STUDIES OF GLUTENIN. VIII. SUBUNIT COMPOSITION AT DIFFERENT STAGES OF GRAIN MATURITY[1]

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ABSTRACT

Glutenins of bread and durum wheats harvested at different stages of maturity showed minor qualitative and quantitative differences in subunit composition determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. In bread wheat glutenin, the 80,000 molecular weight (mol wt) subunit was either absent or present at very low concentration at early stages of maturity; its proportion increased with maturation. In durum wheat glutenin, the 68,000 mol wt subunit showed analogous changes. In other respects, the subunit composition remained the same from early stages of development to full maturity. Relative proportions of some of the subunits changed slightly during maturation. Molecular weights of the largest subunits of bread and durum wheat glutenins were revised downward from 150,000 and 120,000 to 134,000 and 110,000, respectively.

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Glutenin, one of the protein fractions of wheat flour, is important in influencing breadmaking quality (1). Glutenin, in particular, plays an important role in dough development during mixing (2). Its molecules are made up of approximately 15 polypeptide chains (subunits) held together by disulfide bonds (3). The number and location of disulfide bonds and the structure of glutenin molecule(s) remain to be discovered.

Glutenin is synthesized and deposited in wheat grain at the same time as the other proteins during development (4). The present article reports on the subunit

TABLE I
Hard Red Spring Wheat Samples at Different Stages of Maturity

Sample	Harvest Date	Days after Anthesis[a]	Protein Content[b] %	Maturity[c]
M-2	Jul 28	19	12.5	Immature
M-3	Aug 2	24	12.9	Immature
M-4	Aug 7	29	13.6	Immature
M-5	Aug 12	34	14.8	Intermediate
M-6	Aug 17	39	14.5	Partially mature
M-7	Aug 22	44	14.4	Partially mature
M-8	Aug 27	49	14.0	Mature
M-9	Sept 1	54	14.5	Mature
M-10	Sept 6	59	14.5	Mature

[a]Approximate date of anthesis was July 10.
[b]Expressed on a 14% moisture basis.
[c]Assessed by visual examination by experienced wheat grader.

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composition of bread (common or hexaploid) and durum (tetraploid) wheat glutenins at different stages of grain maturity as further evidence for the unique nature of this protein.

MATERIALS AND METHODS

Grain of two wheat varieties of different classes was harvested from field plots at various stages after anthesis and dried by freeze-drying. Canadian hard red spring wheat variety Manitou was selected to represent the common or bread wheat class and the variety Stewart 63 was chosen as the representative of the durum wheat class. The same varieties were used in earlier studies of glutenin in developing grain (4).

Tables I and II list the samples that were harvested together with grain protein contents to provide a more detailed description of the samples. Maturity (last column) was determined visually by an experienced wheat grader of the Inspection Division of the Canadian Grain Commission.

For extraction of glutenin, the grain was ground to a fine meal in the Udy cyclone grinder. Glutenin was prepared from the meal by the pH precipitation method of Orth and Bushuk (5). Gluten (required for preparation of glutenin) could not be washed from the first Manitou sample (see Table I) and the first three Stewart 63 samples (see Table II). Glutenin of these four and a number of more mature (for comparison of methods) samples were isolated by the modified Osborne procedure of Chen and Bushuk (6).

Whole meal and flour were defatted as follows: 10-g samples were extracted twice with 100 ml each of n-hexane, filtered on a Buchner funnel, extracted two more times with 100 ml each of petroleum ether, filtered, and air-dried.

Glutenin was reduced, complexed with sodium dodecyl sulfate (SDS), and examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously (7).

For molecular weight (mol wt) estimation, the following proteins were used to calibrate the gels: bovine serum albumin (Calbiochem), pepsin (Nutritional Biochemicals), α-chymotrypsin (Calbiochem), and cytochrome C (Calbiochem).

