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MACROMOLECULAR STRUCTURE OF WRINKLED- AND SMOOTH-PEA STARCH COMPONENTS*

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ABSTRACT

Starches from wrinkled and smooth pea (*Pisum sativum* L.) have been fractionated quantitatively, using successive thymol or 1-butanol complexation and differential ultracentrifugation. The fine structures of their components were determined by using sequential treatment with isoamylase and/or beta-amylase, followed by gel-permeation chromatography. Amylose (70.9%) and amylopectin (7.8%) of wrinkled-pea starch are similar structurally to their smooth-pea equivalents (33.2% and 64.7%, respectively). The average molecular weights were higher for the smooth-pea components (amylose: $\overline{M}_n$ 170,000, $\overline{M}_v$ 697,000; amylopectin: $\overline{M}_w$ 80.6 × 10^6) than for the wrinkled-pea components (amylose: $\overline{M}_n$ 125,000, $\overline{M}_v$ 430,000; amylopectin: $\overline{M}_w$ 19.4 × 10^6). An intermediate fraction (18.9%) of low molecular weight ($\overline{M}_n$ 25,100), isolated only from wrinkled-pea starch, was composed of the same short (S, d.p. 15) and long (L, d.p. 45) linear chains as amylopectin, but the ratio S/L was 3.6 in contrast to 9.6 for wrinkled-pea and 8.1 for smooth-pea amylopectins.

INTRODUCTION

A few starches, namely those of genetic variants of the pea, *Pisum sativum*[1], maize, *Zea mays*[2], and barley, *Hordeum vulgare*[3], have a high content of amylose in comparison with those of normal genotypes. Our recent work[4] on the behaviour of wrinkled-pea starch granules after mild hydrolysis with acid, such as lintnerisation, suggests that the crystalline phase of this starch could be related to the structures of the amylose and/or amylopectin components. When compared with their normal genotypes, these starches are characterised not only by their high content of amylose, but also by (*a*) longer branches in the amylopectin component[5,6], (*b*) short chains of the amylose components[7–9], and (*c*) intermediate material not fitting the definition of amylopectin or amylose[6,10]. The existence of these three components is not well recognised. These discrepancies are due mainly to the fact

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**Pisum sativum* and *Vicia faba* carbohydrates, Part V. For Part IV, see ref. 4.

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that fractionation of the starch into these macromolecules has been carried out by various investigators, using methods which were not always quantitative. In relation to biosynthesis[9,11] and granule structure[12], it is important to know the exact structures of the components of native starch.

We have investigated the macromolecular characteristics of the components of the starches from wrinkled- and smooth-pea seeds, using gel-permeation chromatography coupled with sequential enzymic hydrolysis[5,13,14], and the classical techniques of starch fractionation[15–17].

EXPERIMENTAL

Materials. — Starches from the smooth pea (*Pisum sativum* L., cv Frimas) and wrinkled pea (*Pisum sativum* L., cv Frogel) were purified as described previously[18]. Isoamylase (EC 3.2.1.68) of *P. amyloderamosa* (Hayashibara Biochemical Laboratories Inc.), sweet-potato beta-amylase (EC 3.2.1.2) (Koch–Light), *Aspergillus niger* amyloglucosidase (EC 3.2.1.3) (Merck), *A. niger* D-glucose oxidase (EC 1.1.3.4) (Grade II) and horse-radish peroxidase (EC 1.11.1.7) (Grade I) (Sigma), ABTS [2,2′-azino-di(3-ethylbenzothiazoline sulphonic acid)] (Boehringer Mannheim), Sepharose CL-2B, and Sephacryl S-200 and S-1000 (Pharmacia) were commercial materials. Industrial amylose (AVEBE, Veendam, The Netherlands) was used as the standard amylose. T.l.c. was performed on Silica Gel 60F 254 (Merck). All other reagents were of analytical grade.

Fractionation of starches. — Starch was fractionated into amylose, amylopectin, and intermediate material, using essentially the procedure of Banks and Greenwood[17]. Amylose was purified by five successive complex formations with thymol or 1-butanol. Solutions containing amylopectin and the intermediate material (corresponding to the supernatant after the first precipitation with thymol) were centrifuged[15] at 90,000*g* (34,000 r.p.m.) for 60 min at 20° (Beckman Ultracentrifuge SW 40 rotor). The supernatant liquid was removed down to the sharply defined interface, and the sediment was redissolved in water and recentrifuged as above.

Leaching of a 0.5% aqueous suspension of wrinkled-pea starch granules was conducted[19] for 60 min, at either 60°, 75°, or 90°.

All of the fractions were stored in a desiccator after freeze-drying.

Enzymic methods. — Debranching of polysaccharide was performed[13] in 40% (v/v) methyl sulfoxide–acetate buffer (50mM, pH 3.5). However, the digest contained a larger amount of enzyme (25.5 nkat of isoamylase/mL)[20]. All other determinations were performed as described previously[4,21].

Chemical determinations. — Iodine-binding capacities were determined by amperometric[22] titration using a Radiometer A/S RTS 822 Autotitrator, with two platinum electrodes, following an automatic dead-stop end-point titration, at 25° or 2.5°.

