), ae-RWB-2 and ae-RWB-3, and W23xL317 hybrid line ae-PP, ae-Bol 561 and ae-emll were uniquely ae type by B type X-ray diffractograms and high gelatinization temperatures by differential scanning calorimetry (DSC), together with poor starch-granule digestibility to amylase, and high apparent amylose (37-45%) and high intermediate fraction (13-18%) contents and low ratios (about 1) of long chains to short chains of amylopectin determined by gel permeation chromatography (GPC) of isoamylase-debranched starches [5].

This paper describes TSK gel permeation chromatography of non-granular starches, the amylopectin chain-length distribution measured by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) and thermal properties measured by high performance DSC of starches obtained from mature kernels of standard ae, ae-RWB-1, ae-RWB-2 and ae-RWB-3 mutants in Oh43 inbred line of maize (Zea mays L.), and ae-PP, ae-Bol 561, and ae-emll mutants in a maize hybrid W23xL317, and their normal counterparts.

Materials and methods

Maize Seeds and Preparation of Starches

Several independently occurring alleles at the ae locus were used. Namely, ae–RWB-1, ae–RWB-2, and ae–RWB-3 are different mutational events found several years ago by R. W. Briggs (formally of Funk Seeds International, Inc.) in different hybrid backgrounds by chemical mutagenesis. The ae-emll allele is a mutation found by D.V. Glover by inducing with the Ds-Ac controlling element system. The ae-PP and ae-Bol 561 alleles are ae mutational events in the Pastrostrum Pearl and Bolivian strains of maize, respectively. All of these ae alleles are allelic to the standard ae locus (unpublished data, D.V. Glover). An inbred Oh43, and a hybrid, W23xL317, served as the basis for development of near-isogenic series, ae–RWB-1, ae–RWB-2, and ae–RWB-3, and ae-PP, ae-Bol 561, and ae-emll, respectively. Maize materials of single mutants and their normal counterparts were grown at the Purdue Agronomy Research Center.

Endosperm starches were prepared from mature maize kernels according to the Schoch's method [6]. Commercial ae starches, Hylon V (ae V) and Hylon VII (ae VII) (National Starch and Chemical Company, Bridgewater, NJ, USA) and a commercial normal maize starch were gift from Nihon Shokuhin Kako Company, Fuji, Japan.

General Methods

GPC of non-granular starch (starch samples gelatinized in 1 N NaOH at 5°C for overnight) were performed through 4 TSK gel columns connected in series, Toyopearl HW75S (300×20 mm)×2, Toyopearl HW65S (300×20 mm), and Toyopearl HW55S (300×20 mm). The method for recording absorption spectra of starch-iodine complexes and the phenol-sulfuric acid method for carbohydrate determination were reported previously [5]. The procedure for Dionex chromatography (HPAEC-PAD) were reported earlier [7, 8] except for the following minor change in the procedure. Namely, PAD-SC cell was used instead of PAD-standard cell and 0.1 M $NaNO_3$ was used in the elution solution instead of 0.5 M CH_3COONa. The starch samples (10–15 mg) were solubilized in 100 µl of 90% (v/v) DMSO (H_2O) at 100°C for 10 min. Differential scanning calorimetry (DSC) has been described elsewhere [8, 9].

Results and discussion

TSK gel permeation chromatography of ae maize starches and normal maize starch

Figure 1A and 1B show elution profiles of non-granular starches through Toyopearl columns. Elution profiles for the Oh43 and commercial normal maize starches were similar ones started at the elution volume (EV) 120 ml with the first fraction (in general, amylopectin) near the void volume with iodine absorption λ_{max} 550 nm followed by the second fraction (in general, amylose) with iodine absorption λ_{max} 650 nm at the peak. These size exclusion patterns were similar to those for rice [10] and maize [11] normal starches through Sepharose CL-2B columns. The elution profiles for the ae starches through Toyopearl columns were different from those of the normal maize starches. Namely the profiles started with EV 125 ml and over with the first fraction with iodine absorption λ_{max} 560 nm followed by the second fraction with iodine absorption λ_{max} 630–640 nm at the peak. These size exclusion patterns were different from those for the normal maize starches, moreover, different from those for ae starches through Sepharose column [11]. The results suggest smaller molecular sizes for the ae amylopectins comparing with the normal amylopectins. The elusion profiles for 5 ae mutants, Oh43 ae, ae-RWB-2 and ae-RWB-3, and W23xL317 ae-PP and ae-Bol 561, were similar to ae and different from ae V and normal maize starches. The elution profile for W23xL317 ae-emII was similar to ae and different from ae and normal maize starches.