TABLE II
Durum Wheat Samples at Different Stages of Maturity

Sample	Harvest Date	Days after Anthesis[a]	Protein Content[b] %	Maturity[c]
63-2	Aug 7	31	...	Immature
63-3	Aug 12	36	12.3	Immature
63-4	Aug 17	41	12.9	Immature
63-5	Aug 22	46	13.3	Intermediate
63-6	Aug 27	51	13.4	Partially mature
63-7	Sept 1	56	14.2	Partially mature
63-8	Sept 6	61	14.3	Mature
63-9	Sept 11	66	14.4	Mature

[a]Approximate date of anthesis was July 8.
[b]Expressed on a 14% moisture basis.
[c]Assessed by visual examination by an experienced wheat grader.

Molecular weights *vs.* mobility curves for these proteins were obtained in the presence and absence of β-mercaptoethanol, a disulfide reducing agent. (Gluten subunits were examined in the presence of excess reducing agent.) Slightly different calibration curves (Fig. 1) were obtained for the two experimental conditions. Addition of reducing agent decreased the mobility. The effect was greatest for the largest molecules. This effect of reduction on mobility (and hence apparent molecular weight) has been reported previously (8,9).

RESULTS AND DISCUSSION

The subunit electrophoretograms of bread wheat glutenin isolated from washed gluten by the method of Orth and Bushuk (5) were qualitatively similar for grain samples harvested from 24 days after anthesis through to 59 days after anthesis (Fig. 2). These samples ranged from highly immature to fully mature. Approximately 14 bands can be distinguished for each sample by detailed examination of the stained gels, although not all bands reproduced clearly in the photograph of Fig. 2.

The electrophoretograms of subunits of bread wheat glutenin isolated by the Osborne procedure showed apparent qualitative differences between samples of different maturity (Fig. 3). The 80,000 mol wt subunit was absent in M-2 and M-3 and faintly visible in M-4, but stained quite strongly in M-5 and M-8, the more mature samples.

A major difference between the glutenins prepared by the two methods used is the intense band corresponding to the 80,000 mol wt subunit obtained for the

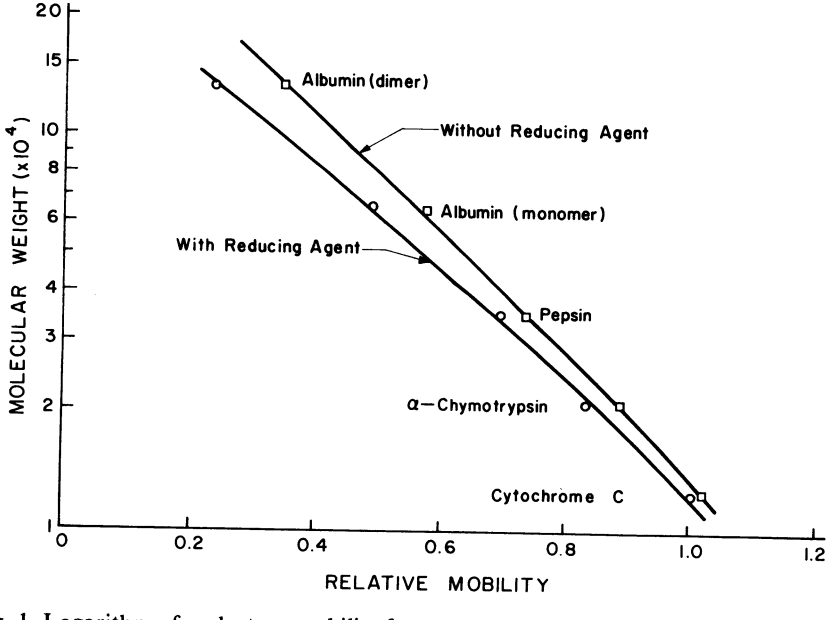

Fig. 1. Logarithm of mol wt *vs.* mobility for marker proteins in the presence and absence of β-mercaptoethanol, a disulfide reducing agent.

