Changes in Amino Acid Composition of Maturing Hiproly Barley[1]

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ABSTRACT

Protein content and amino acid composition were determined at five stages of maturity in seven barley populations including the high-lysine/low-lysine pair, Hiproly and CI 4362. During maturation, concentrations (in the protein) of lysine, aspartic acid, alanine, and valine decreased more rapidly and concentration of glutamic acid increased to higher levels in CI 4362 than in Hiproly. However, amounts of some of the amino acids (i.e., lysine and aspartic acid) per kernel were identical in mature kernels of the pair. The results indicate that differences in concentrations of certain amino acids of the barleys may have resulted from differences in kernel development and protein deposition in the whole kernel or in specific kernel tissues.

Changes in amino acid composition of maturing barley have been the subject of several investigations. Danilova and Pleshkov (1) determined 17 amino acids in proteins of maturing barley grown on different soils. Soil type did not affect amino acid composition. During ripening, glutamic acid, proline, and glycine increased but lysine, histidine, arginine, and alanine decreased. Somewhat different results were found in investigations from our laboratory on changes in amino acids of three cultivars grown for 2 years (2). During maturation, increases were largest in glutamic acid, proline, and cystine; and decreases were largest in alanine, lysine, aspartic acid, and threonine. Ivanko (3), who reported similar changes, has shown that these changes occurred mainly as a result of increases in concentration of barley prolamines (hordein) which are characterized by high concentrations of glutamic acid and proline and low concentrations of aspartic acid, alanine, and lysine.

Recent reports on the improved nutritional value of high-lysine, high-protein Hiproly (*Hordeum vulgare* L.), CI 3947 (4,5,6),have stimulated barley research in many locations. Hiproly is of Ethiopian origin, two-rowed, erectoid, naked, shrivelled, and requires a long photoperiod. A morphologically similar line, CI 4362, has nonshrivelled seed. Both Hiproly and CI 4362 are rich in protein, but Hiproly has substantially more lysine in the protein and a higher nutritional value than CI 4362. Munck et al. (5) reported that the high-lysine and high-protein characters segregated independently.

Previous studies (2) indicated no significant differences in amino acid patterns among hulled cultivars harvested at comparable stages of maturity. The

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availability of a pair of barleys of similar protein content and differing in amino acid composition suggested additional studies. We compare here changes during maturation in amino acid composition of the pair Hiproly and CI 4362 and related barleys.

TABLE I. CERTAIN CHARACTERISTICS OF MATURE BARLEY KERNELS FROM SEVEN TWO-ROWED BARLEY POPULATIONS GROWN AT ABERDEEN, IDAHO IN 1971

Population	Character	Kernel Weight mg.	Protein[1] %	Lysine[2] %
Hiproly	Hull-less	38.2	21.5	4.1
CI 4362	Hull-less	55.2	20.7	2.8
61Ab4965-A	Hulled	57.3	14.6	3.4
M118	Hull-less	54.0	19.9	3.1
M219	Hull-less	35.7	21.5	4.2
M305	Hull-less	45.8	20.1	3.2
M360	Hull-less	38.2	18.4	4.2

[1]Percent, N × 6.25, dry matter.
[2]Percent in protein.

MATERIALS AND METHODS

Seven barley populations (described in Table I) were grown under irrigation at Aberdeen, Idaho in 1971. They included, in addition to the pair, Hiproly and CI 4362, a hulled cultivar designated 61Ab4965-A. 61Ab4965-A originated at Aberdeen as an F_{11} selection from "Piroline"/"Australian." The populations designated M118, M219, M305, and M360 were F_4 populations each derived from single F_2 plants from 61Ab4965-A/Hiproly. Selection of the latter four populations for the study was based, in part, on unpublished amino acid analyses for a series of F_2 plants grown at Mesa, Arizona in 1969-70. M219 and M360 averaged 4.06% lysine at Mesa, compared with 3.63% for Hiproly. M118 and M305 averaged only 2.61% lysine.

