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THE α(1-4)(1-6) GLUCANS FROM SWEET AND NORMAL CORNS

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Abstract—After removal of granular starch at low centrifugal force, the centrifugation, at increasing forces, of aqueous extracts of su_1 corn gave a series of α-glucan precipitates that contained amylose. The amylose content decreased as the force increased. In contrast, in normal corn all the α-glucan precipitated as starch granules at low forces. In the sweet corn precipitates, apart from the granular starch, the branched α-glucan was phytoglycogen. The MW of this decreased as the proportion of amylose decreased. It appears that, as well as starch granules and soluble phytoglycogen, sweet corn contains granules, smaller than starch, of a range of sizes, and these are made up of phytoglycogen and amylose. As granule size decreases, so does the MW of the phytoglycogen and the content of amylose. A method of quantitative extraction of starch giving minimal depolymerization is described. The isopotential iodine absorption of a quantitative extract of sweet corn flour indicated that the total ratio of linear (amylose) fraction to branched (amylopectin + phytoglycogen) fraction was near the normal value of 1:4.

INTRODUCTION

The α(1-4)(1-6) glucans of sweet corn have been extensively examined [1–11]. Morris and Morris [1] extracted a water-soluble glucan after soaking the seeds for several days, macerating and filtering. It produced a colour with iodine similar to that of glycogen. Hassid and McCready [2] extracted the flour after soaking overnight and filtering through a cloth. The filtrate was separated into two fractions. One was insoluble in 66% AcOH and was called starch as it gave a blue colour with iodine and had an average chain length ($\overline{CL}$, determined by methylation analysis) of 25. The soluble fraction was similar to glycogen ($\overline{CL}$ 12). Sumner and Somers [3] suggested the names glycoamylose for the first fraction to distinguish it from granular starch and phytoglycogen for the glycogen. Cameron [4] reported both red and blue staining particles in glycoamylose. Dvonch and Whistler [5] pointed out that amylose is not readily water-soluble. They extracted sweet corn flour with 10% trichloroacetic acid, centrifuged at 40000 rev/min and fractionated the supernatant into material insoluble in 67% AcOH (fraction 1) and a soluble fraction (2). Fraction 1 contained some blue staining material and gave an opalescent solution, whereas fraction 2 gave a clear solution and a purple–grey colour with iodine. Periodate oxidation indicated that the average chain length of fraction 1 was slightly higher than fraction 2 (12 and 11 glucose units). Fraction 1 was considered to contain a small amount of amylose. They also noted the widely differing yields of the two fractions reported in the literature. Peat *et al.* [8] extracted the water-soluble glucans, using Hg^{2+} to inhibit enzymic hydrolysis, and filtered the mixture through a celite pad. They found the chain length of fraction A to be higher than B (13 to 7) as also was the MW, and considered that amylose was not a significant constituent of either fraction and that acetic acid effected an arbitrary fractionation of a polymer-homologous series. Greenwood and Das Gupta [10,11] further examined

Table 1. Fractionation of α(1-4)(1-6) glucans from sweet corn and normal corn

	Fraction (*g*)	Corn variety and time after pollination (days)			
		n, 16	su_1, 16	*n*, 40	su_1, 40
Glucan isolated (% of total)	600 *g* ppt + fraction retained by muslin	99	55	99	39
	2000 ppt	≪1	2	0	5
	7000 ppt	0	4	0	7
	14000 ppt	0	6	0	10
	28000 ppt	0	7	0	10
	28000 supernatant	<1	26	<1	29
Absorbance of	600	0·03	0·82	0·04	0·51
supernatant	2000	0·01	0·53	0·00	0·36
(0·20 cm	7000	0·01	0·28	0·00	0·24
soln	14000	0·01	0·17	0·00	0·22
at 400 nm)	28000	0·01	0·07	0·00	0·16

the starch and water-soluble glucan. The starch was isolated by settling under gravity and the water-soluble glucan by ethanol precipitation after passage through a Sharples centrifuge. The two fractions of the water-soluble glucan showed a small difference in $\overline{CL}$ (13 and 11). Fraction A showed two peaks in ultra-centrifugation. They suggested, on the basis of the small amount of fraction B obtained, that the polysaccharide is essentially homogeneous. The granular starch had an iodine affinity of 5·5 g/100 *g* (28% amylose).

