STUDIES OF PROTEINS OF DEVELOPING WHEAT ENDOSPERM: FRACTIONATION BY ION-EXCHANGE CHROMATOGRAPHY

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Summary

Protein fractions were extracted from endosperm isolated from developing grains of Gabo, Insignia, and Dural varieties of wheat. The changes in total nitrogen and in the distribution of nitrogen in the various extracts were studied.

During development there was a rapid increase in the total endosperm nitrogen. Most of this increase in nitrogen was due to an increase in the acetic acid-soluble proteins.

Proteins present in the sodium pyrophosphate and acetic acid extracts were chromatographed on diethylaminoethyl (DEAE)- and carboxymethyl (CM)-cellulose respectively. The chromatographic elution patterns remained relatively constant for each variety. The amount of each component per grain increased throughout development.

I. INTRODUCTION

This paper reports part of an investigation of protein synthesis and accumulation during development of wheat grain. Changes in protein composition of endosperm have been determined by differential extraction of the proteins and subsequent fractionation of the extracts by ion-exchange chromatography. Three wheat varieties of different genotype and having different baking characteristics were used.

II. MATERIALS AND METHODS

(*a*) *Plant Material*

The three wheat varieties selected were *Triticum vulgare* cv. Gabo and Insignia, and *T. durum* cv. Dural. The plants were grown in field plots at the Waite Institute, Adelaide. For the analytical studies, heads were harvested during 1959 and 1960 at selected time intervals between 12 days after flowering and maturity. At each harvest more than 1000 heads were collected by hand from randomized sites; edge effects were eliminated and sampling errors were reduced, as far as possible, by the use of standard statistical procedures. The heads were transferred to a room at 2°C, mixed, and then stored at −15°C. The frozen grain was separated from the heads by a mechanical thresher at −15°C and the chaff removed by suction by using a vacuum cleaner.

Endosperm was isolated from frozen grain by hand-dissection for the harvests up to 39 days after flowering, and was stored at −15°C (Jennings and Morton 1963). For extraction of proteins the endosperm was freeze-dried and then ground to a fine powder in a mortar.

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Grain from the later harvests (33 days after flowering to maturity) was milled on a Buhler mill to approximately 70% extraction. It was necessary to reduce the moisture content of the immature grain to approximately 13% by freeze-drying before milling.

(*b*) *Extraction and Ion-exchange Chromatography of Proteins*

The endosperm preparations and the flour samples were extracted after the method of Coates and Simmonds (1961) by the standard procedure previously described (Graham 1963), except that four additional extractions were made with 0·1N NaOH. The proteins soluble in sodium pyrophosphate were separated by chromatography on diethylaminoethyl (DEAE)-cellulose (Coates and Simmonds 1961; Simmonds 1962), and those soluble in acetic acid by chromatography on carboxymethyl (CM)-cellulose (Simmonds and Winzor 1961). Three or six columns (1·8 by 15 cm) were used and were eluted simultaneously with buffer pumped from a common reservoir by a bank of micro-pumps.

Protein in the initial extracts was estimated from the nitrogen content determined by a microKjeldahl procedure, and that in column effluents generally from the optical density at 280 mμ. The protein content of turbid fractions which occur when eluting the acetic acid-soluble proteins from CM-cellulose with sodium phosphate and sodium hydroxide was determined by the Lowry procedure (Lowry *et al.* 1951).

III. Results

The total endosperm nitrogen present per grain during development, together with the amounts of nitrogen extracted into sodium pyrophosphate, acetic acid, and sodium hydroxide were measured, and the results obtained for the 1959 and 1960 growing seasons are shown in Figure 1. In all three varieties there is a rapid increase in endosperm nitrogen during development. Although the nitrogen present in both the sodium pyrophosphate and sodium hydroxide extracts increases, the greater part of the increase in total nitrogen is due to the changes in the acetic acid-soluble proteins. Nitrogen present in the n-butanol extracts and remaining in the residue after extraction with sodium hydroxide is omitted as this never exceeded 5 μg per grain.

The chromatographic elution patterns for the three different wheat varieties were similar to those reported previously (Simmonds and Winzor 1961; Simmonds 1962). The elution patterns for three stages of development of Insignia, expressed on a per grain basis are shown in Figure 2. From the sodium pyrophosphate extract eight fractions were obtained as reported by Simmonds (1962), although in both the Insignia and Dural samples fraction D appeared as two peaks. From the acetic acid extracts 10 fractions were obtained as described by Simmonds and Winzor (1961).

In determining the relative distribution of nitrogen in each fraction, the total absorbance of each was calculated and this was expressed as a percentage of the total absorbance recovered from the column. The protein content of the turbid fractions determined by the Lowry method was expressed in terms of equivalent absorbance readings. Chromatographic separations of each extract were carried out in triplicate; variations in the distribution of nitrogen in each component did not exceed ±5%.

