Variation in Starch Granule Size Distribution and Amylose Content During Wheat Endosperm Development

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ABSTRACT

This study confirmed that the amylose content of endosperm starch increases during grain development in wheat. Changes in the percent amylose content of endosperm starch granules separated into different size classes were then followed over the developmental period. At all stages of development, the amylose content of the smaller granules, expressed as a percentage of their total starch content, increased as starch granule size increased. Amylose content of the other separated size classes also increased somewhat in the later stages of development. This was most marked with those less than 7 μm in diameter. The largest granules (25–30 μm) accounted for only 0.4% of total granule numbers at 14 days after anthesis but accounted for about 3.7% by 56 days. At all stages of development, more than 78% of the granules were less than 10 μm.

Grain development in barley (Banks et al 1973), wheat (Matheson 1971), and maize (Boyer et al 1976) appears to be characterized by a steady increase in the amount of amylose synthesized, relative to the total amount of starch. In developing sween corn (Duffus and Jennings 1978), this is considered to be due both to changes in the relative numbers of the different sizes of starch granules and to changes in their amylose content. A similar situation may exist in developing barley endosperm (Williams and Duffus 1977), where the starch composition of small and large starch granules varies independently during development. In starch granules from mature maize (Boyer et al 1976) and barley (Goering and De Haas 1974) and from immature sweet corn (Duffus and Jennings 1978) and barley (Williams and Duffus 1977) endosperms, amylose content decreases as granule size decreases. However, in wheat at maturity (Bathgate and Palmer 1972) there was little difference in amylose content between large and small granules.

This article discusses the changes in starch granule composition and in relative numbers of the different size classes in relation to the reported (Matheson 1971) increase in amylose content during wheat endosperm maturation.

MATERIALS AND METHODS

Plant Material

Wheat (*Triticum aestivum* L.) var. Maris Huntsman was grown under commercial conditions on the University of Edinburgh farms. Ears were harvested at 14, 21, 25, 32, 40, 49, and 56 days after anthesis and stored at −18° C until use.

Fractionation of Starch Granules

The outer layers of pericarp and the embryo were removed from intact grains. The resulting endosperms were then suspended in ice-cold 20% (w/v) sucrose solution. The number of endosperms used varied from 20 in 4.0 ml of 20% sucrose at 14 days after anthesis to 5 in 40 ml at 56 days. Endosperms were homogenized manually in a Pottertype all-glass, ice-cooled homogenizer. After filtration though two layers of muslin, the filtrate was centrifuged at 10,000 × *g* for 10 min. The pellet, referred to as an unfractionated pellet, consisted of starch granules of all sizes. It was resuspended in 2.0 ml of 20% sucrose, and 1.0 ml of this sucrose was layered on top of two discontinuous sucrose gradients, each consisting of 2.0 ml each of 70, 65, and 50% sucrose in a 15-ml polypropylene centrifuge tube. This was centrifuged for 10 min at 500 × *g* in a swing-out head. After centrifugation, the starch granules could be seen as thin white layers concentrated at the interfaces and additionally as a pellet at the bottom. The layers were removed using a Pasteur pipette. The two pellets were resuspended in 1.0 ml each of 70% sucrose, layered on two further gradients of 2.0 ml each of 90, 85, 80, and 75% sucrose, and centrifuged as above.

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The layers between 50 and 65% sucrose and between 65 and 70% sucrose were combined (size class 1). Similarly, the layer between 70 and 75% sucrose is size class 2; that between 75 and 80%, size class 3; those between 80 and 85% and between 85 and 90%, size class 4; and the combined pellets, size class 5.

After dilution with an equal volume of distilled water and sedimentation of the starch granules by centrifugation, the pellets were washed four times in ice-cold distilled water followed by centrifugation. This removed all soluble carbohydrates. Because the time between sampling and obtaining the initial washed pellet generally was not more than 20 min and because all operations were performed at about 2–4° C, any starch degradation by amylase contamination was assumed to be minimal. No increase in reducing sugar content of washed fractions was observed with time.

Fractions were combined in order to reduce the numbers for subsequent analysis. Final fractions were not a quantitative recovery of all starch granules of a single size but were representative of all starch granules in that size range.

Starch Granule Number

The method was a modification (Duffus and Jennings 1978) of that described by May and Buttrose (1959). Only granules stained with iodine were counted.

Starch Granule Size

Starch granules were stained with iodine and examined under a magnification of 1,000 diameters. Measurements of size were made with an ocular micrometer. Samples contained between 1.7×10^3 starch granules (14 days) and 3.5×10^3 starch granules (49 days).

α-Glucan and Amylose Determinations

Total α-glucan was determined in starch granule fractions using the anthrone-sulfuric acid reagent (McCready et al 1950) and amylose by the method of Gilbert and Spragg (1964). Amylose content was determined by reference to a standard curve using pure amylose. All results were averages of three separate experiments, that included duplicate amylose and total α-glucan determinations.