The difference in size and characteristics of fractions could be due to the different methods of extraction. Several authors noted the opalescence of the water extract. In the earliest studies some enzymic degradation may have occurred. In this paper a comparison has been made of the starch and water-extracted glucan from *n* and su_1 lines of similar genetic background.

RESULTS AND DISCUSSION

Normal and sugary corn seeds were macerated in dilute NaCl containing Hg^{2+} and toluene and filtered through muslin. After centrifugation, at 600 *g*, of the filtrate from samples collected both at 16 days after pollination and maturity, the supernatants of the two lines had a very different appearance. In the normal corn all the glucan precipitated, leaving a transparent solution. The su_1 supernatant was translucent and at a series of increasing centrifugal forces produced precipitates. The transparency of the supernatants at each stage in the centrifugations increased (Table 1). When these precipitates were dissolved, under N_2, in hot NaCl solution after prior dispersion in dimethyl sulphoxide (DMSO) and their iodine spectra compared, the wavelength of maximum absorption and the value of this maximum decreased as the centrifugal force producing the precipitate increased (Fig. 1). Similar behaviour was

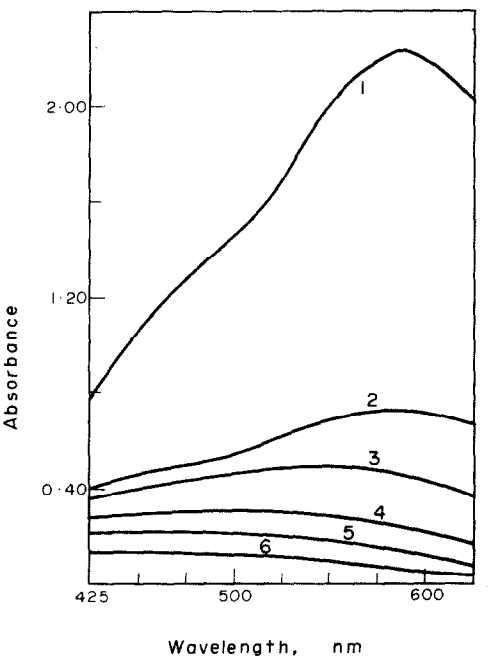

Fig. 1. Absorption spectra of glucan-iodine complexes of su_1 corn fractions at 40 days: 1–600 *g* ppt; 2–2000 *g* ppt; 3–7000 *g* ppt; 4–14000 *g* ppt; 5–28000 *g* ppt; 6–28000 *g* supernatant.

Table 2. Isopotential iodine absorption and apparent amylose content of fractions from corn (40 days)

Corn type	Fraction (*g*)	*I* absorption (g/100 *g*)	Apparent* amylose content (%)
n	600 ppt (starch granules)	5·36	28
su_1	600 ppt (starch granules)	5·77	30
su_1	2000 ppt	1·51	8
su_1	7000 ppt	0·85	4
su_1	14000 ppt	0·39	2
su_1	28000 ppt	0·04	<1
su_1	28000 supernatant	0·02	<1

* Calculated by assuming that amylose absorbs 19·2 g iodine per 100 *g* [12].

shown by extracts of sweet corn sampled 16 days after pollination, showing that this was not due to degradative processes associated with the ripening stages of the grain. The spectra suggested that the samples contained differing amounts of amylose. Measurements of isopotential iodine absorption (Table 2) were in agreement with this and the higher the centrifugal force producing the precipitate the lower the amount of amylose. Samples collected at 2000 *g* and 7000 *g* had definite amylose contents and even at 14000 *g* some was present. A possible interpretation of the ultra-centrifuge patterns shown by phytoglycogen A in a previous study [11] is that the more slowly moving peak is amylose.

When the dissolved precipitates were treated with *n*-butanol to remove amylose and the iodine spectra of the supernatants after centrifugation examined, the reduction in absorption was consistent with the removal of amylose. The absorptions from the 2000 and 14000 *g* samples were much lower than amylopectin and characteristic of phytoglycogen.