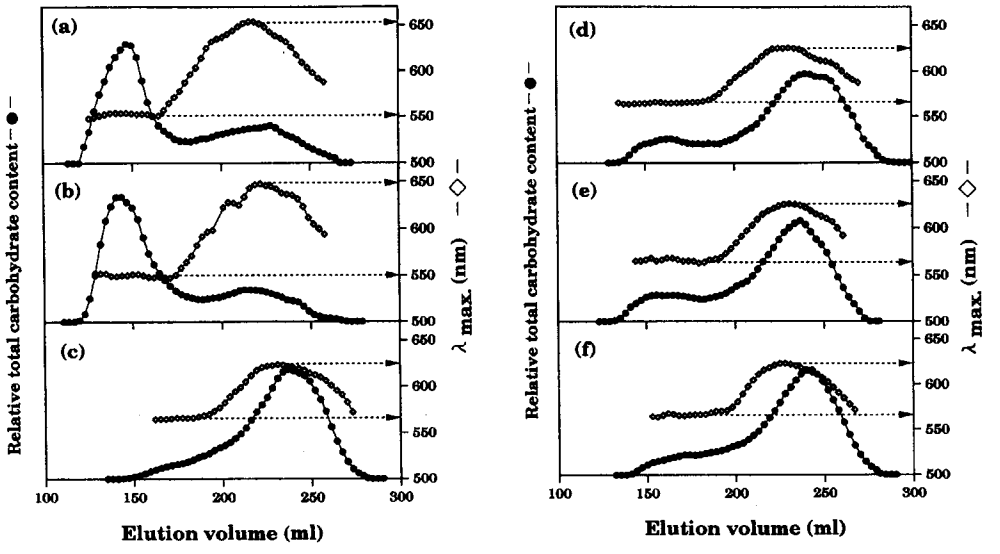

Fig. 1a. Elution profiles of ae starches and normal maize starches separated by TSK gel permeation chromatography: (a) Oh43 normal, (b) Commercial normal maize, (c) Hylon VII, (d) Oh43 ae, (e) Oh43 ae-RWB-2, and (f) Oh43 ae-RWB-3.

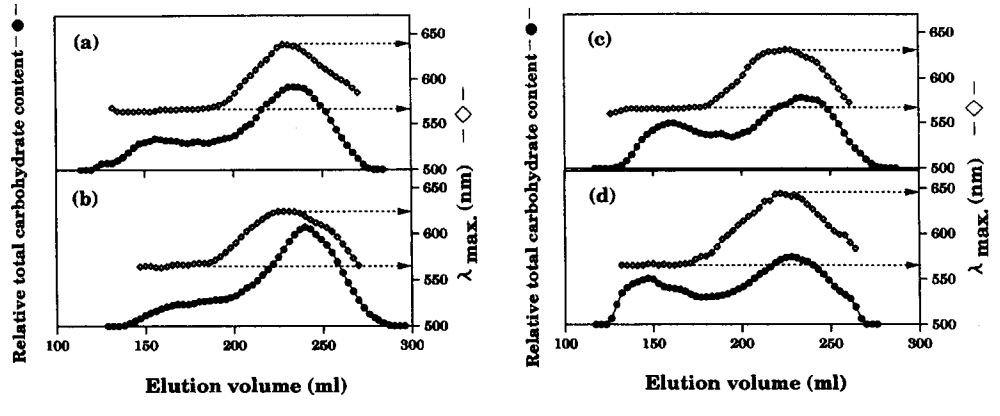

Fig. 1b. Elution profiles of ae starches and normal maize starches separated by TSK gel permeation chromatography: (a) W23 x L317 ae-PP, (b) W23 x L317 ae-Bol 561, (c) W23 x L317 ae-emll, (d) Hylon V.