The populations were space-planted in May 1971 at 18-cm. intervals in adjacent rows 366 cm. long and 36 cm. apart. Each population was composed of 40 rows or about 1,000 plants. The first spike was tagged on each plant prior to anthesis. Tagged spikes of randomly selected rows were harvested at five stages of maturity beginning about 7 days after anthesis and continuing at 7-day intervals until maturity. Spikes from 16 rows of each population were harvested at the first stage of maturity. Spikes from 10, 6, 5, and 3 rows were harvested at the second, third, fourth, and fifth stages, respectively. The five stages of maturity included three at early stages of development (7, 14, and 21 days after anthesis), one at physiological maturity (28 days after anthesis), and one combine-ripe (35 days after anthesis). Harvested spikes were air-dried and hand-threshed. In 1972, only Hiproly and CI 4362 were grown, harvested, and processed in the same manner as the samples from 1971.

Moisture and protein were determined according to the American Society of Brewing Chemists' *Methods of Analysis* (7). Protein is expressed as Kjeldahl N × 6.25, % on a dry matter basis.

Amino acid analyses were performed on a Beckman 121 automatic amino acid analyzer. Detailed descriptions of the acid hydrolysis, the amino acid assay, and

the computations have been given elsewhere (8). Unless stated otherwise, results of amino acid assays are expressed in g. amino acid recovered. Kjeldahl-N average recovery for all samples was 89.6%. However, average recovery for the samples from the earliest maturity stages was only 82.6% compared with 90.6% for the combine-ripe samples. The results indicated substantial amounts of nonprotein nitrogenous compounds in the unripe samples.

RESULTS AND DISCUSSION

Kernel weights of air-dried samples from various stages of maturity are compared in Fig. 1. Hiproly, M219, and M360 attained much lower final kernel weights than CI 4362, 61Ab4965-A, and M118; M305 was intermediate. Excluding hulled 61Ab4965-A, the hull-less kernels at the first maturity stage (7 days after anthesis) attained 7.1 to 18.0% (average 10.5%) of their final dry weight. The ranges and averages were 28.6 to 52.8% (41.2%), 66.5 to 83.3% (77.6%), and 93.4 to 98.9% (96.3%) at stages 2, 3, and 4, respectively.

Moisture contents of hull-less populations were 73.5 to 79.5% (average 77.4%), 63.4 to 73.5% (69.4%), 55.0 to 65.5% (59.0%), 30.4 to 53.4% (42.4%), and 3.3 to 17.3% (7.4%) at maturity stages 1, 2, 3, 4, and 5, respectively. The results indicate that maturation patterns of the seven populations, insofar as kernel weights and moisture contents are concerned, were similar.

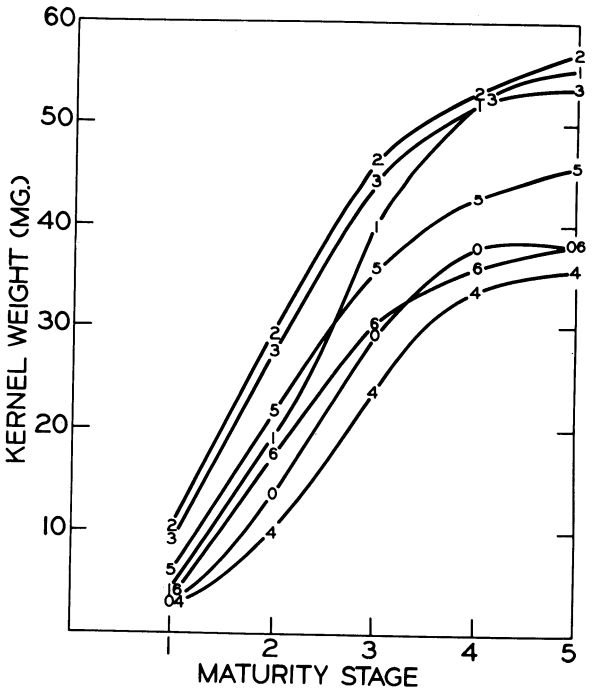

Fig. 1. Kernel weights of air-dried barleys harvested at five stages of maturity in 1971. 0 = Hiproly; 1 = CI 4362; 2 = 61Ab 4965-A; 3 = M118, 4 = M219; 5 = M305; and 6 = M360.

