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Note

The action of isoamylase on the surface of starch granules*

Andrew Lynn and J. Roger Stark†
International Centre for Brewing and Distilling, Department of Biological Sciences, Heriot-Watt University, Edinburgh EH14 4AS (Great Britain)

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There is an apparent conflict regarding the distribution of amylose and amylopectin within cereal-starch granules. When starch was damaged in a laboratory mill, amylopectin was preferentially extracted which cold water[1,2]. The relatively mild damage which occurs during the milling of wheat[2] resulted in almost pure amylopectin being solubilised into cold water. On more severe damage, which results from the use of laboratory milling, starch granules were shown by scanning electron microscopy to crack and craze[2] and, although amylopectin was the major component solubilised by cold water, significant amounts of amylose were also present in the cold-water extract[2,3]. However, experiments on the developing kernels of barley[4] showed that, within the large (A-type) granules of barley starch, there was a relatively higher concentration of amylopectin in the core with increasing amounts of amylose towards the periphery. In order to reconcile these apparent differences, it was speculated[3,5] that there was a small number of clusters of amylopectin that protruded from the otherwise relatively uniform "hairy-billiard-ball" surface described by Lineback[6].

The purpose of the present study was to examine the surface of the starch granule, using enzymic techniques. Undamaged starch granules were washed free of any cold-water soluble materials, then incubated with bacterial isoamylase, and the material solubilised was assayed for total carbohydrate and for interaction with iodine. The results (Table I) showed that a small proportion of carbohydrate material was released. On interaction with iodine, the blue value[7] and the λ_{max} were consistent with data for linear oligosaccharides from debranched amylopectins.

The unit chains released by the action of isoamylase on wheat-starch granules were subjected to gel filtration chromatography on Sephadex G50SF. In Fig. 1, a peak associated with fractions 78–82 did not stain with iodine and corresponded to a mixture of malto-oligosaccharides with an average d.p. of ~ 15. The carbohydrate in fractions 48–70 interacted strongly with iodine and corresponded to a d.p. of about 40–45. Amylose, if present, would have been excluded from the gel, would have appeared in

* Dedicated to Professor David Manners.
† Author for correspondence.

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TABLE I

Action of isoamylase on starch granules

Source of isoamylase treated starch	*Yield of solubilised carbohydrate (%)*	*Action of solubilised iodine on carbohydrate*	
		Blue value	λ_{max}
Wheat	0.24	0.066	512
Barley (A-Granules)	0.14	0.048	510
Corn	1.22	0.103	536
Rice	0.53	0.068	525
Damaged wheat starch[a]	7.7	0.036	497
Damaged wheat starch[b] (no isoamylase)	0.29	0.612	630

[a] Micronised, washed, dried, then treated with isoamylase. [b] Micronised, washed, dried, then re-hydrated in buffer.

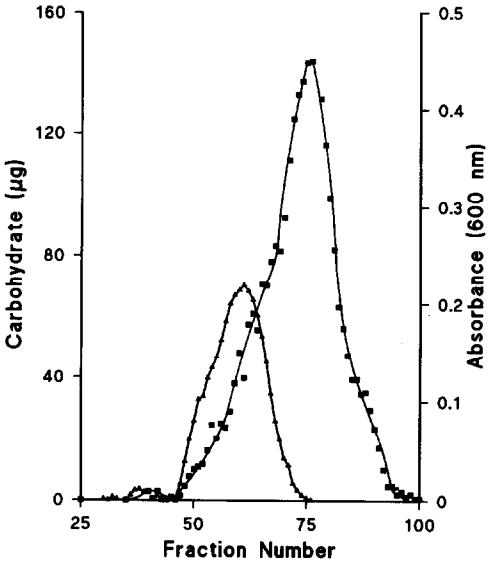

Fig. 1. Chromatography on Sephadex G50 SF of the products of the action of isoamylase on undamaged starch granules: ■, total carbohydrate (µg) per fraction; ▲, iodine-staining absorbance at 600 nm.

fractions 35–40, and given an intense colour with iodine. As can be seen from Fig. 1, there was little or no such material in this region.

Wheat-starch granules were damaged in a laboratory mill (microniser), washed with water, and dried. The yield of carbohydrate then solubilised on treatment with isoamylase was 7.7%, and this material was shown by blue value and by gel permeation

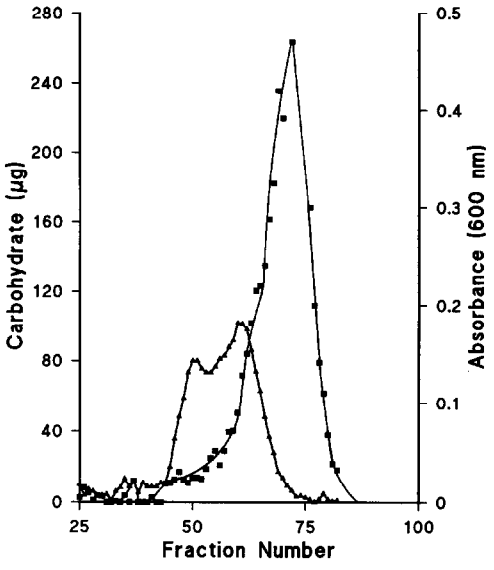

Fig. 2. Chromatography on Sephadex G50 SF of the products of the action of isoamylase on an aqueous extract of micronised starch granules: ■, total carbohydrate (µg) per fraction; ▲, iodine-staining absorbance at 600 nm.