Scanning Electron Microscopy

Starch granule fractions and an unfractionated pellet from wheat endosperm 27 days after anthesis were prepared for scanning electron microscopy. After dehydration in an acetone series, the granules were mounted on stubs by means of double-sided sticky tape, then coated with gold, and examined in a Cambridge stereoscan electron microscope.

RESULTS AND DISCUSSION

The amylose content of the unfractionated starch granules increased steadily over the developmental period (Table I), thus confirming previous results (Banks et al 1973, Boyer 1976, Matheson 1971).

Starch granules from the sucrose gradients increased in size as the sucrose density increased. Dimensions of all size classes are shown in Table II. A range of sizes rather than a mean diameter is given in order to emphasize that the fractions are not uniform. Some overlap was observed between size classes 1 and 2. Significant numbers of granules were obtained in each size class throughout the development period (Table III), indicating that the two types of granules exist in a continuous range of sizes in wheat.

Scanning electron micrographs (Fig. 1) showed that the starch granules were undamaged in the isolation procedure and that each size fraction fell broadly within the size ranges as estimated by light microscopy. The smallest size fraction (Fig. 1B) contained a mixture of spherical (A-type) and small lenticular (B-type) granules. These B-type granules may be precursors of the large granules more clearly seen in size class 5 (Fig. 1F). This suggests that pure fractions of the A-type granules may be impossible to obtain.

The amylose content of the smaller starch granules was less than that of the larger granules at all stages of development (Table II), confirming previous results (Boyer et al 1976, Duffus and Jennings 1978, Goering and De Haas 1974, Williams and Duffus 1977). This finding is not surprising, at least at early stages, because the small starch granules are presumably synthesized first and therefore account for the major amount of polysaccharide present at that time. In wheat, barley, and maize, the polysaccharide is presumably amylopectin.

The increase in amylose content of whole grain starch is clearly not caused by continuous change in the amylose content of granules with identical starch compositions but is caused by variation in the rates of appearance of granules with different starch compositions. As can be seen in Tables I and II, relatively more of the granules in size class 1 must account for an overall amylose content of 6% at 14 days, and relatively more of the granules in size class 5 must account for an overall amylose content

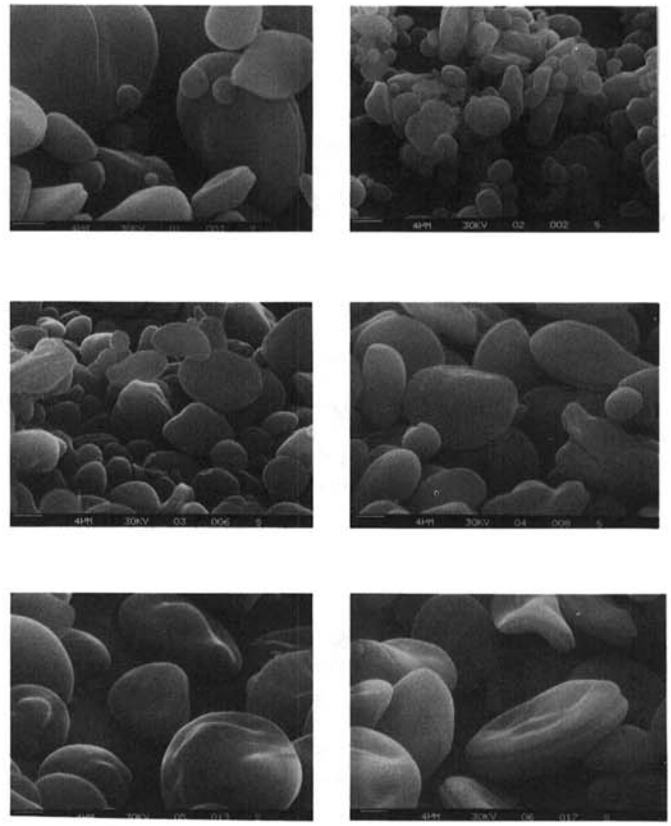

Fig. 1. Scanning electron micrographs of wheat endosperm starch granules at 27 days after anthesis, before and after separation on sucrose density gradients. Bar markers represent 4 μm. **A,** unfractionated pellet; **B,** size class 1; **C,** size class 2; **D,** size class 3; **E,** size class 4; **F,** size class 5.

TABLE I
Changes in Amylose Content of Unfractionated Starch Granules during Endosperm Development in Wheat[a]

Days After Anthesis	Amylose Content of Starch Granules (% w/w)	Total α-Glucan in Starch Granules (mg/endosperm)
14	6.0 ± 1.1	2.5 ± 0.5
21	8.5 ± 1.9	3.1 ± 0.9
25	10.2 ± 4.1	13.1 ± 3.5
32	13.7 ± 2.1	22.2 ± 1.6
40	18.3 ± 1.4	19.2 ± 1.4
49	22.8 ± 2.3	29.6 ± 2.0
56	28.3 ± 5.4	27.7 ± 2.5

[a]Results are the mean ± standard deviation.

