Variation in Protein Composition within the Endosperm of Hard Wheat

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ABSTRACT

Concentrations of glutamic acid and proline were higher, but those of aspartic acid, alanine, lysine, and arginine were lower, (a) in the protein of subaleurone endosperm than in the protein of inner endosperm derived from HRW wheat and from a predominantly hard, mixed grist; and (b) in an interstitial protein concentrate than in an adherent protein concentrate from the mixed grist. Starch-gel electrophoresis showed a higher gliadin-albumin ratio in subaleurone than in inner endosperm, and in the interstitial than in the adherent protein concentrate. It is concluded that storage protein represents a larger proportion of the total protein in subaleurone endosperm than in inner endosperm, and in interstitial than in adherent protein.

The outermost cellular layer ("subaleurone layer") of the starchy endosperm in a HRW wheat (13.7% protein) has been shown (1) to have 33 to 54% protein in comparison with 8 to 15% for the remainder of the starchy ("inner") endosperm. Protein contents of corresponding endosperm regions of a HRS wheat (14.7% protein) were estimated at 31 to 35% and 9 to 18%, respectively (2). Although the wheat grain contains only about 11% of subaleurone endosperm by volume (3), this layer, because of its high protein content, accounts for almost 25% of the total protein of the starchy endosperm in these wheats. It is therefore important to know whether the composition and quality of the subaleurone endosperm protein differ significantly from those of inner endosperm protein.

As part of an investigation of this problem, amino acid analysis has been carried out on the protein of samples of almost pure subaleurone and inner endosperm obtained from HRW wheat and from a commercial mixed grist (90% hard wheats). To elucidate the results, similar analyses have been carried out on concentrates of interstitial protein and adherent protein (4) sep-

arated from the mixed grist. Protein in the endosperm samples from the mixed grist has also been fractionated according to solubility, and has been examined by starch-gel electrophoresis.

MATERIALS AND METHODS

Preparation of Endosperm Fractions from HRW Wheat

Flour (71.5% extraction) was milled in the laboratory from HRW wheat (13.7% protein; all protein data are calculated as N times 5.7, and expressed on 14% moisture basis), the various machine flours being kept separate. The later-break flours, rich in subaleurone endosperm, were subsequently bulked together and air-classified on a Bahco air elutriator at 35 μm cut size.[1] Particles of free interstitial protein and the free starch granules entered the finer fractions, leaving the entire endosperm cells, groups of cells, and substantial cell fragments in the coarse fraction. A blend of the head reduction flours which contained relatively little subaleurone endosperm was similarly air-classified at 35 μm. From the two coarse air-classified fractions, after they were defatted with petroleum ether, samples of a few g. of subaleurone endosperm and inner endosperm, respectively, were obtained by differential sedimentation in mixtures of carbon tetrachloride (density: 1.594 g./ml. at 20°C.) and benzene (density: 0.875 g./ml. at 20°C.) adjusted to particular densities, as previously described (1). Fractions floating at a density of 1.39 g./ml. were subaleurone endosperm containing $< 5\%$ of inner endosperm (as assessed microscopically); fractions sinking at a density of 1.44 g./ml. were inner endosperm containing $< 2\%$ of subaleurone endosperm. The subaleurone and inner endosperm fractions were further purified, to remove traces of interstitial protein, free starch granules, and aleurone cell contents, as described (1), and the purified fractions were used for amino acid analysis. The protein contents of the fractions analyzed were: subaleurone endosperm 55.5%, inner endosperm 9.5%.

Preparation of Fractions from Mixed Grist

To obtain larger quantities of subaleurone and inner endosperm for more extensive tests, a fresh fractionation was made, starting with 280 lb. of a third-break flour commercially milled from a mixed grist (13.1% protein) consisting of 55% Manitoba No. 2, 35% HRW, and 10% Australian wheats. The third-break flour had 15.1% protein. The fractionation employed air classification and flotation-sedimentation procedures similar to those used in the fractionation of the HRW flour (except that tetrachloroethylene, density 1.61 g./ml. at 25°C., and toluene, density 0.868 g./ml. at 20°C., were used instead of carbon tetrachloride and benzene for the suspending medium to reduce the toxicity hazard). Protein contents of the separated subaleurone and inner endosperm were 40.5 and 13.2%, respectively.