Osborne procedure glutenin from mature grain (compare Figs. 2 and 3). Further investigation showed that the stained band for the 80,000 mol wt subunit faded gradually during destaining. This fading was particularly rapid in the pattern for glutenin isolated from gluten washed from undefatted wheat meal; defatting the wheat meal decreased the rate of fading (results not shown). Furthermore, the fading was slow for glutenin isolated by the Osborne procedure compared with glutenin from washed gluten. Restaining of completely faded gels produced a weakly stained band for the 80,000 mol wt subunit, while the other bands restained to original intensity. It appears that wheat lipids interfere with the binding of the dye by the 80,000 mol wt subunit. This observation merits further investigation. For the present, it should be emphasized that for best results, the electrophoretograms of reduced glutenin should be photographed immediately after destaining.

As was found for the bread wheat samples, the subunit patterns of durum wheat glutenin isolated from washed gluten were also qualitatively similar for the stages of maturity examined (Fig. 4). Approximately 11 bands can be identified in these patterns.

Subunit electrophoretograms for durum wheat glutenin obtained by the Osborne procedure showed that the 68,000 mol wt subunit was absent in immature samples 63-2, 63-3, and 63-4 (Fig. 5). These results are analogous to

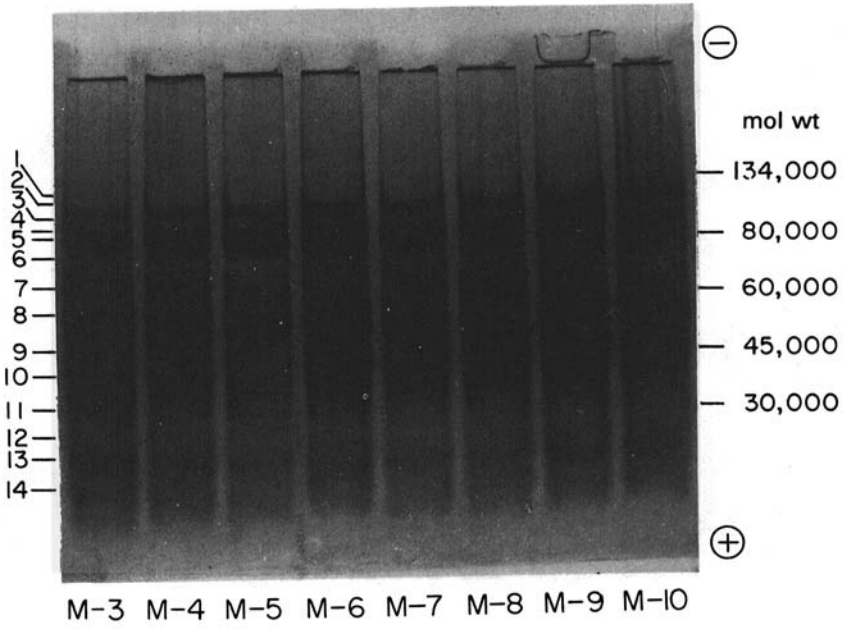

Fig. 2. SDS-PAGE electrophoretograms of reduced bread wheat glutenin isolated by the method of Orth and Bushuk (5) from grain harvested at different times after anthesis: M-3, 24 days; M-4, 29 days; M-5, 34 days; M-6, 39 days; M-7, 44 days; M-8, 49 days; M-9, 54 days; and M-10, 59 days.

TABLE III
Molecular Weights of Glutenin Subunits by SDS-PAGE Calibrated with and without β-Mercaptoethanol (Disulfide Reducing Agent)

Subunits	Molecular Weight	
	With reducing agent	Without reducing agent
Bread wheat		
1	134,000	150,000
2	132,000	140,000
3	110,000	120,000
4	98,000	105,000
5	90,000	98,000
Durum wheat		
1	110,000	120,000
2	90,000	105,000