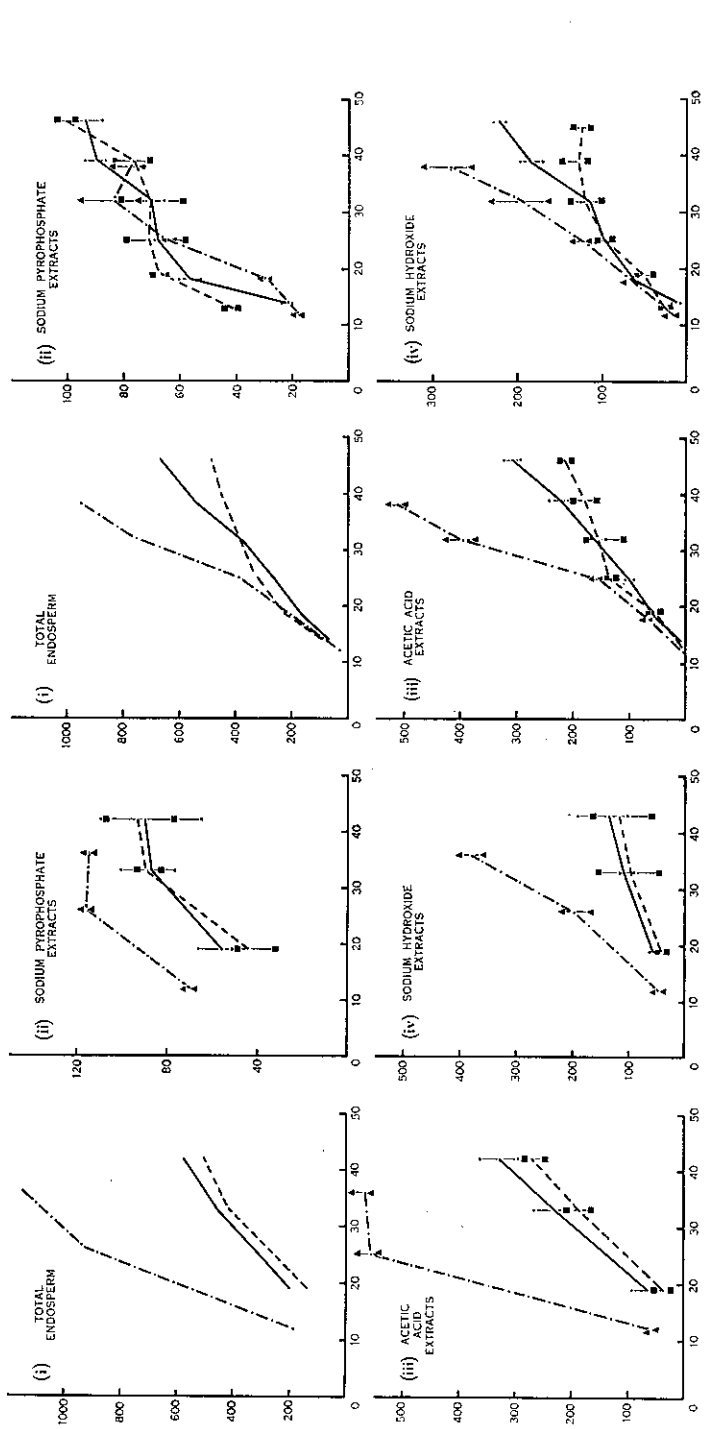

Fig. 1.—Changes in nitrogen content expressed as μg per grain, of developing endosperm of *T. vulgare* cv. Gabo and Insignia, and *T. durum* cv. Dural with time from flowering: (i) total endosperm nitrogen; (ii) nitrogen extracted with 0·01M sodium pyrophosphate, pH 7·4; (iii) nitrogen extracted with 0·05N acetic acid, pH 3·5; (iv) nitrogen extracted with 0·1N sodium hydroxide. (*a*) 1959 growing season; mean values and range of three separate extractions for each variety are shown. (*b*) 1960 growing season; mean values and range of two separate extractions for each variety are shown. Values for 32 days and for 39 days (Gabo and Insignia) after flowering are the means of duplicate extractions on both hand-dissected and milled endosperm material.

From the nitrogen per grain extracted by sodium pyrophosphate and by acetic acid, and from the distribution of nitrogen in each of the fractions separated from these extracts, the amount of nitrogen present in each fraction at each stage of development of the endosperm was calculated.

Tables 1 and 2 show the nitrogen present per grain in each fraction of the sodium pyrophosphate and acetic acid extracts at each stage of development of the three varieties in the 1959 harvest. The results show that all the fractions are present throughout development.

