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Note

Some structural properties of amylopectin from sugar cane

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Whayman and co-workers[1–3] studied starch isolated from varieties of cane grown in Queensland and from raw sugar. They related its properties to other starches and determined its influence on milling technology. Cane starch occurs in irregular granules which vary from 1 to 5 μm in maximum diameter, and has an amylose content of 14 ±1% when measured on starches sampled throughout the cane-growing regions of the state. The amylopectin fraction absorbs less iodine in its complex than amylopectins from potato and waxy maize, and λ_{max} is at a lower wavelength. These properties were attributed to a higher degree of branching in the cane amylopectin.

Our studies were primarily to verify this hypothesis on the structural character of cane amylopectin. Amylolysis of starch isolated from raw sugar frequently indicates a residual component resistant to enzymic degradation but large enough to absorb iodine, as in the colorimetric method for starch analysis[4]. Our secondary objective was to determine whether amylase-resistant macrodextrins were produced during amylolysis of cane starch.

Amylopectins were prepared from starch recovered from cane variety NCo310 and potatoes grown locally. Amylopectin from waxy maize was purchased from ICN Pharmaceuticals (Cleveland, Ohio). Amylose was removed by complexing with 1-butanol, using the method of Schoch[5]. A second treatment of the amylopectin fractions with 1-butanol ensured no carry-over of amylose. Failure of the cane amylopectin to form a complex with 2-nitropropane confirmed the absence of an intermediate fraction[6]. The amylopectins were considered to be free of amylose, using the criteria of repeated treatment with butanol and similar spectral properties to those reported by Whayman and Willersdorf[2].

Properties of average chain-length, light absorption by the iodine complex, and beta-amylolysis limit are shown in Table I.

Average chain-lengths were determined by periodate oxidation and ^{1}H-n.m.r. spectroscopy. The amount of formic acid produced was the basis of the

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TABLE I

PROPERTIES OF AMYLOPECTINS FROM CANE, POTATO, AND WAXY MAIZE

Amylopectin	*Average chain-length*		*Iodine complex* (λ_{max}, *nm*)	*% Conversion into maltose by beta-amylase*
	Periodate oxidation[a]	*^{1}H-N m.r. data*[b]		
Cane	19	19	530	59
Waxy maize	23	22	555	61
Potato	N D.	22	563	60

[a]Determined from the formic acid produced [b]Ratio of intensities of the signals for H-1 in (1→4)- and (1→6)-linked units

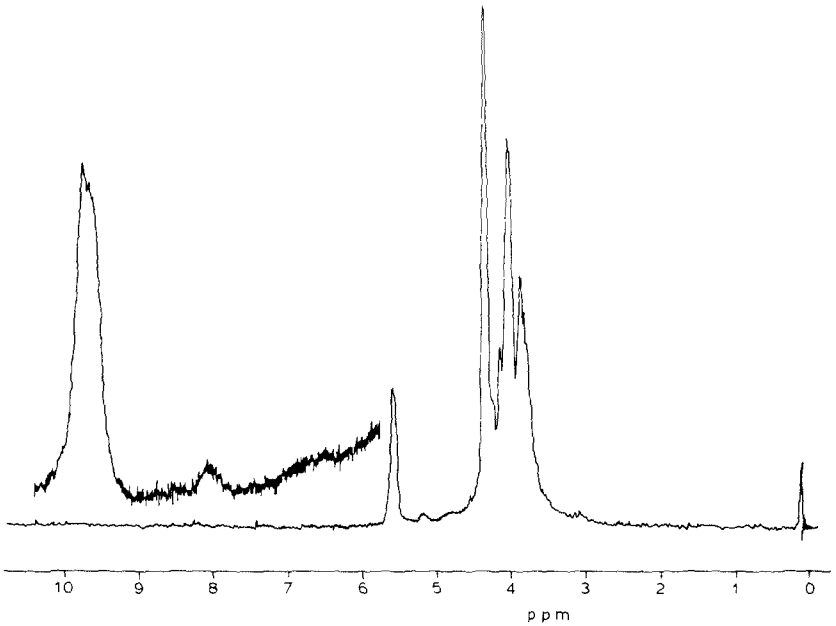

Fig. 1. ^{1}H-N m.r. spectrum (100 MHz, 90°) of cane amylopectin. Signals were integrated by excision of the peaks and weighing.

periodate calculations. The low strength of the signal for H-1 in the (1→6)-linked units limited the accuracy of the ^{1}H-n.m.r. method (Fig. 1). This signal could be amplified by boosting the signal-to-noise ratio or by the use of Fourier-transform n.m.r. The results were of the same order of magnitude as those reported in the literature[7,8].