The reducing residues of amylose and the intermediate material were determined in quintuplicate by the Park–Johnson[23] procedure, as modified by Hizukuri *et al.*[24]. The absorbance at 715 nm was linearly related to the reducing concentration in the range 0–5 μg/mL.

Gel-permeation chromatography. — Sephacryl S-200 in aqueous 40% methyl sulfoxide and Sepharose CL-2B in 0.1M KOH were used[4,25]. The calibration of Sephacryl S-200 was checked with debranched waxy-maize amylopectin and confirmed previous findings[4]. A column (2.5 × 95 ±1 cm) of Sephacryl S-1000 was eluted with an upward flow of degassed, aqueous 40% methyl sulfoxide at 13 mL/h at room temperature.

High-performance t.l.c. — Three upward elutions with acetonitrile–water (85:15) were used with subsequent detection with the thymol–sulfuric acid reagent[26].

Physicochemical determinations. — (*a*) *Light-scattering.* The intensity of scattered light from aqueous 50% methyl sulfoxide solutions of polysaccharides was measured between 30°–150°, using a commercial Sofica photometer at room temperature with vertically polarised light (546 nm), and calibration with distilled benzene. The specific refractive index increment dn/dc of amylopectin at 25° was taken[27] as 0.11 $m^{-3}.Kg^{-1}$. Stock solutions of polysaccharides were prepared by shaking polymer (2–3 mg) with methyl sulfoxide (25 mL) for 1 h at room temperature and then slowly diluting with an equal volume of water. Further solutions were prepared by successive dilution with aqueous 50% methyl sulfoxide. The solutions were passed through 0.45-μm Millipore filters directly into a dust-free cell. Data from light scattering were processed by the Zimm method[28].

The second virial coefficient A_2 was calculated according to the equation

$$\left[\frac{Kc}{R_\theta}\right]_{\substack{c\to 0\\ \theta\to 0}} = \frac{1}{M_w} + 2\,A_2C,$$

where θ is the scattering angle, and R_θ is the reduced scattering intensity which depends on the ratio of incident and scattered light, but is independent of θ.

(*b*) *Viscometry.* Measurements of intrinsic viscosity $|\eta|$ were carried out for solutions in 0.2M KOH at 25°, using an automatic Ubbelohde viscometer and concentrations in the range 1.5–3 mg/mL (solvent flow-time, 380 s).

RESULTS

Starch fractionation. — (*a*) *Dispersion of the granule.* Yields were calculated for five small-scale (5 g) fractionations of wrinkled-pea starch and three of smooth-pea starch. The properties of the various fractions are shown in Table I. The purity of the starch components is based on their iodine affinity, which is related to their fine structure, and on gel-permeation chromatography, which indicates the distribution of molecular weights.

TABLE I

RELATIVE DISTRIBUTION AND PHYSICOCHEMICAL CHARACTERISTICS OF WRINKLED- AND SMOOTH-PEA STARCH COMPONENTS

	Fractionation yield (% of original starch)	*Iodine-binding capacity (mg of I_2 bound per 100 mg of polysaccharide)*	*Iodine complex λ_{max} (nm)*	*Beta-amylolysis limit (%)*	*Intrinsic viscosity*	
					$[\eta]$ ($mL.g^{-1}$)	*Huggins constant (λ_H)*
Wrinkled pea						
Starch	100	14.7 ±1.8	605	79	N.d.[a]	N.d.
Amylose	70.9 ±3.0	18.8 ±0.4	630	85	172	0.53
Amylose impurities	2.4 ±1.8	6.7 ±1.9	570	N.d.	N.d.	N.d.
Intermediate components						
Fraction 1	16.3 ±2.1	9.5 ±2.8	580	72	19	0.33
Fraction 2	2.6 ±1.1	8.7 ±1.9		69		
Amylopectin	7.8 ±1.2	1.7 ±0.7	560	61	114	0.65
Smooth pea						
Starch	100	6.7 ±0.3	595–600	68	N.d.	N.d.
Amylose	33.2 ±3.2	19.1 ±0.2	625	82	264	0.41
Amylose impurities	1.7 ±1.4	N.d.	565	N.d.	N.d.	N.d.
Intermediate components						
Fraction 1	0.4 ±0.3	1.2	560	N.d.	N.d.	N.d.
Fraction 2	N.d.	N.d.	N.d.	N.d.	N.d.	N.d.
Amylopectin	64.7 ±2.0	0.9 ±0.2	560	57	126	0.64

[a] N.d., not determined.