The precipitates were then dispersed in DMSO and dissolved, under N_2, in hot NaCl solution and chromatographed on agarose gel (Sepharose 2B) [13]. Aliquots were also treated with *n*-butanol to remove amylose prior to chromatography. The 2000, 7000 and 14000 *g* precipitates contained material that from the iodine spectra and elution behaviour on Sepharose 2B could be described as amylose (Figs. 2 and 3). It was removed by *n*-butanol. There was also a phytoglycogen fraction with an absorption maximum below

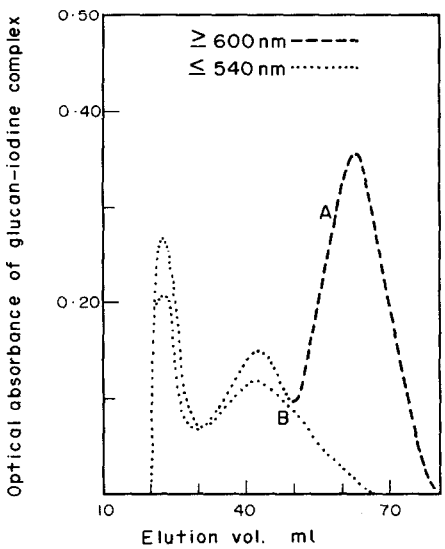

Fig. 2. Chromatography on 2% agarose gel of 2000 *g* ppt from su_1 corn at 40 days: A—before *n*-BuOH treatment; B—after *n*-BuOH treatment.

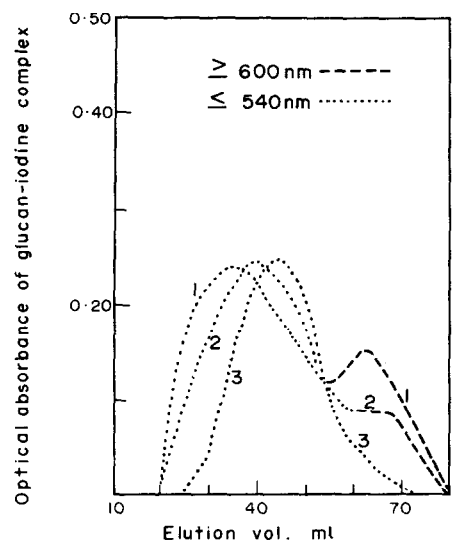

Fig. 3. Chromatography on 2% agarose gel of fractions from su_1 corn at 40 days: 1–7000 *g* ppt; 2–14000 *g* ppt; 3–28000 *g* ppt.

Table 3. Sedimentation values and average chain lengths of fractions not precipitated by *n*-butanol

Fraction (*g*)	$10^{13} \times S_{20}$	$\overline{CL}$ (D-Glc units)
su_1 28000 ppt	85	14·2
su_1 14000 ppt	88	n.d.*
su_1 7000 ppt	94	13·8
su_1 2000 ppt	103	13·8
su_1 600 ppt	n.d.	19·0
n 600 ppt	n.d.	19·7

* Not determined.

540 nm. The elution volumes of this indicated that the MW decreased as the centrifugal force at which the precipitated fraction was isolated increased. This was confirmed by separation of these phytoglycogen fractions using *n*-butanol complexing and their examination in the ultracentrifuge (Table 3). The sedimentation showed an absence of concentration dependence characteristic of phytoglycogen but not amylopectin. Determination of the $\overline{CL}$ from the formic acid released on periodate oxidation gave values consistent with a phytoglycogen structure (Table 3).

Thus, the α(1-4)(1-6) glucans of sweet corn do not divide simply into a water-soluble phytoglycogen and a granular starch. There is a significant fraction of small particulate material that contains phytoglycogen and amylose and this precipitates at 2000–14000 *g* after starch granules have been precipitated at 600 *g*. Then, in any fractionation procedure, as long as amylolysis is stopped, the properties of the fractions will be dependent on the methods used e.g. the type of filtration or the force applied in centrifugation.

A method of quantitative extraction of α(1-4)(1-6) glucan that caused minimal depolymerization was developed. This involved initial dispersion with DMSO [14,15], and precipitation into ethanol, followed by dissolution in NaCl solution under N_2 and precipitation of the glucan–iodine complex. This was dissociated with arsenite ion in buffered solution to avoid alkaline conditions. The decolorized solution was dialysed and the glucan precipitated by EtOH. The elution patterns on gel chromatography on Sepharose 2B of starch extracted from normal corn as granules and by this method were practically identical, indicating that any degradation is slight. The granules and the extracted starch were dispersed in DMSO prior to dissolution in NaCl solution for chromatography.