The amylopectins were debranched using isoamylase from *Cytophaga*, and the products were chromatographed on Sephadex G-50. The profiles shown in Fig. 2 compared favourably with those of amylopectins from maize and potato chromatographed on Sephadex G-75 after debranching[9].

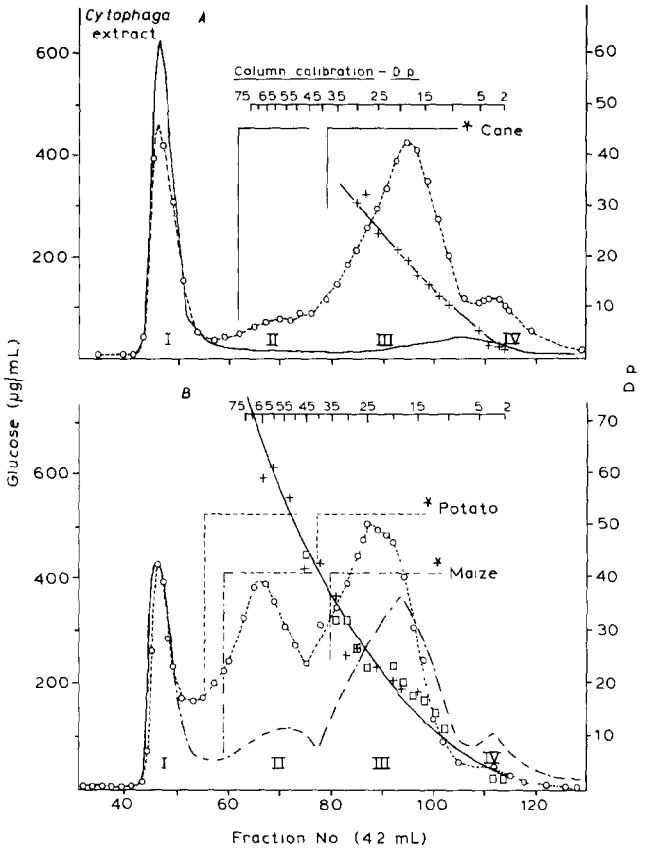

Fig. 2. Chromatography on Sephadex G-50 of the products obtained by debranching amylopectins with isoamylase: *A*, (·——·), the enzyme preparation; (⊙– – –⊙), cane amylopectin; *B*, (⊙– – –⊙), potato amylopectin; (·–·–·), waxy-maize amylopectin; + and ⊡ are the d.p. values measured on the collected fractions in the separate chromatographic analyses; *d.p. ranges for peaks II and III.

The excluded fraction (Peak I) is due to a component in the enzyme preparation. Gunja-Smith and co-workers[10] achieved a 10-fold purification of the enzyme by chromatography on DEAE-cellulose and eliminated this component. Since the component did not interfere in the work reported here, its removal was considered to be unnecessary.

In this discussion, the terminology of A and B chains is used[11]. An A chain is a linear unit attached through its reducing end to a B or C chain; a B chain carries a branch chain at position 6 of one or more of its glucosyl residues and is linked through its reducing end to a B or C chain.

Akai and co-workers[9] noted the existence of B chains in amylopectins, with d.p. in the ranges 40–80 and 20–40. They speculated that the longer B chains were present in the inner structure of the molecule, whereas the shorter chains were located in the outer branching of the molecule.

Potato amylopectin has an average chain-length which is longer than that of waxy maize[12], and this is understandable when the distribution pattern of chain lengths (Fig. 2) is examined. The greater content of longer B chains generates a higher average chain-length. However, the measured differences in average chain-lengths are usually small because of the common contribution of A and shorter B chains in the second size-distribution. Thus, the differences in structure of amylopectins are best determined by debranching the molecule and subjecting the products to gel-permeation chromatography.

As seen in Fig. 2, cane amylopectin is significantly different from potato and waxy-maize amylopectin in that it lacks a pronounced bimodal distribution of chain lengths. It possesses only a small portion of longer B chains (d.p. 45–80), whereas the majority of chains are in the range d.p. 10–40 with an apex at 19.