Amylose obtained in the first thymol precipitation was purified further by two recrystallisations from hot 1-butanol and then two with cold solvent. For wrinkled-pea starch, the four by-products that contaminated the amylose were combined; the variable amount of this fraction, during the five assays (standard deviation/mean, $\sigma/\mu = 0.75$), was due to the non-reproducible quantity of amylopectin which co-precipitates with amylose in the first thymol precipitation. Consequently, this fraction (2.4% of the total starch) was not studied. With smooth-pea starch, the same phenomenon (σ/μ 0.82) was observed for the amylose impurities (1.7% of total starch). Recovered amylose (70.9% for wrinkled pea, 33.2% for smooth pea) was pure, as indicated by the high iodine-binding capacities (18.8 mg of iodine bound per 100 mg of polysaccharide for wrinkled pea, and 19.1 mg for smooth pea). The profiles on Sepharose CL-2B of these native amyloses (Fig. 1, for wrinkled pea) contained two peaks, the first located at the void volume of the gel (18.1% for wrinkled-pea, 23.0% for smooth-pea amylose); the iodine complex of this peak had λ_{max} ~615 nm, whereas that of the second, broader peak [K_{av} 0.1–0.9, with a maximum at K_{av} 0.47 (wrinkled pea) or 0.44 (smooth pea)] had λ_{max} 630–640 nm.

On differential ultracentrifugation, the material which did not form a complex with thymol was separated into a very thin sediment and a non-sedimentable

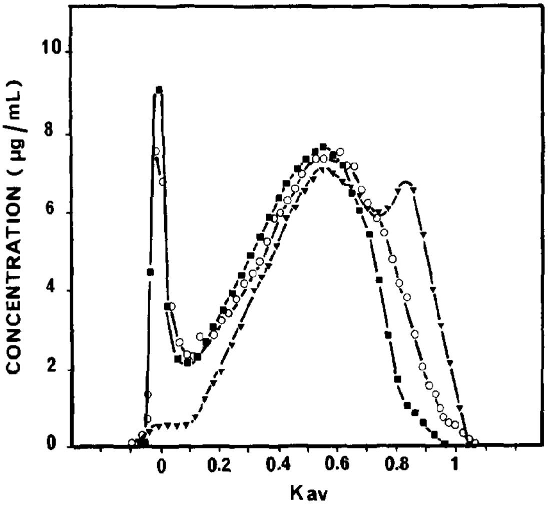

Fig. 1. Elution profiles on Sepharose CL-2B of amylose from wrinkled-pea starch: native, ■; debranched, ▼; beta-limit dextrin, O.

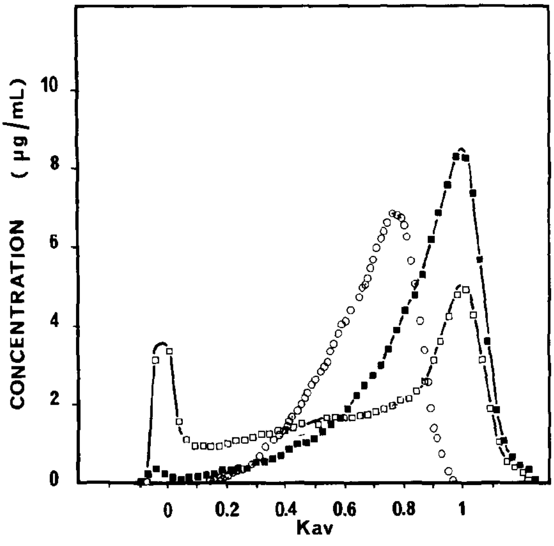

Fig. 2. Elution profiles on Sepharose CL-2B of intermediate material from wrinkled-pea starch: first supernatant, ■; second supernatant, □; beta-limit dextrin, O.

fraction. For wrinkled pea, the two non-sedimentable fractions (16.3 and 2.6%) had intermediate iodine-binding capacities of 9.5 and 8.7, respectively, and therefore were combined to give the intermediate material. When chromatographed on Sepharose CL-2B (Fig. 2), the first supernatant was characterised by an asymmetrical peak at K_{av} 0.90 preceded by a low edge, whereas the second supernatant showed, in addition, the presence of material (7.3% of the second supernatant) at

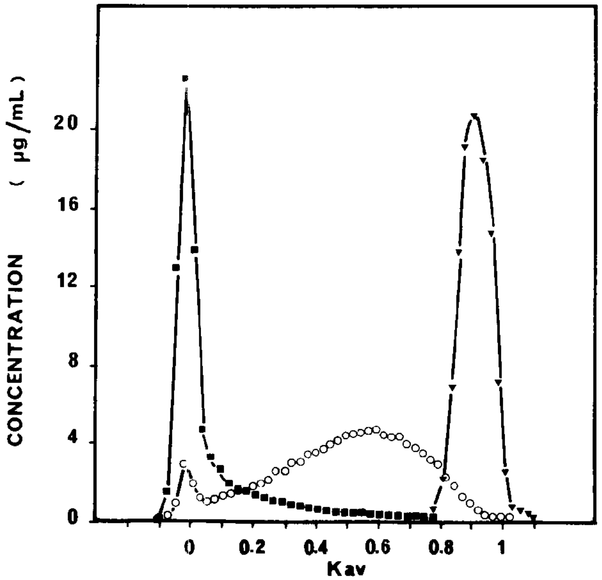

Fig. 3. Elution profiles on Sepharose CL-2B of amylopectin from wrinkled-pea starch: native, ■; debranched, ▼; beta-limit dextrin, O.

