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CHARACTERISTICS OF STARCH IN DEVELOPING PEA SEEDS

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Abstract—Changes in the physical and chemical properties of pea starch in developing seeds have been investigated. Increase in the iodine-binding capacity of starch is due to an increase in the amount of the amylose component. The fine structure (average chain length and chain distribution) of the amylopectin molecules did not change throughout the growth period.

INTRODUCTION

Changes in composition, morphology and properties of starch at different stages of maturity have been reported for several cereal grains [1–8], potato tubers [9] and legume seeds [10, 11]. These studies showed that there is an increase in granule size and iodine affinity (I.A.) of the starch during seed development. The latter trend has often been attributed to an increase in the amylose content [7–11]. However, suggestions were made [1, 3] that such increase may reflect changes in the iodine-binding capacity of the amylose component *per se*. Furthermore, although three investigations, to date, have focused attention on changes in the fine structure of starch during growth [7, 9, 10] there have been no structural studies by employing the currently existing enzymic methods for the molecular characterization of α-D-glucans. The qualitative information obtained by using these techniques have important bearing on the mechanism of biosynthesis of these polysaccharides [12]. This report examines the changes in I.A. as well as the physical properties of pea starch during seed growth. In addition, the structures of isolated amylopectin fractions were investigated using hydrolytic enzymes and gel filtration chromatography.

RESULTS AND DISCUSSION

The gelatinization temperature of starch increased during seed growth (Table 1), implying the development of a more rigid granular structure at later stages of maturity. Such behaviour is not general among starches, since the gelatinization temperature of potato starch decreases towards maturity [9] and that of barley starch remains constant throughout development [7]. The values for the enthalpy changes associated with the gelatinization showed negligible differences among the three samples. Therefore, it is difficult to detect differences in the starch granule organization by simply considering the ΔH. Apparently, it has been suggested [13] that in addition to crystallite disruption other processes such as granule swelling and hydration of starch molecules during the order → disorder transition may also contribute to the enthalpy of the gelatinization process.

The I.A. of starch increased from 4.96 to 7.27 during seed development, confirming previous reports on cereal [2–7], tuber [9] and legume [10, 11] starches and thus indicating that a common biosynthetic trend exists for most starches. To investigate if this increase in I.A. is due to changes in the iodine-binding capacity of the amylose

Table 1. Characteristics of pea starch and its amylopectin fractions during seed growth

		Starch			Amylopectin		
Days after anthesis	Starch content of seeds (%)	Gelatinization (°)	Gelatinization enthalpy, ΔH (cal/g)	I.A.	I.A.	$\overline{CL}$	$P\beta_{+}^{\ddagger}$ (%)
17	20	58*57†	4.0	4.96	0.8	22	97
27	46	66*66†	3.8	6.89	1.3	22	96
46	48	70*68†	3.8	7.27	1.6	22	97

*Temperature at which 50% of the granules lose their birefringence.
†Peak temperature of the gelatinization endotherm.
‡ %β-Amylolysis of pullulanase-debranched amylopectins.

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or from increasing proportions of amylose to amylopectin, the MW distribution of the components of these samples was determined by gel chromatography. The elution profiles (Fig. 1) showed a very high and narrow exclusion peak and a lower curving section within the range of 0.4–1.0 K_{av}. Chromatography of waxy maize starch (*ca* 100% amylopectin) revealed only one peak at the V_0, indicating that amylopectin molecules are excluded from the Sepharose 2B and that no degradation took place during dispersion of granular starch with $HClO_4$. The retarded peaks in the chromatograms of Fig. 1 were comprised of the amylose components as suggested by the λ_{max} values (620–640 mm) of their iodine–polysaccharide complexes. Their elution patterns provided strong evidence that the amount of the amylose does in fact increase with seed maturity. An attempt to isolate quantitatively pure amyloses from these starches was not successful. Although the obtained fractions were pure (as assessed by both chromatography through Sepharose 2B and I.A. determination) their yields were only between 65 and 71% of the apparent amylose content of the corresponding starches. Nevertheless, the similar I.A. of these fractions (I.A.: 19.1, 18.9 and 18.7) suggested that the iodine-binding capacity of the amylose did not change significantly. The latter is also supported by the similar λ_{max} profiles of the polysaccharide–iodine complexes (Fig. 1) which implies that no major structural differences exist among the eluted amyloses. It appears, therefore, that the increase in I.A. of the whole starch, as the pea seeds mature, is due to an increase in the amount of amylose and is not due to major changes in the structure or molecular size of this starch fraction.