The presence of a small but significant group of chains in Peak IV for amylopectins from cane and waxy maize may represent maltosyl and maltotriosyl stubs formed by beta-amylase activity during extraction of starch granules. The conditions of isolation from waxy maize, a commercial product, are unknown. In the isolation of starch granules from cane, ~10 min elapsed from cellular disruption by milling to extract the juice to the addition of mercuric ion inhibitor. It is generally accepted that amylolysis of intact starch granules is quite slow[13], a fact acknowledged in the production of cane sugar where the removal of starch by indigenous enzymes requires a definite incubation period at temperatures that reflect a compromise between the rates of enzyme denaturation and the gelatinisation of starch granules.

Akai and co-workers[14], in studies of the debranching of glycogen, noted a component similar to Peak IV and identified maltotriose, maltotetraose, and traces of maltopentaose, but no maltose. These small chains were thus an integral part of the glycogen. Unfortunately, in these studies, insufficient material was available for chromatographic identification of the components of the peak.

After digestion of amylopectin from cane with bacterial alpha-amylase to constant reducing activity, chromatography of the resultant dextrins on Sephadex G-25 did not reveal a macrodextrin component of high molecular weight that was resistant to enzymic degradation. The profile (Fig. 3) revealed that no material was large enough to be excluded from the gel, but 12% of the digest had d.p. >8 as measured by its mobility during paper chromatography.

Thus, the amylopectin from cane starch was typically amylopectin in character. It possessed a greater abundance of smaller chains of fairly uniform length than amylopectins from potato and waxy maize, and these contributed to a slightly smaller average chain-length for the molecule. Their preponderance also explained its lower iodine absorption at a wavelength slightly lower than for the complexes from potato and waxy maize.

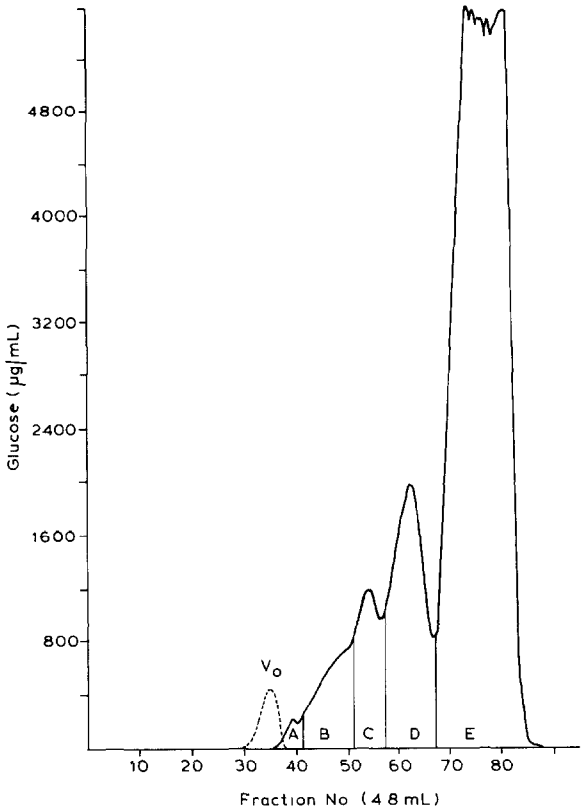

Fig. 3. Gel-permeation chromatography on Sephadex G-25 of the products obtained by digestion of cane amylopectin with alpha-amylase of *B. subtilis*. Fractions as marked were combined and analysed by p.c. The dotted line is a Blue Dextran marker for the void volume of the column.

EXPERIMENTAL

Starches. — Cane starch was isolated from second ratoon NCo310 harvested in late October in the Mackay district. Juice was extracted by a double pass through a laboratory mill and filtered through a 125-μm screen before being made ~0.01M in mercuric chloride to inhibit amylase activity[15]. The operation from cutting to treatment with mercuric chloride was concluded in 2 h. The time from the extraction of juice to addition of inhibitor was ~10 min.

The juice was centrifuged at 1,800*g* for 15 min at 4°, and starch granules were recovered as outlined by Stevenson and Whayman[1].

Potato starch was isolated from potatoes grown locally; the botanical and agricultural history was unknown. The potatoes were homogenised in saline, using a Jefco disintegrator, and the extract was processed by the standard procedure.