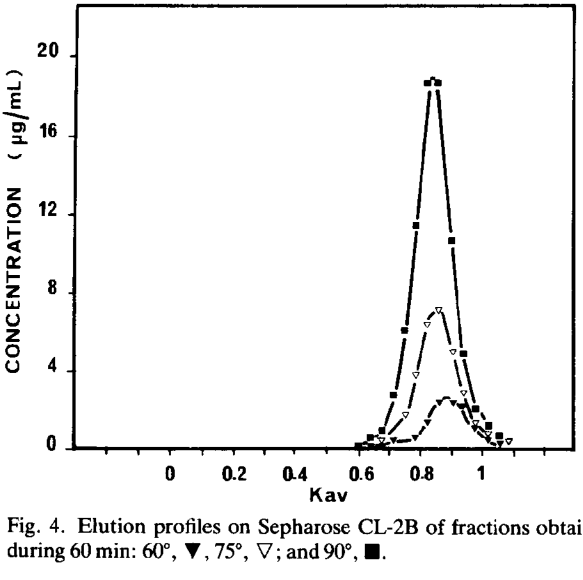

Fig. 4. Elution profiles on Sepharose CL-2B of fractions obtained by leaching of wrinkled-pea starch during 60 min: 60°, ▼, 75°, ▽; and 90°, ■.

the void volume. The iodine complex of the material with K_{av} 0.6–1.0 had a constant λ_{max} of 580 nm, demonstrating its homogeneity, whereas that of the excluded fraction had a lower value (565 nm), indicating possible contamination with amylopectin. This procedure gave 64.7% of a sediment with smooth-pea starch with 0.4% of non-sedimentable material, whereas the sedimentable fraction represented 7.8% of the initial wrinkled-pea starch.

Each sediment had a low iodine-binding capacity (1.7 ±0.7 and 0.9 for wrinkled pea and smooth pea, respectively), demonstrating their amylopectin-like nature. Their elution profiles on Sepharose CL-2B (Fig. 3 for wrinkled pea) showed mainly a sharp peak (79.2% and 90.8% for wrinkled and smooth peas, respectively) at the void volume, and the iodine complexes had λ_{max} 560 nm for wrinkled pea and 555 nm for smooth pea, and a very low tail up to K_{av} 0.70–0.75.

(*b*) *Aqueous leaching.* When wrinkled-pea starch was leached with neutral water for 60 min, 1.9% was removed at 60°, 5.1% at 75°, and 9.3% at 90°. These three fractions had identical iodine complexes (λ_{max} 580–590 nm), iodine-binding capacities (10.5 ±2.3), and behaviour on Sepharose CL-2B (Fig. 4), with a peak maximum at K_{av} 0.8–0.9.

(*c*) *Amylose.* The properties of wrinkled-pea amylose (Table I) (iodine-binding capacity, 18.8; iodine complex, λ_{max} 630 nm; beta-amylolysis limit, 85%) were similar to those observed for smooth-pea amylose (19.1, 625 nm, 82%). A better procedure than the iodine-titration method[17] for testing the purity of amylose is gel-permeation chromatography on Sepharose CL-2B (Fig. 1), where any contaminating amylopectin would be in the void volume. Indeed, for wrinkled-pea amylose, the λ_{max} of the iodine complex of the material in the first peak was 615 nm, whereas, in the second part of the profile, the λ_{max} of that for each fraction was always in the range 620–640 nm, except after K_{av} 0.7 where a decrease to 600 nm suggests contamination by some intermediate material. The same observations were made with the iodine complex of smooth-pea amylose, with only a decrease to 615 nm after K_{av} 0.75. The intrinsic viscosity of wrinkled-pea amylose is 172 $mL.g^{-1}$ (Huggins constant λ_H 0.53), leading[29] to a viscometric-average molecular weight, $\overline{M}_v$, of 430,000. The number-average molecular weight, $\overline{M}_n$, measured by the reducing-end-group analysis, was only 125,000 ±3,000. Therefore, the polydispersity ($I = \overline{M}_v/\overline{M}_n$) was 3.4. For smooth-pea amylose, the molecular weights are higher: $\overline{M}_v$ 697,000, $\overline{M}_n$ 170,000, and I 4.1. For each amylose, the distribution of molecular weights was wide, as confirmed by the chromatographic profiles on Sepharose CL-2B.

Complete debranching of wrinkled-pea amylose, achieved in aqueous 40% methyl sulfoxide by isoamylase, was shown by the high beta-amylolysis limit (103%). After debranching, the intrinsic viscosity was reduced to 85 $mL.g^{-1}$ (λ_H 0.34), indicating $\overline{M}_v$ to be 175,000, whereas $\overline{M}_n$ was 55,000 and I was not greatly modified (3.2). Based on the d.p. values, these results demonstrate that 1.3 reducing glucose units were liberated for each initial reducing glucose unit by debranching amylose. The foregoing data indicate that amylose is composed of 2–3 chains. The same conclusions are reached from the changes in $\overline{M}_v$. For smooth pea, amylose molecules comprise an average 3.2 chains, which become linear after debranching (beta-amylolysis limit, 104%).