Amylopectin chain-length distributions measured by HPAEC-PAD of isoamylase-debranched materials of ae maize starches

Amylopectin chain-length distributions were measured by HPAEC-PAD of isoamylase-debranched starches. We calculated the peak area percentage of each unit chains with DP 6–48 for total area percentage assigned to unit chains with DP 6–48 as

100%. The ae starch had decreased amounts of chains with DP 9-17 and increasing amounts of chains with DP 20 and over compared with a commercial normal maize starch. Fig. 2 shows comparison of unit chain-length distributions of isoamylase-debranched 10 sample maize starches to the ae starch. The starches of 5 ae mutants, Oh43 ae, ae-RWB-2 and ae-RWB-3, and W23xL317 ae-PP and ae-Bol 561 were uniquely ae type similar to ae starch, and different from ae starch. W23xL317 ae-emll had the amylopectin chain-length distribution similar to aeV. Mature kernels of Oh43 ae-RWB-1 mutant showed tarnished and translucent phenotype characteristics of the ae genotype but contained endosperm starch having 21–22% of apparent amylose which was lower than that of Oh43 normal counterpart [5]. The ae-RWB-1 starch showed similar amylopectin chain-length distribution to the normal counterpart.

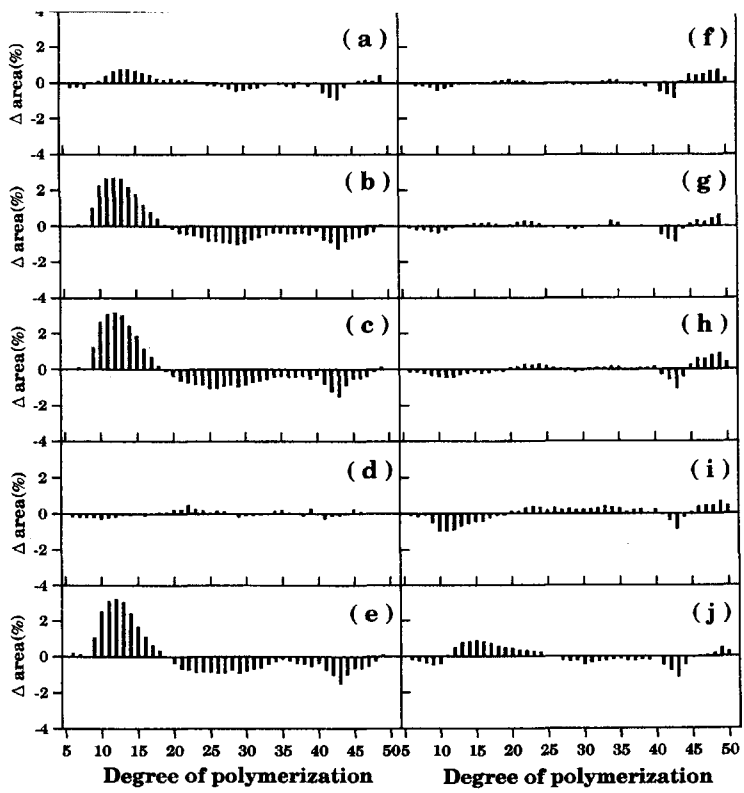

Fig. 2. Differences in chain-length distributions measured by HPAEC-PAD of isoamylase-debranched starches of ae mutants and their normal counterparts of maize with comparison to Hylon VII starch: (a) Hylon V, (b) Oh43 normal, (c) W23 x L317 normal, (d) Oh43 ae, (e) Oh43 ae-RWB-2, (f) Oh43 ae-RWB-3, (g) W23 x L3317 ae-PP, (h) W23 x L317 ae-Bol 561, and (i) W23 x L317 ae-emll.

DSC characteristics of ae maize starches

Fig. 3 and Table 1 show DSC characteristics of the ae starches and their normal counterparts. DSC thermograms for the normal counterparts and Oh43 ae-RWB-1 starches shown in Fig. 3 had 2 separate endothermic peaks, namely native starch gelatinization peak (68–72°C) and amylose-lipid complex melting peak (105°C), however, those for other ae starches had incomplete separation of endothermic peaks. Accordingly, we compared top temperatures of gelatinization (Tp) for these starches shown in Table 1. The ae starches except for Oh43 ae-RWB-1 had higher Tp (78–88°C) compared with their normal counterparts as reported previously [5]. The Oh43 ae-RWB-1 starch had similar thermal properties to those of the Oh43 normal. Thermal properties of retrograded starches of the ae starches are shown in Fig. 4. It was very difficult to estimate Tp from the DSC thermograms for the retrograded ae starches except for Oh43 ae-RWB-1. Accordingly, we could not compare thermal properties of the retrograded ae starches with those of the retrograded normal counterparts. Here again the retrograded Oh43 ae-RWB-1 starch had similar thermal properties to those of the retrograded Oh43 normal counterpart. DSC characteristics of starches were affected by amylopectin properties and not by amylose contents [11, 12].