Amylopectin from waxy maize was purchased from ICN Pharmaceuticals (Cleveland, Ohio).

Starch components. — *(a) Isolation.* The various starch samples were fractionated by complexing[5] with 1-butanol. This procedure, when applied to amylopectin from waxy maize, yielded a small proportion of insoluble complex.

(b) Periodate oxidation. Amylopectin (200–300 mg) was dissolved in water (200 mL), and sodium periodate was added to a final concentration of 25mM. 1-Propanol (10 mL) was added as an oxygen scavenger to inhibit depolymerisation[16]. Samples were kept at 20° in the dark for 70 h, and the reduction of periodate was monitored using an ion-exchange procedure[17]. The same amount of resin equilibrated with acetate was used for the blank and the periodate analysis in order to avoid variation in the absorbance background of acetate anion eluted by iodate.

Formic acid was measured by titration against standard alkali. The results are shown in Table I.

(c) ^{1}H-N.m.r. spectroscopy. Deuterated amylopectins were analysed as 4% solutions in D_2O at 90°, using a 5-mm probe and a JEOL JNM MH100 spectrometer on external lock. Sodium 4,4-dimethyl-4-silapentane-1-sulphonate was used an internal reference. Signals were integrated using a sweep width of 270 Hz. Peak areas were also measured by excision and weighing.

The signal for H-1 in (1→4)-linked glucosyl residues was located at δ 5.44 ($J_{1,2}$ 3.0 Hz) and that for H-1 in (1→6)-linked glucosyl residues was at δ 5.05. The spectrum of the cane amylopectin is shown in Fig. 1 and the results are given in Table I.

(d) Enzymic debranching. Amylopectin (50 mg) was incubated in 25mM acetate (pH 5.5, 5 mL) with *Cytophaga* isoamylase (B.D.H., 50 mg) for 48 h at 37°. The incubate was then heated for 5 min at 100° and centrifuged at 20,000*g* for 20 min at 4°.

The clear supernatant solution was applied to a column (96 × 2.6 cm) of Sephadex G-50 and eluted with 10mM sodium acetate at 23 mL/h. Fractions (4 mL) were analysed for total carbohydrate by the phenol–sulphuric acid method, and for reducing activity by the Nelson–Somogyi method[18]. The d.p. was obtained as the ratio of total carbohydrate, expressed as glucose, to the number of reducing end-groups also equated to a glucose standard.

Profiles for Sephadex G-50 are shown in Fig. 2.

Digestion by amylase. —*(a) Beta-amylase.* Amylopectins (30 mg) were dissolved in boiling water and the solutions were autoclaved for 20 min before adjusting to a concentration of 0.5% (w/v) in 50mM acetate buffer (pH 4.8). Beta-amylase (Calbiochem, 1% in acetate) was added in portions (0.5 mL, 6000 units) at 0, 18, and 26 h. The enzyme was free from alpha-amylase.

Reducing sugar was determined after 24 and 51 h. The change in concentration at the second sampling was barely significant. The maltose concentrations obtained are reported in Table I.

(b) Alpha-amylase. A dispersion of cane amylopectin (720 mg) in water was autoclaved for 30 min before adjustment to 25mM in citrate buffer (pH 6.0) and a final volume of 50 mL. The solution was equilibrated to 39.5°, and a 0.4% solution

(1.5 mL) of enzyme was added. The enzyme, from *Bacillus subtilis,* was purchased from Calbiochem as a crystalline solid with 10.9% of sodium acetate and 21% of calcium acetate. Measured reducing-strength of the digest was constant in 6 h.

The sample was centrifuged at 27,000*g* for 20 min at 5° and the clear solution was eluted from a column (90 × 2.6 cm) of Sephadex G-25 superfine with water at 0.5 mL/min. The resulting chromatogram is shown in Fig. 3. Fractions were combined as indicated, and 663 mg were recovered. The relative percentage distribution in weight of the fractions was A, 0.5; B, 5.6; C, 6.3; D, 15.6; and E, 71.9.

Dextrins from these fractions were subjected to p.c. for 65 h, using 1-butanol–ethanol–water (2:1:1) with maltodextrins from partially hydrolysed amylose as standards.

Fractions A–C remained virtually at the origin, D was composed of maltodextrins of d.p. 4–8, and E contained mainly maltose and maltotriose with some glucose.

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