All populations showed a decrease in protein content in samples from the second and third stages of maturity. The protein content increased thereafter and either reached or exceeded the protein content of samples from the first stage.

To simplify presentation of results of amino acid analyses, only the data for Hiproly, CI 4362, and 61Ab4965-A at three stages of maturity in 1971 are presented in Table II.

The shrunken kernels of M219 and M360 resembled Hiproly in high protein content and amino acid composition. In all three populations (Hiproly, M219, and M360), proteins of mature kernels contained over 4.0% lysine and about 7.5% aspartic acid.

The well-developed kernels of M118 and the intermediate-sized kernels of M305 had only about one percentage point less protein than Hiproly but their amino acid compositions differed substantially from that of Hiproly. Analyses showed, for instance, M118 and M305 had consistently (at all maturation stages) low concentrations of lysine and aspartic acid.

Hiproly and CI 4362 had comparable protein contents at all stages of maturity (Table II). The two cultivars had comparable levels of lysine at early stages of kernel development but at subsequent stages there was a much more marked decrease in CI 4362 than in Hiproly. This, presumably, resulted from more extensive biosynthesis of lysine-poor proteins (hordein) in CI 4362 than in Hiproly. The larger decrease in lysine in CI 4362 was also accompanied by decreases to lower levels (than in Hiproly) of aspartic acid, alanine, and valine and of higher levels of glutamic acid. CI 4362 proteins contained more cystine than proteins of Hiproly.

TABLE II. PROTEIN CONTENT (%) AND AMINO ACID COMPOSITION[1] OF THREE BARLEYS HARVESTED AT VARIOUS STAGES OF MATURITY IN 1971

Parameter	Days after Anthesis.								
	7	21	35	7	21	35	7	21	35
	Hiproly			CI 4362			61Ab4965-A		
Protein	20.5	19.6	21.5	20.2	18.5	20.7	13.9	13.3	14.6
Lysine	5.7	4.1	4.1	5.6	3.5	2.8	5.3	3.7	3.4
Histidine	2.0	1.9	2.1	1.8	2.0	1.9	2.0	2.1	2.0
Ammonia	3.2	3.0	2.9	3.2	3.3	3.0	2.8	3.1	2.8
Arginine	4.3	4.2	4.9	5.3	4.0	3.6	5.4	4.7	4.6
Aspartic acid	17.4	6.7	7.5	10.8	5.9	5.4	10.6	6.3	6.2
Threonine	3.5	3.3	3.2	3.6	3.1	2.9	4.1	3.4	3.2
Serine	3.8	3.7	3.7	3.7	3.6	3.5	4.3	3.9	3.6
Glutamic acid	12.9	26.3	25.2	19.8	27.9	30.5	18.0	26.5	26.1
Proline	11.1	12.0	12.1	7.3	13.1	15.2	9.1	10.8	13.1
Cystine	0.4	0.7	0.8	0.5	1.0	0.9	0.7	1.1	1.3
Glycine	4.8	4.1	4.1	4.5	3.8	3.4	4.9	4.0	4.0
Alanine	6.9	4.8	4.2	9.0	3.6	3.2	6.4	3.9	4.0
Valine	5.5	5.2	5.2	5.6	4.7	4.4	6.0	5.1	5.2
Methionine	2.2	2.4	2.4	2.8	2.4	2.0	2.7	2.3	2.5
Isoleucine	3.8	3.7	3.6	3.9	3.6	3.4	4.0	3.7	3.7
Leucine	6.3	6.5	6.3	6.4	6.5	6.1	6.8	7.0	6.7
Tyrosine	2.1	2.3	2.5	2.4	2.3	2.1	2.5	2.9	2.3
Phenylalanine	4.0	5.2	5.3	3.8	5.7	5.9	4.4	5.4	5.3

[1]g. Amino acid per 100 g. recovered.