The third-break flour was also classified at 17 μm cut size and from the fine, 0 to 17 μm air-classified fraction was obtained, by flotation, a portion (density below 1.41 g./ml.) having 44.3% protein and consisting of a con-

[1]Editor's Note: The British μm (micrometer) is equivalent to 1 μ (micron) or 10^{-6} meter.

centration of interstitial protein, most of it derived from the inner endosperm. From the intermediate (17- to 35-μm) air-classified fraction was obtained a portion (density 1.43 to 1.47 g./ml.) having 4.9% protein. The protein in this fraction, which consisted mostly of starch granules, was a concentration of adherent protein, much of it derived from the inner endosperm.

Amino Acid Analysis

Nitrogen was determined by the Kjeldahl method. For the amino acid analysis, samples were weighed into screw-capped tubes fitted with Teflon seals and constant-boiling hydrochloric acid added (1,000 times wt. of sample). After flushing with nitrogen, hydrolysis was for 24 hr. at 105°C. The acid was removed by rotary evaporation at room temperature and the residue taken up in 0.1N HCl containing nor-leucine before loading into a Technicon analyzer. Correction factors were applied for loss of serine and threonine and incomplete liberation of valine and isoleucine. Separate analyses were carried out on samples previously subjected to performic acid oxidation by the method of Hirs (5), except that the acid was removed by freeze-drying without dilution and the materials were subsequently treated with water and again freeze-dried; this latter process was repeated three times. Values for CyS-2 and Met (when higher than for the unoxidized samples) were incorporated into the results.

Starch-gel Electrophoresis

The method previously described by Ewart (6) was used. Solutions containing about 15 mg. of protein (N times 5.7) per ml. were made up from all samples except the adherent protein concentrate, for which the protein solution contained 25 mg. per ml. Approximately 0.1 ml. of solution was applied to the sample slot of the starch gel. Mercuric chloride, 50 p.p.m., was incorporated into the protein solutions to inactivate beta-amylase (7). Density of the patterns was measured by scanning on a Chromoscan densitometer (Joyce, Loebl & Co., Ltd.), and the areas under the parts of the curves corresponding to gliadins and albumins, respectively, were computed and expressed as a ratio.

Fractionation of Protein According to Solubility

Appropriate weights of the endosperm fractions from the mixed-grist third-break flour, chosen so that each contained 1 g. of protein, were slurried with 100 ml. of 1% NaCl solution and dialyzed against 1% NaCl solution for 36 hr. at 0°C.; a viscose semipermeable membrane was used. Each slurry was then centrifuged for 20 min. at 400 times *g*. The supernatant was collected and the centrifugate was redispersed in 50 ml. of 1% NaCl solution and centrifuged again. The supernatant was added to the first supernatant.

The centrifugate was dispersed in 100 ml. of 70% ethanol, allowed to extract for 18 hr. at room temperature, and centrifuged. The centrifugate was re-extracted, this time with 50 ml. of 70% ethanol, and again centrifuged, and the two supernantants were bulked. The residue was air-dried and ground in a mortar.

Nitrogen was determined on the extracts and residue. The dialysates were not recovered.

RESULTS

Amino acid analyses are shown in Table I. While the compositions of the proteins from the subaleurone and inner regions of the endosperm are generally similar, there are some important differences shown by the fractions from both wheats. The proportions of glutamic acid and proline are higher in the subaleurone protein than in that from the inner endosperm, whereas the inner endosperm has the higher proportions of aspartic acid, alanine, lysine, and arginine.

TABLE I. AMINO ACID COMPOSITION OF PROTEIN FROM INNER AND SUBALEURONE REGIONS OF WHEAT ENDOSPERM AND OF INTERSTITIAL AND ADHERENT PROTEIN CONCENTRATES

Amino acid	Hard Red Winter Wheat Endosperm		Manitoba-HRW-Australian Mixed Grist			
	Subaleurone	Inner	Subaleurone endosperm	Inner endosperm	Interstitial protein	Adherent protein
	g. amino acid N per 100 g. total N					
Asp	1.93	2.69	1.86	2.25	2.14	3.59
Thr	2.11	2.31	1.88	2.10	2.05	2.15
Ser	5.04	4.97	4.81	4.78	5.05	4.67
Glu	22.36	18.02	21.08	18.99	21.49	15.91
Pro	10.43	8.61	9.20	8.26	8.81	7.02
Gly	3.88	3.89	3.48	3.63	3.48	3.83
Ala	2.47	3.15	2.27	2.62	2.53	3.00
Val	2.94	3.25	2.68	2.87	2.89	2.88
CyS	1.81	1.78	1.24	1.24	1.42	0.89
Meth.	0.78	0.78	0.62	0.65	0.80	0.54
Ileu	2.67	2.53	2.17	2.33	2.44	2.18
Leu	4.59	4.52	4.11	4.36	4.53	4.02
Tyr	1.67	1.51	1.44	1.42	1.57	1.19
Phe	2.98	2.56	2.66	2.52	2.65	2.26
Lys	1.59	2.51	1.77	2.15	1.97	2.72
His	3.35	3.54	3.02	3.05	3.10	3.14
Arg	6.53	7.62	6.05	6.93	6.92	7.76
Total	77.13	74.24	70.34	70.15	73.84	67.75
Protein (N × 5.7, 14% m. b.):	55.5	9.5	40.5	13.2	44.3	4.9