Further experiments were conducted only on the debranched, wrinkled-pea amylose. No oligosaccharide with d.p. <6 was detected by high-performance t.l.c. The profile of debranched amylose (Fig. 1), compared to that of native amylose in

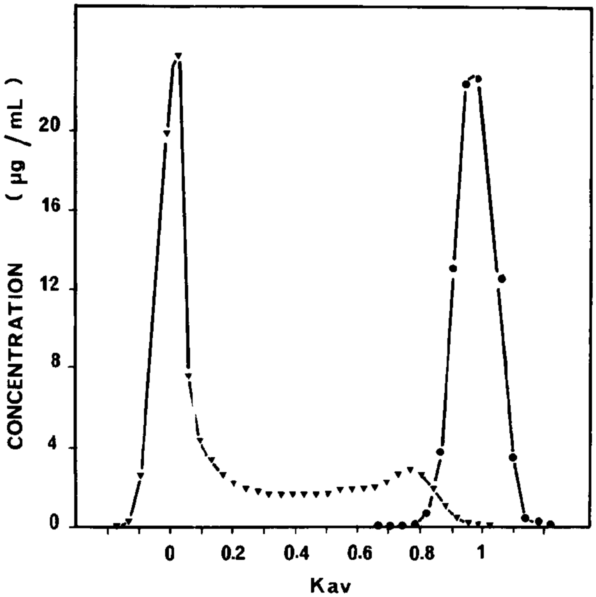

Fig. 5. Elution profiles on Sephacryl S-200 of wrinkled-pea starch amylose after debranching (▼) and debranching followed by hydrolysis with beta-amylase (●).

gel-permeation chromatography on Sepharose CL-2B, shows 4.5% of excluded material, a broad peak between K_{av} 0.1 and 0.8 (slightly shifted towards lower molecular weight), and a third peak at K_{av} 0.9. When chromatographed on Sephacryl S-200 (Fig. 5), 67.5% of the debranched amylose was eluted at the void volume, followed by a long tail and a small peak (7.4%) at K_{av} 0.75. After beta-amylolysis, there was no material of high molecular weight, and glucose, maltose, and maltotriose were the only products located at the total volume (Fig. 5).

The beta-limit dextrin of wrinkled-pea amylose, corresponding to only 15.5% of the original amylose, was isolated after exhaustive treatment with beta-amylase and had properties (iodine-binding capacity, 18.9; $|\eta|$ 127 mL.g^{-1}; λ_{max} of iodine complex, 610 nm) comparable to those of the native amylose. The same trends were observed for smooth-pea beta-limit dextrin (iodine-binding capacity, 19.3; $|\eta|$ 215 mL.g^{-1}; λ_{max} of iodine complex, 620 nm). The behaviour of wrinkled-pea beta-limit dextrin on Sepharose CL-2B was similar to that of native amylose (Fig. 1), with 13.4% of material eluted at the void volume, followed by a broad peak, with a maximum at K_{av} 0.62 and ending at K_{av} 1.0. The smooth-pea beta-limit dextrin gave a profile with 15.1% of excluded material, and a second peak at K_{av} 0.58.

(*d*) *Amylopectin.* Wrinkled-pea amylopectin, corresponding to only 7.8% of the total starch (iodine-binding capacity, 1.7 ±0.7; λ_{max} of iodine complex, 560 nm) is similar to smooth-pea amylopectin (0.9, 560 nm). Measurement of the light-scattering intensity gave $\overline{M}_w$ 19,400,000. A typical Zimm plot is shown in Fig. 6; no deformation was observed with angle, due to the good solvent power of methyl sulfoxide, which is confirmed by a positive second virial coefficient $A_2 = 1.7 \times 10^{-4} m^3.mol.Kg^{-2}$. Smooth-pea amylopectin had a higher $\overline{M}_w$ (80,600,000), but a lower A_2 value ($1.7 \times 10^{-5} m^3.mol.Kg^{-2}$). The elution profile on Sephacryl S-1000 of amylopectins showed a single peak for each starch (Fig. 7), with a maximum at K_{av} 0.27 for smooth-pea amylopectin and at K_{av} 0.53 for wrinkled-pea amylopectin, confirming the difference in their molecular weights. Nevertheless, for each amylopectin, the chromatogram was homogeneous in that there was no variation

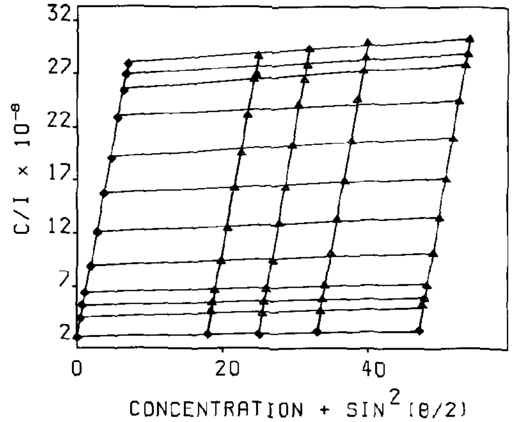

Fig. 6. Zimm plot of wrinkled-pea starch amylopectin in 1:1 methyl sulfoxide–water at 25° and concentrations of 47, 33, 24, and 18 μg/mL.