The high concentration of aspartic acid in Hiproly is of particular interest as aspartic acid is known to be a key intermediate in the biosynthesis of lysine in bacteria, algae, and higher plants (9).

Interpretation of the changes in amino acid composition of proteins in 61Ab4965-A is complicated by two factors: the relatively low protein content and the presence of hulls. The correlation coefficient between protein and lysine of hulled barley cultivars is known to be negative (about −0.4 in 15 cultivars grown for 2 years at two locations) (10). In addition, proteins of the hulls of mature barley contain substantially more lysine than the whole kernel or the dehulled and degermed kernel (8). This might explain the fact that lysine decreased to 3.4% in proteins of mature, hulled, low-protein 61Ab4965-A but to 2.8% in proteins of mature, hull-less, high-protein CI 4362.

The results summarized in Table II were confirmed by analyzing Hiproly and CI 4362, harvested at five stages of maturity, from the 1972 crop. Kernel weight, lysine, and aspartic acid data for the five maturity stages in 1972 are summarized in Fig. 2.

The results in Table II were calculated as concentration of amino acids in the protein. The pair Hiproly and CI 4362 had comparable protein contents, but differed in kernel size. Amino acids expressed as percentage concentrations in the total protein, therefore, would differ from those expressed as mg. per kernel. Calculations on a kernel basis are particularly important in following changes in

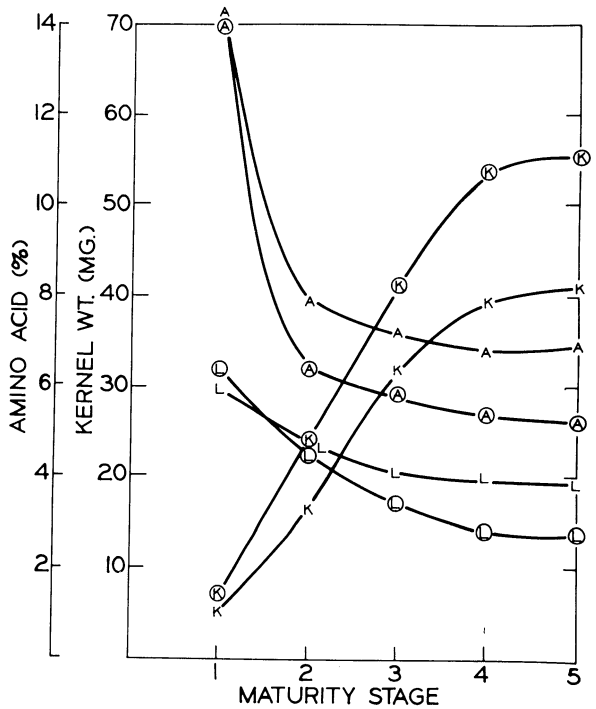

Fig. 2. Kernel weights (K), lysine (L), and aspartic acid (A) in proteins of maturing barleys harvested at five stages of maturity in 1972. Circled figures = CI 4362; others = Hiproly.

maturing grain which increases in weight during development and in mature cultivars varying widely in kernel weight. The data in Table II, therefore, were recalculated in terms of mg. protein or amino acid per kernel. Thus, for instance, a mature kernel of Hiproly contained 8.8 mg. protein of which 0.33 mg. was lysine and 0.62 mg. was aspartic acid. A mature kernel of CI 4362 contained 11.2 mg. protein of which 0.33 mg. was lysine and 0.58 mg. aspartic acid. Thus, while the weight percentage concentrations of lysine and aspartic acid in proteins of Hiproly and CI 4362 differed, amounts of those amino acids per kernel were almost identical. Mature kernels of CI 4362 and Hiproly also contained similar amounts of alanine and valine. On the other hand, whereas a kernel of Hiproly contained only 2.30 mg. glutamic acid, a kernel of CI 4362 contained 3.46 mg. of this amino acid which is a major component of storage proteins synthesized in later stages of grain development. This indicates that the high concentrations of lysine and aspartic acid in proteins of Hiproly may be attributed, in part at least, to differences in kernel development and protein deposition in the whole kernel or in specific kernel tissues.

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