A similar difference in ratio of glutamic acid + proline to aspartic acid + alanine + lysine + arginine exists between interstitial and adherent protein concentrates, although the adherent protein has a more extreme amino acid composition than any other fraction. As would be expected, amino acid composition of inner endosperm protein is intermediate between those of interstitial and adherent protein concentrates, the latter fractions being derived mainly from inner endosperm.

Starch-gel electrophoresis patterns of the four fractions from the mixed grist are shown in Fig. 1. The ratio of gliadin to albumin is greater in subaleurone endosperm than in inner endosperm, and greater in the interstitial than in the adherent protein concentrate. This conclusion is supported by the densitometer readings, which were as follows:

	Ratio of Gliadin to Albumin		
	Pattern 1	Pattern 2	Mean
Subaleurone endosperm	1 : 0.20	1 : 0.33	1 : 0.26
Inner endosperm	1 : 0.88	1 : 0.64	1 : 0.76
Interstitial protein conc.	1 : 0.46	1 : 0.44	1 : 0.45
Adherent protein conc.	(1 : 1.61)	1 : 0.77	1 : 0.77

The value in parentheses has been excluded, as the very weak gliadin bands appear to be abnormal.

The tailing in the electrophoresis patterns is somewhat heavier in the subaleurone than in the inner endosperm, and this is believed to be chiefly the fraction of the glutenin which has a sufficiently low molecular weight to penetrate the pores of the starch gel.

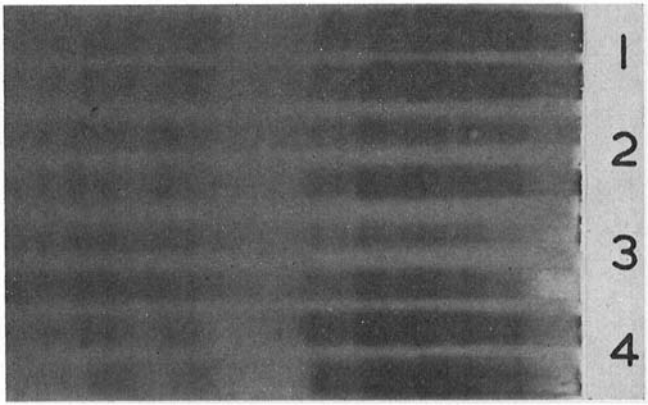

Fig. 1. Starch-gel electrophoresis patterns of the proteins of endosperm fractions separated from mixed-grist third-break flour: 1, subaleurone endosperm (40.5% protein); 2, inner endosperm (13.2% protein); 3, adherent protein concentrate (4.9% protein); 4, interstitial protein concentrate (44.3% protein).

TABLE II. FRACTIONATION OF ENDOSPERM PROTEIN ACCORDING TO SOLUBILITY (FRACTIONS FROM MANITOBA-HRW-AUSTRALIAN MIXED GRIST)

	Subaleurone endosperm	Inner endosperm	Interstitial protein	Adherent protein
		% of total protein		
Soluble in 1% NaCl	10.4	12.7	10.5	14.3
Soluble in 70% EtOH	34.0	38.2	37.7	32.7
Insoluble	46.2	48.2	41.2	47.9
Recovered	90.6	99.1	89.4	94.9

Results of the protein fractionation of the endosperm samples from the mixed grist according to solubility are shown in Table II. There was a larger proportion of salt-soluble protein in the adherent protein concentrate and in inner endosperm than in the interstitial protein concentrate and subaleurone endosperm.

DISCUSSION

The results presented for protein solubility and starch-gel electrophoresis are in line with findings of other workers who have reported a higher proportion of glutenin-gliadin and a lower proportion of "water-soluble" proteins

in the portions of endosperm having higher protein content than in those with lower (8), and in high-protein air-classified flour fractions than in low-protein fractions (9). Higher ratios of alcohol-soluble to salt-soluble protein (10) and of gliadin to albumin (11,12) have also been reported in interstitial protein than in adherent protein.