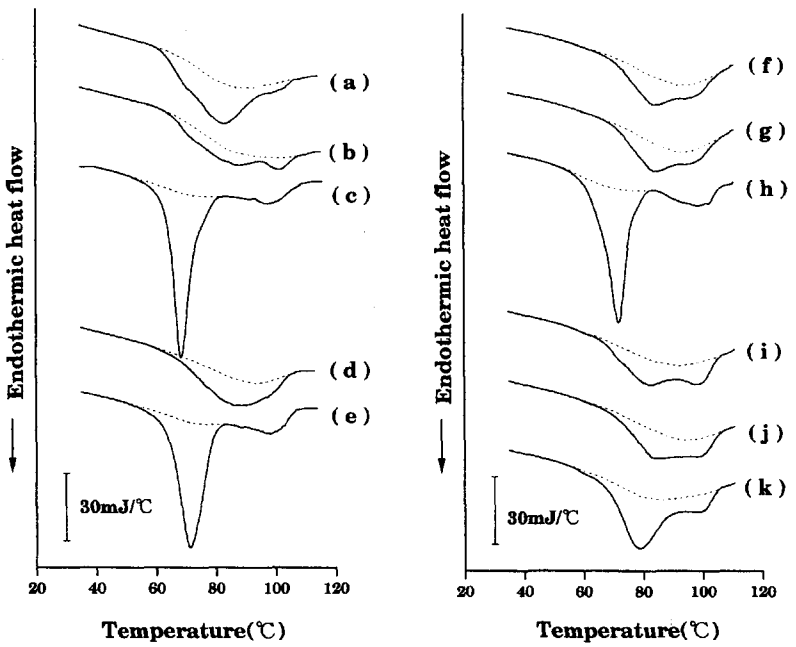

Fig. 3. DSC thermograms of native starches of ae mutants and their normal counterparts of maize: (a) Defatted Hylon V, (b) Defatted Hylon VII, (c) Oh43 normal, (d) Oh43 ae, (e) Oh43 ae-RWB-1, (f) Oh43 ae-RWB-2, (g) Oh43 ae-RWB-3, (h) W23 x L317 normal, (i) W23 x L317 ae-PP, (j) W23 x L317 ae-Bol 561, and (k) W23 x L317 ae-emll.

Table 1

Peak temperatures of gela.tinization *(Tp)* measured by DSC of *ae* and normal maize starches.

Starch sample	Tp (°C)
Nylon V *(ae V)*	81.8
Nylon VII *(ae* VII)	87.6
Normal maize	68.7
Oh43 *ae*	87.8
Oh43 *ae* - RWB-1	71.3
Oh43 *ae* - RWB-1	84.1
Oh43 *ae* - RWB-1	83.3
W23 x L317 normal	72.0
W23 x L317 *ae* - PP	81.7
W23 x L317.*ae* - Bo1.561	84.1
W23 x L317 *ae* - emll	78.0