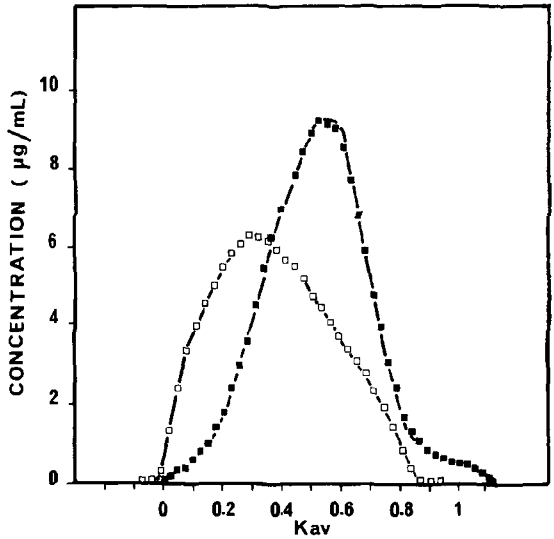

Fig. 7. Elution profiles on Sephacryl S-1000 of wrinkled-pea (■) and smooth-pea (□) starch amylopectins.

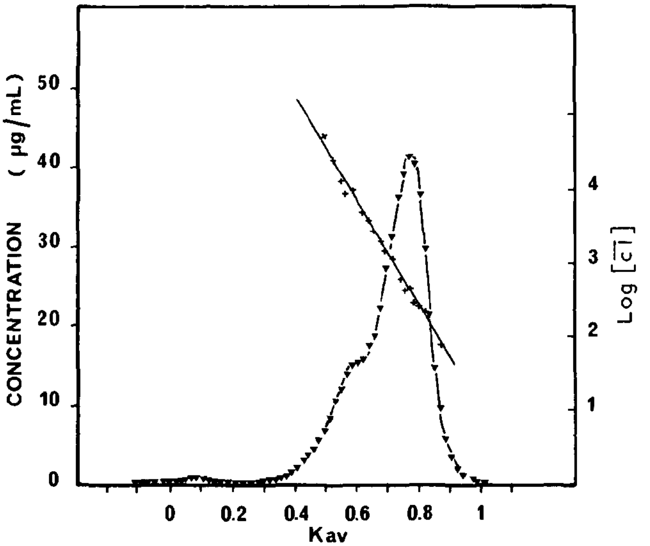

Fig. 8. Elution profiles on Sephacryl S-200 of debranched amylopectin (▼) from wrinkled-pea starch and the corresponding log chain-length (c.l.) (+) for each fraction.

in λ_{max} of the iodine complex (after dilution to <5% of methyl sulfoxide) observed along the chromatograms. Furthermore, the intrinsic viscosities of smooth-pea ($|\eta|$ 126 mL.g^{-1}) and wrinkled-pea ($|\eta|$ 114 mL.g^{-1}) amylopectins suggest a higher $\bar{M}_w$ for smooth-pea amylopectin.

Total debranching of wrinkled-pea amylopectin by isoamylase, as shown by further beta-amylolysis (limit, 102%), gave material with an average chain-length of 20.5. The debranched material, located at the total volume on the Sepharose CL-2B column (Fig. 3), was fractionated into three peaks on Sephacryl S-200 (Fig. 8); the excluded peak represented 2% of the total material, there was a shoulder located at K_{av} 0.57, corresponding to a material of d.p. ~45, and the main peak was at K_{av} 0.75 with chains of d.p. ~15. Based on the short (S, d.p. 15) and long (L, d.p. 45) constitutive chains of amylopectin (described by Robin *et al.*[40] as A and B) when calculated on a molecular weight basis, the S:L ratio for wrinkled-pea amylopectin was ~9.6. With an excluded material of 4.1% on Sephacryl S-200, the debranched, smooth-pea amylopectin had an S:L ratio of 8.1. For both starches, the minor peaks remaining at the void volume decreased greatly on further beta-amylolysis, giving 1.1% and 1.7%, respectively, of material at V_0 for wrinkled-pea and smooth-pea amylopectins.

The limits of beta-amylolysis of smooth- and wrinkled-pea amylopectins were 57% and 61%, respectively, and the iodine complexes of the corresponding beta-limit dextrins had λ_{max} at 530 nm. The elution profile on Sepharose CL-2B of wrinkled-pea beta-limit dextrin (Fig. 3) shows excluded material (7.4%) followed by a broad peak, ending at K_{av} 0.90, with a maximum at K_{av} 0.55.