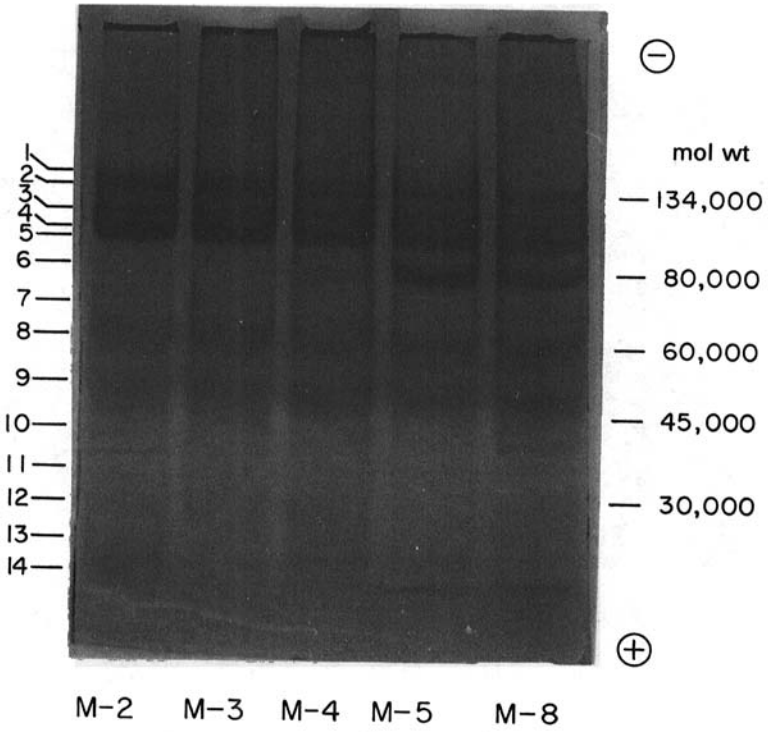

Fig. 3. SDS-PAGE electrophoretograms of reduced bread wheat glutenin isolated by the modified Osborne fractionation procedure from grain harvested at different times after anthesis: M-2, 19 days; M-3, 24 days; M-4, 29 days; M-5, 34 days; and M-8, 49 days.

those obtained for the bread wheat, except the subunit involved here has a lower molecular weight. The possible involvement of lipid in the staining intensity of the 68,000 mol wt subunit of durum wheat glutenin was not investigated. An 80,000 mol wt subunit was present in durum wheat glutenin at all stages of development examined. The equivalent subunit of bread wheat glutenin was present only in the electrophoretograms for the mature grain samples.

The electrophoretograms for the two types of glutenin (washed gluten and Osborne fractionated) at different stages of maturity were examined to determine if it might be possible to estimate the relative amounts of subunits. There is some indication that the relative proportion of the high-molecular-weight subunits increased as the grain approached full maturity. In the case of bread wheat, the 80,000 mol wt subunit appears to be absent (or present but at very low concentration) in immature glutenin, but is clearly visible as a strong band in the patterns of mature glutenin. In durum wheat glutenin, the 68,000 mol wt subunit shows similar changes with maturation. To what extent these qualitative and quantitative differences in glutenin subunit composition are related to functional properties remains to be investigated.

In previous SDS-PAGE studies of glutenin subunits in this laboratory (7), it

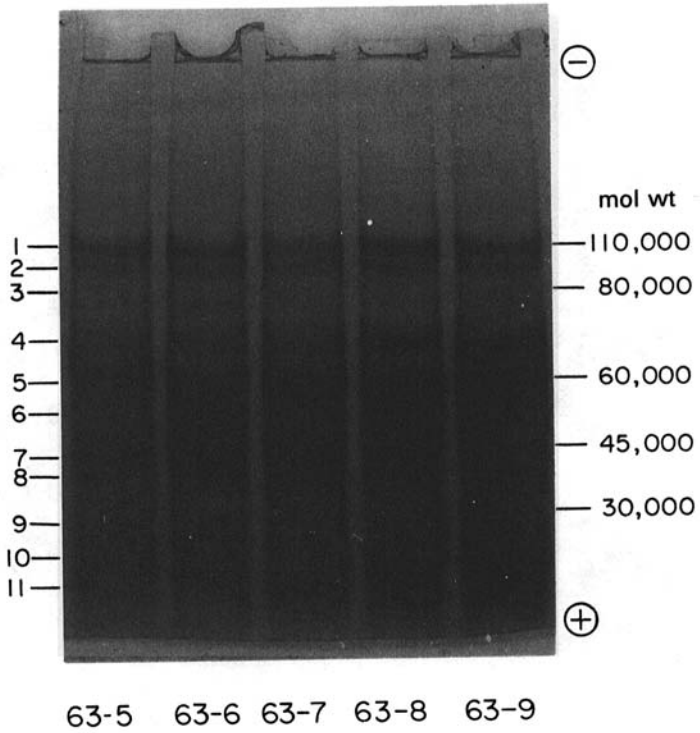

Fig. 4. SDS-PAGE electrophoretograms of reduced durum wheat glutenin isolated by the method of Orth and Bushuk (5) from grain harvested at different times after anthesis: 63-5, 46 days; 63-6, 51 days; 63-7, 56 days; 63-8, 61 days; and 63-9, 66 days.