When the residue from aqueous extraction of sweet corn was treated by this method, the extract had an apparent amylose content estimated from iodine absorption of 43%. Chromatography on agarose gel (Sepharose 2B) of this material and the fraction not precipitated as the amylose *n*-butanol complex also indicated a high amylose content. This suggested that water-soluble phytoglycogen is extracted from an original glucan mixture where the ratio of amylose to amylopectin plus phytoglycogen may be similar to the usual starch values i.e. about 1:4. Also, in the elution pattern of the non-amylose fraction the elution volume and wavelength of maximum absorption of the iodine complex (<540) showed that aqueous extraction leaves some branched glucan, of lower MW than is usually found for amylopectin, with a glucan–iodine spectrum similar to phytoglycogen.

Quantitative extraction of sugary corn using the DMSO-arsenite method gave a glucan with an isopotential iodine absorption of 4·07 g/100 *g*, i.e. an apparent amylose content of 21%, showing that su_1 corn maintains a ratio of linear to branched polysaccharide within the usual range. Gel chromatography of the DMSO-arsenite extract and solubilized granules from sugary corn gave the patterns shown in Fig. 4. The low overall

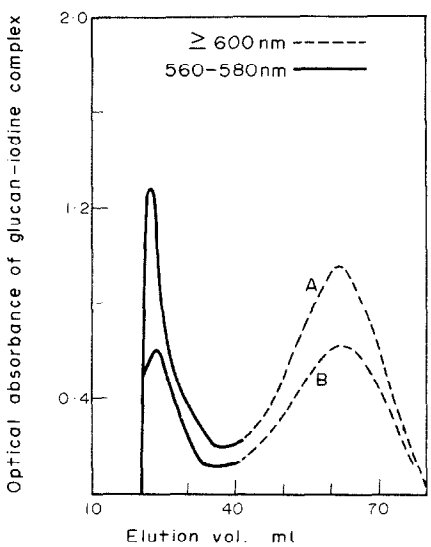

Fig. 4. Chromatography on 2% agarose gel of solubilized granules (A) and DMSO extract of whole flour (B) from su_1 corn.

colour of the flour extract is due to the low absorption of the phytoglycogen–iodine complex.

The increasing tendency for granule formation as the MW of the phytoglycogen increases suggests that the very high MW of amylopectin may be a factor in starch granule formation.

EXPERIMENTAL

Plant material. Samples were harvested at 16 and 40 days after hand pollination. The normal variety was inbred W22 and the su_1 was a back-crossed sub-line of W22.

Extraction and fractionation of starch granules and water soluble glucan. All plant material was macerated within one hour of harvesting. For samples collected at 16 days whole cobs were macerated whereas at 40 days the grains were removed from the husk prior to maceration. After maceration in 0·01 M $HgCl_2$, 0·1 M NaCl and toluene (5% of aq. vol) and filtering through muslin the residue was macerated and filtered 2×. Combined filtrates were centrifuged at 600 *g* for 30 min at 25°. The ppt of starch was collected and the supernatant, after 18 hr, separated into a toluene lipid layer and aq. lower layer. The aq. layer was again centrifuged at 600 *g* for 30 min and the ppt combined with the previous one, washed with EtOH, Me_2CO, Et_2O and dried. The optical density of the aqueous layer at 400 nm was measured. This layer was then centrifuged at 2000 *g* for 30 min at 25° and the precipitate washed with EtOH, Me_2CO, Et_2O and dried. The OD of the supernatant was measured. This process was repeated at 7000, 14000 and 28000 *g*. Final supernatant was poured into 3 vols of EtOH and the ppt washed with EtOH, Me_2CO and Et_2O and dried. The ppt at 14000 and 28000 *g* appeared more as a phase separation than a particulate precipitate.

Extraction of glucan with DMSO. Plant material was macerated in hot EtOH and soln boiled, filtered and washed with Me_2CO and Et_2O and dried. The flour was suspended in DMSO (to give a starch concentration of approx. 1–2%) and kept at 35° for 3 days. The mixture was agitated by a stream of N_2 gas free of O_2. After pouring into 3–4 vol. of EtOH, centrifuging and washing the precipitate with EtOH, Me_2CO and peroxide-free Et_2O, this precipitate was suspended in peroxide-free Et_2O. The mixture was heated at 100° in M NaCl (to give a starch concn of 1%) and agitated by a stream of N_2 (free of O_2). After cooling, the soln was centrifuged and re-extracted 2× with hot M NaCl ($\frac{1}{2}$ and $\frac{1}{3}$ of original vol.). Extracts were combined and the NaCl concn increased to 15 g/100 ml and 3% I_2/KI added and the soln stored at 0°. The starch–I_2 ppt was centrifuged at 14000 *g* for 30 min at 2° and decomposed by stirring in M/10 $NaAsO_3$ soln in 0·2 M PO_4 buffer (pH 6·0). The final starch concn was about 1%. The soln was dialysed for 24 hr, centrifuged at 14000 *g* for 30 min at 25° and the supernatant poured into 3–4 vols of EtOH, centrifuged, washed with EtOH, Me_2CO and Et_2O and dried in a vacuum desiccator. Methods for the *dispersion of fractions* and *fractionation with n-butanol* and *chromatography on agarose gel* have been described [13].