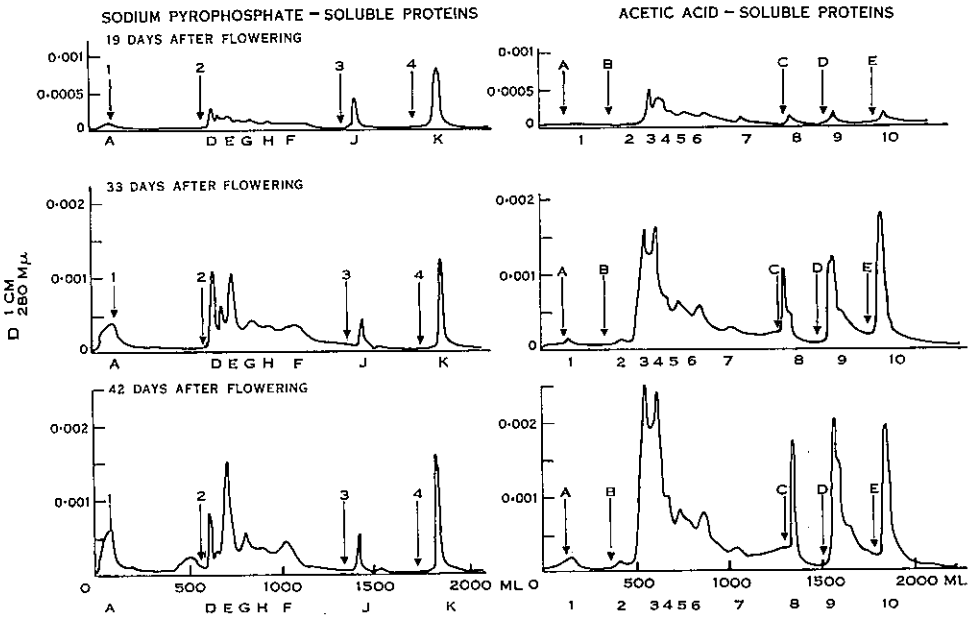

Fig. 2.—Changes in the chromatographic patterns of protein fractions of developing endosperm of *T. vulgare* cv. Insignia. Sodium pyrophosphate-soluble proteins were separated by chromatography on DEAE-cellulose, and the fractions were eluted as follows: *1*, 500 ml 0·006M glycine, pH 9·2; *2*, 1 l. gradient of 0·3M NaCl in glycine buffer; *3*, 400 ml 0·05N acetic acid; *4*, 400 ml 0·1N NaOH. Acetic acid-soluble proteins were separated by chromatography on CM-cellulose and the fractions were eluted as follows: *A*, 250 ml 0·005M sodium acetate containing 1·0M dimethyl formamide (DMF), pH 4·1; *B*, 1 l. gradient of 0·2M NaCl in acetate–DMF buffer; *C*, 250 ml 0·005M sodium acetate containing 1·0M DMF and 0·5M NaCl; *D*, 250 ml 0·005M trisodium phosphate containing 1·0M DMF and 0·5M NaCl; *E*, 500 ml 0·1N NaOH.

IV. Discussion

In early investigations with whole wheat grain (Woodman and Engledow 1924; McCalla 1938) it was found that both the salt-soluble nitrogen and the glutenin fraction increased throughout development, whereas gliadin increased rapidly in the later stages. Similarly it has been reported that hordein increased rapidly during the later stages of development of barley (Bishop 1930). Results obtained with wheat plants injected with [^{14}C]acetate were also interpreted as indicating that gliadin is formed at a later stage of development and independently of other protein components (Bilinski and McConnell 1958). However, Figure 1 shows that the storage protein,

represented by the sum of that soluble in acetic acid and that soluble in sodium hydroxide, increases rapidly throughout the period of development studied.

No attempt is made here to correlate the acetic acid-soluble proteins and the sodium hydroxide-soluble proteins with ethanol-soluble protein (gliadin) and dilute alkali-soluble protein (glutenin), the classical subdivision of wheat gluten (Osborne 1907). Separation of ethanol-soluble proteins and acetic acid-soluble proteins by starch-gel electrophoresis (Graham 1963) has shown that these groups of proteins contain identical protein components. The protein extracted into sodium hydroxide may contain some denatured protein which would otherwise be soluble in pyrophosphate and acetic acid. This is indicated in the decrease in the proportion of both the

TABLE I

PROTEIN FRACTIONS SOLUBLE IN SODIUM PYROPHOSPHATE OBTAINED FROM DEVELOPING GABO, INSIGNIA, AND DURAL ENDOSPERM

Nitrogen present in each fraction expressed as μg/grain. Values are means of triplicate determinations