The Biogel P-10 elution profiles of the pullulanase-debranched amylopectin fractions (Fig. 2) revealed the

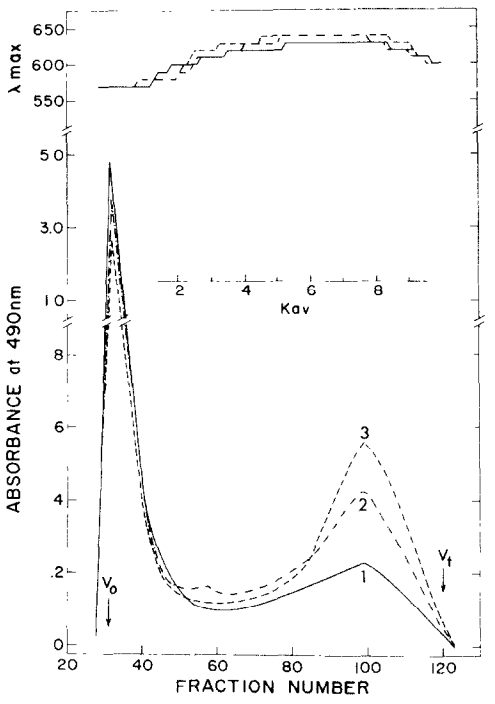

Fig. 1. Elution profiles of smooth pea starches on Sepharose 2B column (2.6 × 68 cm). 1, 2 and 3 correspond to samples harvested at 17, 27 and 46 days after anthesis.

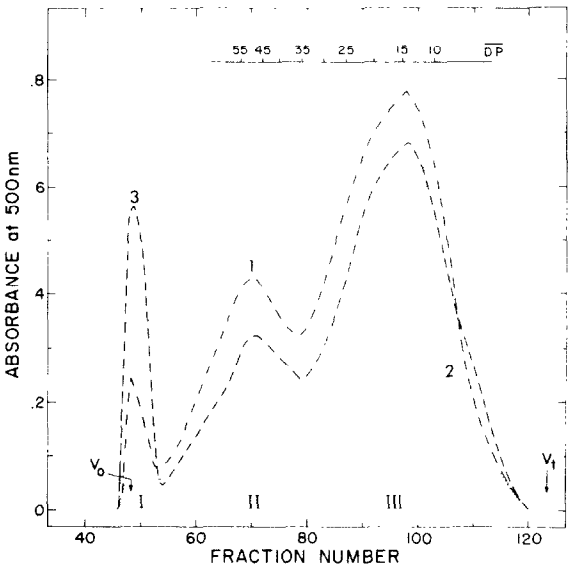

Fig. 2. Elution profiles of pullulanase-debranched amylopectins on Biogel P-10 column (2.6 × 94 cm). 1, 2 and 3 correspond to samples harvested at 17, 27 and 46 days after anthesis.

typical bimodal chain distribution (chains II and III with $\overline{DP}$ of 48 and 14, respectively) previous reported for cereal [14–17] and tuber [18] starches. Clusters of short III-chains have been suggested to constitute the crystalline areas of normal amylose and waxy type [17–19] of starches. Furthermore, their $\overline{DP}$ of 10–15 appears to be the minimum chain length requirement to allow formation of a stable double helix between two singly branched chains and render the complex susceptible to the multiple branching action of the Q-enzyme [20]. The gel-excluded carbohydrate material (I) which was verified to be linear by a further β-amylolysis (Table 1, Pβ column), must be present either as contaminant of the isolated amylopectins and/or may be liberated during debranching from the branched starch molecules (e.g. the long C-chain of the amylopectin [12]). The average $\overline{CL}$ (22), the chain-distributions (Fig. 2) and molar ratios of III-chains/II-chains (8.0–8.4) were similar for all three samples, indicating that the fine structure of these molecules did not substantially change along with seed maturity. These findings suggest that although there is a decrease in the percentage of amylopectin, the biosynthesis of the branched starch molecules is under the same enzymic control throughout seed development. It is likely that such control lies on special structural requirements of the amylopectin-synthesizing enzymes (Q-enzyme, starch synthetase and/or phosphorylase) in terms of both chain length and tertiary structure of precursor glucan molecules (e.g. the above-mentioned substrate specificity of Q-enzyme) so that a constant branching pattern is always maintained.