TABLE II
Amylose Content as % (w/w) of total α-Glucan in Starch Granules of Different Size Classes during Endosperm Development in Wheat[a]

Size Class	Size Range	Age in Days After Anthesis						
	(μm)	14	21	25	32	40	49	56
1	2–7	11.5 ± 4.5	11.2 ± 1.6	11.5 ± 4.6	10.0 ± 3.8	11.2 ± 2.5	13.4 ± 4.1	17.8 ± 3.1
2	5–10	13.4 ± 7.1	14.5 ± 6.0	13.6 ± 6.7	14.9 ± 5.7	16.5 ± 2.9	14.5 ± 7.7	17.7 ± 1.9
3	10–15	21.1 ± 5.8	12.0 ± 3.1	12.3 ± 1.2	16.8 ± 2.1	19.3 ± 1.7	17.1 ± 1.9	23.8 ± 1.4
4	15–25	20.4 ± 6.8	21.6 ± 4.8	17.5 ± 0.2	17.5 ± 4.3	15.5 ± 3.3	22.0 + 8.5	28.2 ± 6.1
5	25–30	21.8 ± 7.5	25.4 ± 5.7	19.7 ± 1.3	22.6 ± 3.1	20.0 ± 0.2	22.7 ± 3.1	30.8 ± 7.3

[a]Results are the mean ± standard deviation.

TABLE III
Percentage of Total Numbers of Starch Granules in Each Size Class During Endosperm Development in Wheat[a]

Size Class	Size Range	Age in Days After Anthesis						
	(μm)	14	21	25	32	40	49	56
1	2–7	64.1 ± 3.9	76.0 ± 2.7	75.9 ± 5.2	68.0 ± 5.2	78.2 ± 2.2	77.7 ± 1.7	76.2 ± 2.6
2	5–10	14.1 ± 1.7	8.25 ± 1.8	8.6 ± 1.8	12.7 ± 2.3	13.6 ± 1.7	13.1 ± 1.5	11.7 ± 1.5
3	10–15	10.7 ± 1.8	6.0 ± 1.3	6.0 ± 0.9	7.4 ± 2.3	3.0 ± 1.0	3.3 ± 0.6	4.7 ± 0.9
4	15–25	10.6 ± 1.9	8.1 ± 1.1	7.4 ± 2.0	7.7 ± 1.6	3.5 ± 0.7	3.6 ± 0.7	3.5 ± 0.5
5	25–30	0.4 ± 0.2	1.6 ± 0.4	2.1 ± 0.8	4.1 ± 0.9	1.6 ± 0.5	2.4 ± 0.5	3.7 ± 1.1

[a]Results are the mean ± standard deviation.

of 28.3% at 56 days. Although the numbers in size class 5 do increase from 0.4 to 3.7% during the developmental period (Table III), the relative numbers in size class 1 also increase. The difference is made up by decreases in the relative numbers of granules in size classes 3 and 4.

At 14 days a major contribution to grain starch is made by the smallest granules, which have the lowest amylose content but account for over 64% of total numbers. In sweet corn, granules less than 1.4 μm account for about 70% of total numbers at this age (Duffus and Jennings 1978). The relative contributions of A-type (0–8 μm) and B-type granules (8–26 μm) to total starch weight in wheat var. Maris Huntsman at maturity vary according to grain size and degree of grain filling (Brocklehurst and Evers 1977), which are influenced by differences in rates and conditions of grain growth. In all types of grain, however, the percentage by weight of granules under 10 μm was about 40%. Certainly our results show clearly that the small granules make some contribution to total wheat starch because, at maturity, about 88% of the total number of granules is made up of those less than 10 μm in diameter. In sweet corn, however, the smallest granules contribute less than 4% of total starch but account for as much as 50% of total numbers (Duffus and Jennings 1978), and in barley (Bathgate and Palmer 1972) at maturity the small granules are 88% of total numbers but contribute only 11% of the starch. Although in maize, the small granules are thought to derive from younger cells (Shannon 1974), the results reported here are difficult to relate to their likely physiological origins because the smaller granules also may occur in large numbers in the interior and hence older cells of the endosperm (Sandstedt et al 1968).

In addition to progressive increase in amylose content as granule size increases, amylose content also may increase in the separated size classes as they mature. This is clearest in size classes 1 and 5 between 40 and 56 days after anthesis. The increasing amylose content of size class 1 as maturity approaches may account for Bathgate's observation (1972) that, composition differs little between granules divided into small and large fractions.

We conclude that the increase in percent amylose of endosperm starch relates mainly to changes in relative numbers of the different size classes present, which show increasing amylose content with increasing size. Changes in the amylose content within a size range, particularly size class 1 (A-type granules), also contribute to the overall increase in percent amylose content of total starch.

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