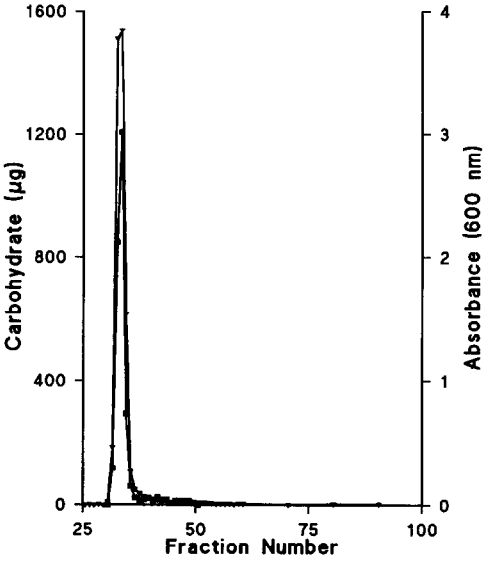

Fig. 3. Chromatography on Sephadex G50 SF of the products from damaged/washed/re-hydrated-starch granules: ■, total carbohydrate (µg) per fraction; ▲, iodine-staining absorbance at 600 nm.

chromatography to be chains derived from amylopectin (Fig. 2). Previous studies with scanning electron microscopy showed that this micronising procedure cracks the surface of the granules. In the present work, the newly exposed surfaces in the micronised granules were accessible to isoamylase, which released more amylopectin chains. When the micronised granules were treated with buffer in the absence of enzyme, a small amount of amylose (<4% of that released by the action of isoamylase on the damaged starch), as indicated by the blue value (Table I) and by gel permeation chromatography, was eluted as a single peak (Fig. 3).

Thus, isoamylase can release a small but significant number of chains from amylopectin molecules at the surface of starch granules. Areas of amylopectin on the surface of cereal-starch granules which are accessible to isoamylase could be the result of enzymic cleavage of protruding clusters of amylopectin or from dislocations in the layering of amylopectin where (1→6)-α-D-glucosidic linkages are exposed. The proportion of amylopectin chains liberated by the action of isoamylase on undamaged starch granules was equivalent to 0.24% of the total carbohydrate and is of the same order of magnitude as that of the pure clusters of amylopectin released from mildly damaged starch granules[8].

EXPERIMENTAL

Materials. — "Undamaged" wheat-starch granules were prepared by the method of Morrison and Gadan[9], as modified by McDonald and Stark[10], from a sample of wheat (variety Mercier). The purified wheat starch contained both large and small granules. Large (A-type) starch granules were isolated from the kernels of barley by extracting the crude starch as described above followed by repeated sedimentations until the number of small granules was negligible. Final purification was by shaking with toluene to remove protein. Using a haemocytometer, the preparation was shown to contain 92.1% of large granules by number (99.9% by weight). Rice and corn starches were standard laboratory samples prepared by the general procedure described above for wheat starch. Isoamylase was obtained from Hayashibara Biochemical Laboratories (Japan).

General methods. — The blue value and λ_{max} of iodine complexes were determined[7] by combining each fraction that contained a known amount of carbohydrate (~2 mg) with iodine solution (0.25 mL; 0.2% of iodine and 2% of potassium iodide) and M hydrochloric acid (4 drops) in a total volume of 25 mL.

Pre-treatment of the starch. — Starch (1 g) was washed with 0.01M acetate buffer (pH 3.8, 30 mL), mixed by rotation for 1 h, and then centrifuged (1800*g*, 10 min), and the supernant solution was discarded. The starch samples were resuspended in the same buffer and mixed by rotation for 1 h. The centrifugation and resuspension procedures were repeated a further two times, with the mixing stages shortened to 30 min.

A similar procedure was used for starch that had been damaged in a McCrone micronising mill for 10 min, except that all the washing times were 1 h in order to ensure the removal of readily extractable carbohydrate. After washing the starch with acetone

and centrifuging, the supernatant solution was discarded, and the starch was allowed to dry overnight. The starch (1 g) was mixed by rotation for 30 min with acetate buffer, then centrifuged (1800*g*, 10 min), and the supernatant solution was discarded. The damaged-washed sample was then treated as for the other samples.

Debranching of starch granules. — To portions of the washed starch were added 0.01M acetate buffer (pH 3.8, 2 mL) and isoamylase (glycogen 6-glucanohydrolase, EC 3.2.1.68) from *Pseudomonas amylodermosa* (50 μL, 3000 U). To the other portions, only buffer (50 μL) was added. Each mixture was then kept under toluene at 30° for 65 h with re-mixing every 24 h.

Distribution of chain lengths. — After incubation, each mixture was centrifuged (1800*g*, 10 min), filtered through glass-fibre filter paper (Whatman GF/A) under reduced pressure, and analysed for total carbohydrate[11]. The filtrate from the isoamylase digest was heated at 100° for 3 min, the denatured protein was removed by centrifugation, and an aliquot of the supernatant solution, containing a known amount (~ 5 mg) of carbohydrate, was applied to a column (0.02 × 0.87 m) of Sephadex G50SF and eluted with 0.02% sodium azide in 0.021M potassium hydroxide[12]. Fractions (3.5 mL) were collected, and aliquots (1 mL) were analysed for total carbohydrate[11]. To the remaining fraction (2.5 mL) was added M HCl (4 drops) and iodine solution (0.1 mL; 0.2% of iodine and 2% of potassium iodide), and the absorbance at 600 nm was measured. The column was calibrated by fractionating debranched waxy-sorghum amylopectin and measuring the reducing power[13] and total carbohydrate[11] for selected fractions. In order to obtain accurate values of reducing power for the longer-chain material, debranched polysaccharide (100 mg) was applied to the column.

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