EXPERIMENTAL

Isolation, physical and chemical properties of starch. Smooth-seeded field peas (*Pisum sativum* var. Trapper) grown on field plots were harvested at 17, 27 and 46 days after onset of anthesis. Subsequently, they were freeze-dried and ground on a Udy mill.

Starch contents were determined as described in ref. [21]. Starch was isolated according to [10], using 0.01 M $HgCl_2$ to inhibit endogeneous amylolytic activity and then deproteinized with toluene (5% of aq. vol.). The purified starches were defatted with boiling aq. 80% MeOH. Calorimetric studies on starch gelatinization were carried out as described in ref. [22], using a Du Pont 990 differential scanning microcalorimeter. Gelatinization enthalpies (ΔH) correspond to the endothermic transitions of a 47% w/v starch–H_2O mixture heated at a rate of 5°/min. The obtained ΔH values had an average s.d. of ± 0.25 cal/g. Gelatinization temp. was determined from the peak temp. of the endotherm as well as according to ref. [23] by recording the temp. at which 50% of the granules lose their birefringence. Iodine affinities were measured by potentiometric titration at 30° ± 0.1° according to [24].

Chromatography of starches on Sepharose 2B. Starch samples (95 mg) were dispersed at 2° in 0.5 ml of 40% aq. $HClO_4$ [25] and diluted first with 5 ml NaOH (0.144% w/v) and then to 50 ml with distilled H_2O. Aliquots of 5 ml were applied to a Sepharose 2B (Pharmacia) (2.6 × 68 cm) and eluted with H_2O (4°) by the ascending method at a flow rate of 13 ml/hr. Collected fractions (3 ml) were analysed for total carbohydrates (PhOH–H_2SO_4 method, [26]) and λ_{max} of iodine–polysaccharide complexes as described in ref. [27]. K_{av} is defined as $(V_e - V_0)/(V_t - V_0)$, where V_e is the elution vol., V_0 is the exclusion vol. and V_t is the total vol. Recoveries from the column were within 93–97%.

Isolation and structural characterization of amylopectin fractions. Amylopectins were isolated from DMSO dispersed granules after selective precipitation of the amylose with thymol, according to [28]. Freeze-dried amylopectins (40 mg) were debranched with 32 I.U. of pullulanase (Hayashibara Biochem. Lab. Inc., Japan) in 5 ml solution (20% DMSO in 0.1 M NaOAc buffer, pH 5.5) at 37°, according to [14]. The debranched digests were heated in boiling H_2O to inactivate the enzyme and then analysed as follows: (a) total carbohydrates (30–250 mg/ml, 0.1 M NaOAc buffer, pH 4.8) by enzymic hydrolysis with 0.5 I.U. glucoamylase (Diazyme L-100, Miles Lab.) and subsequent glucose determination by the D-glucose oxidase–peroxidase method [29] using the Statzyme glucose reagent (Worthington Corp.); (b) total reducing power by the Nelson's reducing sugars method [30]; (c) β-amylolysis by adding 0.5 I.U. of barley β-amylase (Fluka, Buchs, Switzerland) to 1.0 ml polysaccharide soln (1–2 mg/ml, 0.1 M NaOAc buffer, pH 4.7) and expressing the amount of liberated maltose as % total carbohydrates [12]; and (d) gel chromatography on a Biogel P-10 (100–200 mesh, Bio-Rad Lab.) column (2.6 × 94 cm) eluted by the ascending method at 22° with 0.1 M NaOAc buffer, pH 4.8, containing 0.02% NaN_3 at a flow rate of 16 ml/hr. The average degree of polymerization ($\overline{DP}$) of each eluted fraction (4 ml) was determined by dividing the total carbohydrate concn by its reducing capacity [14]. The average chain length ($\overline{CL}$) of the amylopectins was determined using the equation [12]:

$$\overline{CL} = \frac{\text{Total carbohydrate (glucose)}}{\text{Reducing capacity of debranched digest (as glucose)}}.$$

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