was incorrectly assumed that reducing agents would not affect the mobility of the marker proteins used to calibrate the gels for molecular weight estimation. This assumption was tested experimentally and, as already indicated, inclusion of the disulfide reducing agent (as required for examination of reduced glutenin) in the marker proteins decreased their mobilities. This produced a lowering of the previously reported (7) subunit molecular weights by approximately 10%. The revised molecular weights of the subunits in the high-molecular-weight group are given in Table III together with the molecular weights obtained with gels calibrated without the addition of reducing agent to the marker proteins. The revised molecular weights agree better with the values of Bietz and Wall (3) obtained by SDS-PAGE at slightly higher pH (8.9 *vs.* 7.3).

This study of the subunit composition of glutenin of one bread wheat variety and one durum wheat variety at different stages of grain maturity has shown that the composition remains essentially constant from the earliest stage when glutenin can be isolated to full maturity of the grain. The absence of one subunit (80,000 mol wt for bread wheat and 68,000 mol wt for durum wheat) in immature glutenin should be confirmed by other techniques. On the basis of results obtained, it is not possible to establish unequivocally whether the qualitative difference observed is real or whether it is an artifact of the procedure used to

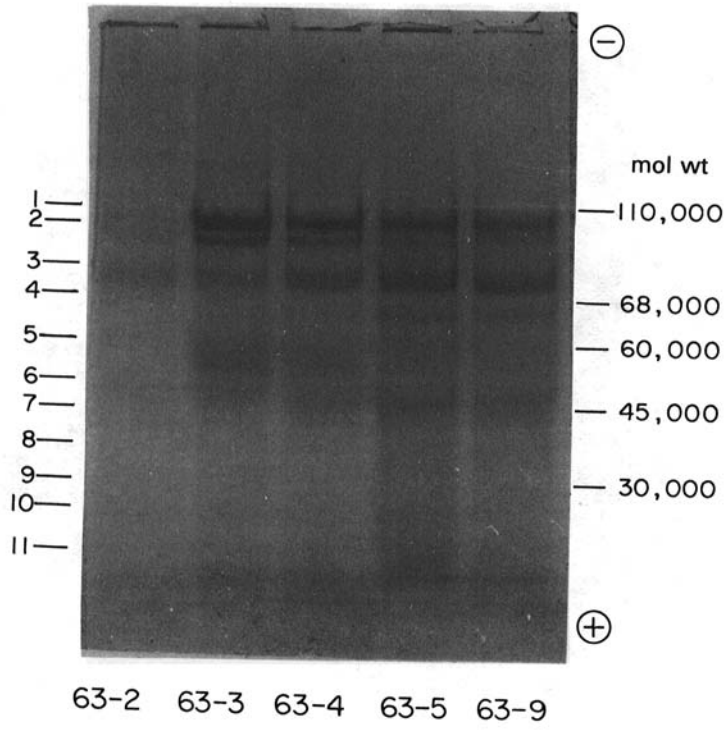

Fig. 5. SDS-PAGE electrophoretograms of reduced durum wheat glutenin isolated by the modified Osborne fractionation procedure from grain harvested at different times after anthesis: 63-2, 31 days; 63-3, 36 days; 63-4, 41 days; 63-5, 46 days; and 63-9, 66 days.

isolate the glutenin. The present study confirms a previous report (10) that the major difference between bread and durum wheat glutenins is in the high-molecular-weight group of subunits. In this group, bread wheat glutenin contains five subunits. Durum wheat glutenin lacks the two largest and the lowest subunits of this group (see Table III).

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