This difference is reflected in the amino acid compositions: the ratio (glutamic acid + proline): (aspartic acid + alanine + lysine + arginine) is much higher for glutenin and for gliadin (13) than for albumin (14) or globulin (15), and a similar ratio difference (although much smaller in magnitude) is shown between subaleurone and inner endosperm proteins, and between interstitial and adherent protein concentrates.

Hess (4) classified wheat endosperm protein as either: "Zwickel" (wedge, interstitial) protein, situated in the interstices between the tightly packed starch granules and released as particles of free protein when endosperm is finely ground; or "Haft" (adherent) protein, present as a thin fibrillar deposit adhering to starch granule surfaces. Hess's "Zwickel" and "Haft" proteins have some similarity to what others have described as "storage" and "membrane" protein, respectively, but the correspondence is not exact.

Considering the protein of the wheat grain physiologically, Jennings et al. (16) have shown that the storage protein of the developing wheat grain accumulates as "protein bodies," said to range up to 15 μm in diameter. The protein bodies are encapsulated by a lipoprotein membrane. As a result of the initiation of new starch granules and the enlargement of existing ones, together with progressive desiccation during ripening, the protein bodies undergo compression and shrinkage. Thus, as Buttrose (17) has suggested, it is probable that the endosperm protein matrix present at maturity consists largely of a conglomerate of dried protein bodies (with their surrounding membranes). If this be so, the relative proportions of storage to membrane protein would depend on the size of the protein bodies.

Buttrose (17,18) has shown that amyloplast membranes surround starch granules at least until the initiation of the smaller types of granule. The existence of a unit membrane-type structure completely enveloping all starch granules at maturity is, however, disputed by many and categorically denied by Badenhuizen (19). In this connection, Jennings and Morton (20) have shown that the absolute amount of lipid phosphorus, reflecting the amount of membrane material, increases in the developing wheat grain up to maximum dry weight stage. It is unlikely, therefore, that the membrane material is metabolized; part of the amyloplast membrane may remain at maturity and contribute to the adherent protein.

Before the rapid accumulation of starch and storage protein in the endosperm, the cells contain a proteinaceous cytoplasm. When the grain dries out, the remains of the cytoplasm, together with the endoplasmic reticulum (of similar structure to the membrane) may be deposited on the interfaces among the cell contents (largely starch granules and protein bodies). This cytoplasmic protein may therefore be a constituent of both the interstitial and the adherent protein.

It therefore seems that storage, membrane, and cytoplasmic proteins all

contribute to interstitial protein, with storage protein predominating. Adherent protein, however, is likely to consist only of membrane or cytoplasmic protein, or both.

Interstitial and adherent proteins (in Hess's sense) differ from one another in amino acid composition, particularly as regards the ratio (glutamic acid + proline): (aspartic acid + alanine + lysine + arginine); this ratio was (Table I: amino acid N basis) 2.24 for interstitial protein concentrate, 1.34 for adherent protein concentrate. Rohrlich and Niederauer (21) published data for amino acid composition from which this ratio (amino acid basis) can be calculated as 4.1 for interstitial protein, 1.4 for adherent protein. Golenkov (10), however, reported no difference between interstitial and adherent protein in amino acid composition, despite a well-marked difference in ratio of salt-soluble to alcohol-soluble components.

During maturation of the wheat grain, the amino acid composition of the total endosperm protein shifts so that the proportions of glutamic acid and proline increase, whereas those of aspartic acid, alanine, lysine, glycine, and valine (22,23) and arginine (22) decrease. The ratio of acetic acid-soluble to salt-soluble protein also increases continuously during development of the grain (22). This is an indication that the proportion of storage protein is increasing relative to the amount of membrane protein present.

The samples described as interstitial and adherent protein, for which data are presented in this paper, represent only a partial separation of interstitial from adherent protein, and the protein in them only a relative concentration of the types of protein named. Nevertheless, the two samples will differ in their ratios of storage to membrane protein, and this difference is shown by the higher gliadin:albumin ratio in the interstitial than in the adherent protein concentrate.

The differences between the protein of subaleurone and inner endosperm reported here are interpreted as indicating that the ratio of storage protein to membrane protein (and of interstitial protein to adherent protein) is higher in subaleurone than in inner endosperm, and that this conclusion is consistent with the observed differences in the gliadin:albumin ratio and in the amino acid composition.

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