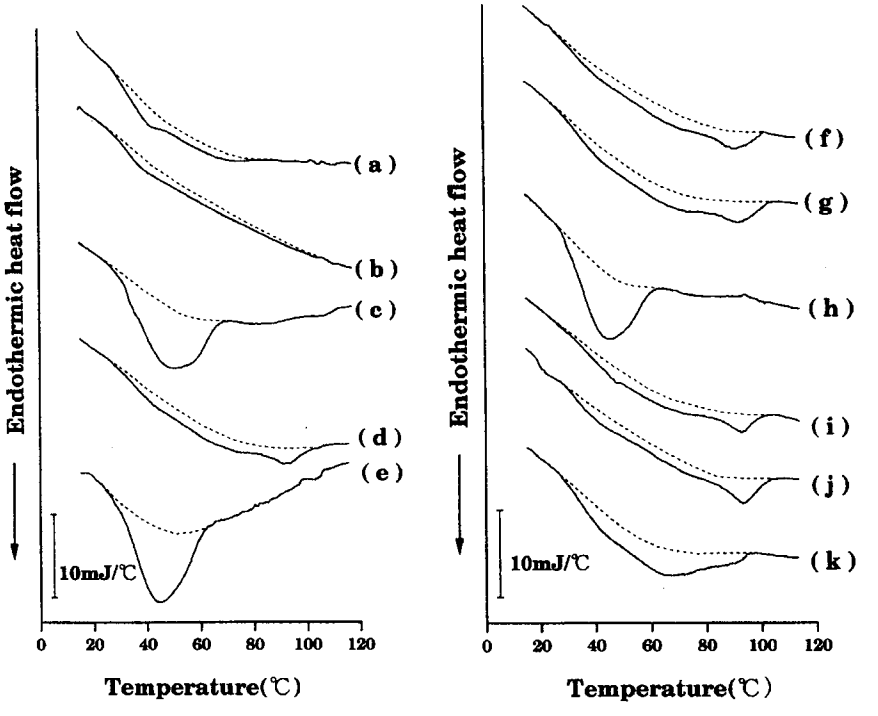

Fig. 4. DSC thermograms of retrograded starches of ae mutants and their normal counterparts of maize: (a) Defatted Hylon V, (b) Defatted Hylon VII, (c) Oh43 normal, (d) Oh43 ae, (e) Oh43 ae-RWB-1, (f) Oh43 ae-RWB-2, (g) Oh43 ae-RWB-3, (h) W23 x L317 normal, (i) W23 x L317 ae-PP, (j) W23 x L317 ae-Bol 561, and (k) W23 x L317 ae-emll.

Conclusions

GPC of non-granular starches showed that elution profiles for 5 ae mutants, Oh43 ae, ae-RWB-2 and ae-RWB-3, and W23xL317 ae-PP and ae-Bol 561, were similar to Hylon and different from Hylon V and normal maize starches. The elution profile for W23xL317 ae-emll was similar to Hylon and different from Hylon and normal maize starches.

HPAEC-PAD of isoamylase-debranched starches showed that 5 ae mutants, Oh43 ae, ae-RWB-2 and ae-RWB-3, and W23xL317 ae-PP and ae-Bol 561 were uniquely ae type similar to Hylon, and W23xL317 ae-emll had the amylopectin chain-length distribution similar to Hylon V.

Gelatinization temperatures (Tp) measured by DSC of the ae starches were high compared with the normal counterpart starches.

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STRUKTURA I WŁAŚCIWOŚCI TERMICZNE SKROBI KUKURYDZIANYCH Z ENDOSPERM POSIADAJĄCYCH RÓŻNE ALLELE W ae-loci

Streszczenie

Skrobie nieziarniste badano za pomocą żelowej chromatografii podziałowej (TSK), zaś udział długich łańcuchów w amylopektynie oznaczono przy użyciu wysokosprawnej chromatografii cieczowej, a skaningową kalorymetrię różnicową wykorzystano do scharakteryzowania skrobi z endosperm ziaren kukurydy, posiadających różne allele w ae-loci. Żelowa chromatografia podziałowa nieziarnistych skrobi na kolumnach Toyopearl pokazała, że profile elucyjne pięciu mutantów ae tj. skrobi z linii ae Oh43 (standard ae), ae-RWB-2 i ae-RWB-3, linia hybrydowa ae-Ppi ae-Bo 561 W23xL317 były podobne do tychże w han-

dlowej skrobi ae Hylon VII i różne od tychże w skrobi Hylon VII i normalnych skrobi kukurydzianych. Wysokosprawna chromatografia cieczowa skrobi pozbawionych odgałęzień za pomocą izoamylazy pokazała, że pięć mutantów ae było wyjątkowo podobnych do skrobi Hylon VII i zarazem różnych od tychże w skrobi Hylon V. W23xL137 ae-emll miała rozkład długich łańcuchów podobny to amylopektyny ze skrobi Hylon V. Temperatura żelowania (T_p) skrobi ae mierzone za pomocą mikrokalorymetru Setram Micro DSC III była wyższe od tychże normalnych skrobi, wyjąwszy skrobię Oh43 ae-RWB-1. Ta ostatnia skrobia miała budowę i termiczną charakterystykę podobne do normalnych skrobi kukurydzianych.