Measurement of isopotential iodine binding of glucans. Glucan (100–200 mg) was wetted with DMSO (3 ml) in a 50 ml volumetric flask and stood 18–24 hr. The gel was heated in a steam bath for 5 min, cooled and 0.05 M KI–0·05 M KCl soln added to the calibration mark. A 20 ml aliquot was transferred by pipette and dil. to 50 ml with 0·05 M KI–0·05 M KCl in a closed titration vessel fitted with a stirrer, platinum electrode and calomel half cell. The soln was titrated with 0·005 M I_2 in 0·05 M KI–KCl at 20°. Bound I_2 was plotted against free I_2 and the plateau region extrapolated to the bound I_2 axis.

Iodine spectra. Glucan (25·0 mg) was treated with DMSO (2·0 ml) and stood 18 hr. After heating at 100° 3 min, H_2O (20 ml) was added followed by M KOH (5·0 ml) and the soln shaken until all the polysaccharide dissolved. Alkali was neutralized with 0·5 M H_2SO_4 (5·0 ml) and the vol. made up to 50·0 ml. An aliquot was mixed with Ac' buffer (pH 4·7–10 ml), 0·1% I_2 in 0·1% KI (2·0 ml) and the vol. made up to 25·0 ml with H_2O. Spectra were recorded from 420–650 nm.

Estimation of average chain length. Amylopectin or phytoglycogen (600 mg) was wetted with Et_2O and dissolved in 0·56 M KCl (200 ml) at 15·0° and $NaIO_4$ (2·10 g) added. The reaction mixture was gently stirred in a bath at 15·0°. Aliquots were taken at 48 hr intervals, treated with ethylene glycol and titrated potentiometrically in a CO_2-free atmosphere against 0·01 M NaOH [16]. The end-point was taken at pH 6·25. The readings, after subtraction of a blank value, were plotted and extrapolated to zero time.

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REFERENCES

1. Morris, D. Z. and Morris, C. T. (1939) *J. Biol. Chem.* **130**, 535.
2. Hassid, W. Z. and McCready, R. M. (1941) *J. Am. Chem. Soc.* **63**, 1632.
3. Sumner, J. B. and Somers, G. F. (1944) *Arch. Biochem.* **4**, 7.
4. Cameron, J. W. (1947) *Genetics* **32**, 459.
5. Dvonch, W. and Whistler, R. L. (1949) *J. Biol. Chem.* **181**, 889.
6. Wolf, M. J., McMasters, M. M., Hubbard, J. E. and Rist, C. E. (1948) *Cereal Chem.* **25**, 312.
7. Meyer, K. H. and Fuld, M. (1949) *Helv. Chim. Acta* **32**, 757.
8. Peat, S., Whelan, W. J. and Turvey, J. R. (1956) *J. Chem. Soc.* 2317.
9. Maywald, E., Christensen, R. and Schoch, T. J. (1955) *Agr. Food Chem.* **3**, 521.
10. Greenwood, C. T. and Das Gupta, P. C. (1958) *J. Chem. Soc.* 707.
11. Greenwood, C. T. and Das Gupta, P. C. (1958) *J. Chem. Soc.* 703.
12. Anderson, D. M. W. and Greenwood, C. T. (1955) *J. Chem. Soc.* 3016.
13. Matheson, N. K. (1971) *Phytochemistry* **10**, 3213.
14. Libby, R. A. (1970) *Cereal Chem.* **47**, 273.
15. Whistler, R. L. and Be Miller, J. N. (1962) *Arch. Biochem. Biophys.* **98**, 126.
16. Anderson, D. M. W., Greenwood, C. T. and Hirst, E. L. (1955) *J. Chem. Soc.* 225.