Variety	Days after Flowering	Total Nitrogen	Chromatographic Fractions						
			A	D	E	G and H	F	J	K
Gabo	19	33·3	2·2	4·3	4·1	2·0	5·7	0·8	14·2
	33	61·6	7·7	10·0	9·9	5·0	13·9	1·7	13·4
	42	72·6	10·2	11·1	15·4	4·8	18·9	4·2	8·3
Insignia	19	27·3	2·3	3·3	2·2	4·7	4·7	1·9	8·2
	33	64·1	8·6	9·4	12·6	11·4	12·6	2·0	7·6
	42	76·0	14·7	5·9	15·3	15·0	11·9	3·6	9·9
Dural	12	37·3	1·9	7·6	2·9	3·1	5·9	2·2	13·6
	26	81·3	10·9	19·8	7·1	10·1	17·2	4·3	11·6
	36	91·7	16·2	11·6	5·0	8·3	34·0	4·4	12·0

pyrophosphate- and acetic acid-soluble nitrogen in 1959 Dural samples between 26 and 36 days, which was probably due to partial denaturation during milling, as considerable difficulty was encountered in the milling of this 36-day material. Variations occur in the proportion of nitrogen present in each extract according to methods of pretreatment of endosperm material; there is an increase in the proportion of sodium hydroxide-soluble protein both in milled samples as compared to hand-dissected samples, and after extraction of lipids with n-butanol. The pyrophosphate- and acetic acid-soluble proteins have been separated further by ion-exchange chromatography. The fractions (A–K of the pyrophosphate-soluble proteins, and 1–10 of the acetic acid-soluble proteins) are heterogeneous when separated by starch-gel electrophoresis (Graham 1963); nevertheless, the patterns are reproducible and therefore measure changes in protein fractions during development.

Figure 2 shows that, during development, the changes in protein composition are quantitative rather than qualitative. Tables 1 and 2 show that the amount of

TABLE 2

PROTEIN FRACTIONS SOLUBLE IN ACETIC ACID OBTAINED FROM DEVELOPING GABO, INSIGNIA, AND DURAL ENDOSPERM

Nitrogen present in each fraction expressed as μg/grain. Values are means of triplicate determinations

Variety	Days after Flowering	Total Nitrogen	Chromatographic Fractions									
			1	2	3	4	5	6	7	8	9	10
Gabo	19	63·4	0·9	0·6	10·0	6·3	4·6	15·4	8·2	2·8	13·4	1·3
	33	231·8	2·5	0·9	47·5	19·0	13·9	45·7	26·0	15·5	50·1	11·1
	42	328·3	14·8	1·3	59·8	25·3	18·4	63·0	39·1	24·0	64·3	18·4
Insignia	19	34·9	0·8	0·6	5·2	8·3	2·7	5·2	7·0	1·2	1·8	2·1
	33	186·7	1·7	1·1	24·4	26·9	11·6	17·0	32·1	11·8	33·2	26·9
	42	271·4	3·5	0·8	36·4	51·0	15·5	22·5	46·7	15·5	43·2	36·4
Dural	12	53·6	2·3	1·4	10·8*		5·3	12·5	1·1	5·5	12·9	1·2
	26	558·4	35·7	3·9	111·1*		19·0	126·2	39·6	39·6	145·2	38·0
	36	568·0	4·0	6·2	131·2*		29·0	126·7	37·5	37·5	166·4	29·0

* Components 3 and 4 were not separated.

each of the protein fractions extracted increases during development. For each variety, the proportion of many of these fractions remains relatively constant. The proportion of fraction K (Table 1), however, declines during development in each variety. This fraction consists of residual material on the chromatographic column and which is soluble only in 0·1N sodium hydroxide. Fraction A (Table 1) and fraction 10 (Table 2) appear to increase in proportion during development in each variety. It can also be seen from Tables 1 and 2 that proportions of several other fractions also vary but it is perhaps premature at this time to interpret the significance of these trends. As there is little change in the amount of dialysable nitrogen per endosperm during development (Jennings and Morton 1963), the increase in pyrophosphate-soluble nitrogen per endosperm (Fig. 1) represents an increase in the pyrophosphate-soluble proteins. The differences in behaviour between the two seasons (Fig. 1) reflect marked differences in rainfall for the growing crop; 1959 was a year of low rainfall in South Australia. The results show that there is a more rapid increase in endosperm nitrogen during development of cv. Dural as compared with cv. Insignia and Gabo. Dural has larger grains and these have a higher protein content, on a dry weight basis, than do Gabo and Insignia.

Although the results described here are reproducible and show the overall changes occurring in the proteins of developing endosperm, ion-exchange chromatography fails to separate individual protein components of endosperm extracts. However, this can be achieved by starch-gel electrophoresis and as described in the the next paper of the series (Graham and Morton 1963), starch-gel electrophoresis gives a more detailed understanding of protein changes during endosperm development.

V. Acknowledgments

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