Intermediate material. The two combined intermediate fractions, correspond-

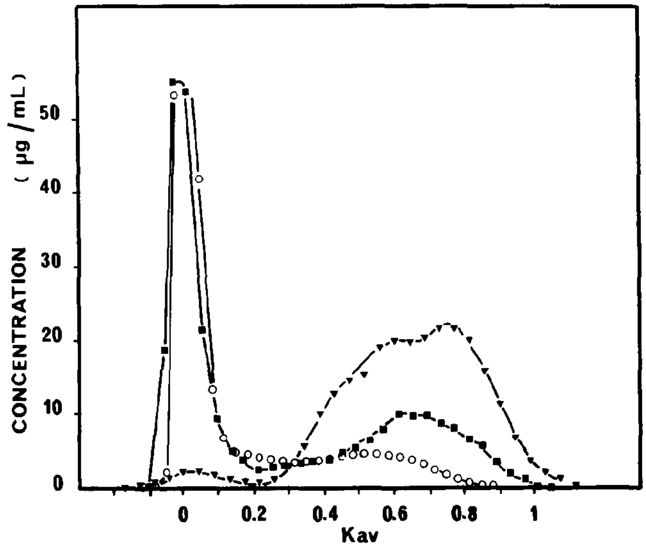

Fig. 9. Elution profiles on Sephacryl S-200 of intermediate material from wrinkled-pea starch: native (■), debranched (▼), and beta-limit dextrin (○).

ing to 19% of the wrinkled-pea starch, had a very low molecular weight ($\overline{M}_n$ 25,100 ±1,100) and intrinsic viscosity (19 mL.g^{-1}, λ_H 0.33) (Table I), and the iodine complex had λ_{max} 585 nm. The iodine-binding capacity could not be determined at 25° due to the absence of a linear portion in the amperometric titration curve, whereas, at 2.5°, the iodine-binding capacities were 9.5 ±2.8 and 8.7 ±1.9 for the first and the second supernatants, respectively. The elution profile on Sephacryl S-200 (Fig. 9) shows an excluded peak corresponding to 61.1% of the intermediate material, followed by a tail up to K_{av} 0.80.

Debranching of this polysaccharide by isoamylase gave a product which had an average chain-length of 28.9: there was a large decrease in the material excluded (4.0%) on Sephacryl S-200 (Fig. 9), compared to the native polysaccharide, and there were two major chain populations at K_{av} 0.58 (43.8%) and 0.76 (52.2%), corresponding to d.p. values of 45 and 15, respectively. Although the peak at K_{av} 0.58 was asymmetrical towards components of higher molecular weight, suggesting that longer chains with d.p. >45 were present, the S:L ratio was calculated on a weight basis and corresponded to 3.6. This material was almost completely degraded by beta-amylase, giving a limit of 100%. However, further chromatography on Sephacryl S-200 showed a small peak (2.1%) at the void volume, and a main one at the total volume (93.2%) preceded by a low edge starting at K_{av} 0.73. This result indicated that debranching was not complete since the debranched material was not transformed by beta-amylase into glucose and maltose.

The beta-amylolysis limit of native intermediate material was 70%. The iodine complex of the residual beta-limit dextrin had λ_{max} 568 nm, indicating that the inner chains were still long enough to form a complex with iodine. The profile on Sepharose CL-2B (Fig. 2) shows a unique and asymmetrical peak at K_{av} 0.75, whereas, on Sephacryl S-200 (Fig. 9), two peaks are present, namely, an excluded peak (43%) and another peak at K_{av} 0.66. Like the precursor intermediate-material, the beta-limit dextrin was polydisperse.

DISCUSSION

The different materials obtained by the fractionation of starch must together represent the whole initial starch. Two intrinsic properties of starch components, namely, iodine-binding capacity and beta-amylolysis limit, when summed for the various fractions (Table I), give values near to those of initial wrinkled- (15.4 instead of 14.7 for iodine-binding capacity, and 78.1 instead of 78.5 for beta-amylolysis limit) and smooth-pea starches (6.9 instead of 6.7 for iodine-binding capacity, and 68.3 instead of 67.5 for beta-amylolysis limit). For each starch, the various fractions were pure, in contrast to those obtained by Biliaderis *et al.*[30,31] by either precipitation or leaching. The same criticism applies to the recent results of Baba *et al.*[10], who sub-fractionated amylomaize starch by selective precipitation with 1-butanol. Their procedure gave a high percentage of amylopectin-contaminating amylose, and the existence of intermediate material in their amylopectin fraction, as proved by a peak eluted at ~145 mL the iodine complex of which had λ_{max} 600 nm, which was quite different from that of the iodine complex of the amylose fraction (λ_{max} 645 nm) eluted at 130 mL (Fig. 1 in ref. 10). This lack of purification prevents any conclusion on the structure of the components. In another way, Greenwood and Thomson[15] found a higher content of amylopectin, which may be explained by either the differential centrifugation step, which is not very reproducible, or cultivar differences. Furthermore, our results indicate that, by leaching wrinkled-pea starch granules, only ~10% of amylose and intermediate material are extractable, and the profiles on Sepharose CL-2B (Fig. 4) demonstrate a behaviour different from that of total amylose, thereby invalidating the use[30,32] of this technique for representative fractionation.

Wrinkled-pea starch contains about twice as much amylose (70.9%) as smooth-pea starch (33.2%), with a broad distribution of molecular weights for each amylose. This polydispersity seems to be a general feature for amylose, as already observed for potato amylose[33]. However, average molecular weights are considerably less than those observed for amyloses of potato[24] or cereals[16,17], although that for smooth-pea amylose is larger than that for wrinkled-pea amylose. According to the results represented here, there are ~2–3 chains per amylose molecule, confirming the previous findings of Potter *et al.*[34,35] based on periodate oxidation. Recently, Hizukuri *et al.*[24] claimed potato amylose to contain 9–20 chains with $\overline{M}_n$ ~810,000. All of these data accord with a branched structure involving 2–4 chains per thousand D-glucose residues. The behaviour of debranched amylose on Sepharose CL-2B (Fig. 1) demonstrates the presence of branched molecules with long side-chains rather than short chains or stubs. The presence of short stubs has been suggested by Hizukuri *et al.*[24], in order to explain inconsistent results obtained on hydrolysis with isoamylase, pullulanase, and beta-amylase. Indeed, when debranching is carried out in aqueous buffer, the long and linear material does not stay in solution. The further aggregation therefore explains the incomplete beta-amylolysis after debranching, which was not observed in the present studies in which aqueous 40% methyl sulfoxide was used as solvent. The assumption of long-chain branching was also proposed by Banks and Greenwood[8] to explain the hydrodynamic behaviour of amyloses of high molecular weight, in comparison with that of linear amyloses. The high intrinsic viscosities of beta-limit dextrins and their corresponding chromatographic profiles on Sepharose CL-2B suggest high molecular weights for the inner part of the macromolecules. Furthermore, the relatively high iodine-binding capacities of beta-limit dextrins, similar to those of native amylose, can be explained only if the branch points are located near the non-reducing ends of the native amylose molecules, thereby protecting the main linear part of the molecule against beta-amylolysis. From these results, amylose must be considered as a mixture of linear and branched chains with 2–3 branch points per molecule. Although the proportions are different in smooth- and wrinkled-pea starches, the similar physicochemical characteristics of amylose suggest similar structure in both genotypes.

Whereas the amylopectin content of smooth-pea starch (64.7%) is slightly lower than that[12] (73–80) of cereals and tubers, amylopectin is a minor component (7.8%) in wrinkled-pea starch. However, both amylopectins have iodine-binding capacities comparable to the values reported by others[15,31,32] but two or three times higher than those for cereal amylopectins[16,17]. This difference cannot be explained by contaminant amylose, as the debranched material gives only one peak at the total volume on Sepharose CL-2B (Fig. 3) in contrast to amylose. The intrinsic viscosities of both amylopectins are similar to those reported[16,32], and the weight-average molecular weights $\overline{M}_w$ are of the same magnitude as those recorded by several workers[27,36–38], but are greatly lower than those obtained by Banks and Greenwood[8]. These discrepancies would result from the solvent used: methyl sulfoxide and 0.2M NaOH[36] are better solvents than 0.2M NaCl[39], which does not disrupt all of the aggregates of amylopectin molecules. For both starches, the isoamylase-debranched amylopectins contain S (d.p. 15) and L (d.p. 45) chain populations, in a ratio which corresponds to the cluster organization described by Robin *et al.*[40] for waxy-maize amylopectin. However, the higher iodine-binding characteristics than for cereals could be explained by longer external chain-lengths[41], since the λ_{max} for the iodine complexes of the respective beta-limit dextrins are identical.

Wrinkled-pea starch contains a high percentage (18.9%) of intermediate material in contrast to smooth-pea starch (0.4%). This fraction is branched and has a low molecular weight, but is very polydisperse. The high content of L chains is probably responsible for the instability in solution during enzymic experiments, which require aqueous 40% methyl sulfoxide; previous studies[5,13] have shown the existence of a material of d.p. ~60. In contrast, the beta-amylolysis limit (70%) is rather lower than the value (100%) reported by Greenwood and Thomson[15]. The difference is important since these authors[15] postulated that the intermediate material is a short-chain amylose. According to Pfannemüller *et al.*[42], the λ_{max} of the iodine complex of this fraction indicated an average chain-length of ~70, whereas the d.p. value was ~150. The debranching experiments demonstrate clearly that this intermediate material is built with the same constitutive S and L chains present in amylopectin, thereby proving the relationship of these two types of molecules. The intermediate material was not studied by Biliaderis *et al.*[32] and Baba *et al.*[10] since their fractionation procedure was not applied to the "amylopectin fraction". However, it may be noted that their intermediate material had an S:L ratio close to those reported for amylopectin. Since the content of true amylopectin is much lower than that of the intermediate material in wrinkled-pea starch, the analysis of these two debranched fractions, when mixed, does not permit assessment of the existence of normal amylopectin.

The two pea starches differ mainly in the amylose:amylopectin ratio and in the presence of an intermediate material of low molecular weight only in wrinkled pea. Both the high content of amylose and the intermediate branched fraction, with a content of L chains relatively higher than that of normal amylopectin, could explain the higher resistance of wrinkled-pea starch to mild hydrolysis with acid[4], as they must have an important function in the constitution of the starch-granule crystallites. Furthermore, the less-branched amylopectin-like material would accord with the lower level of branching enzymes observed[43] in the wrinkled pea. According to Borovsky *et al.*[44], the transglycosylation activity of the Q-enzyme would be modified in order to produce a different ratio of S and L chains.

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