Amino-Terminal Amino Acid Sequence of Hordein

J. A. BIETZ, Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture,[1] Peoria, IL 61604

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ABSTRACT

Hordein, the alcohol-soluble prolamin fraction from barley, was subjected to Edman degradation to investigate its N-terminal amino acid sequence. Although electrophoresis revealed whole hordein to be very heterogeneous, a single major sequence was obtained for 35-40 residues, demonstrating homology among many individual hordein polypeptides. This sequence was, in part, NH_2-Arg-Gln-Leu-Asn-Pro-Ser-Ser-Gln-Glu-Leu-Gln-Ser-Pro-Gln-Gln- and represents primarily hordein's C components having molecular weights of approximately 53,000. Little sequence similarity was evident, however, between hordein and the prolamins from wheat or corn. These observations suggest that gene duplication followed by mutation is the most likely explanation for the occurrence of homologous prolamins in barley, as it is in other cereals.

Hordein, the alcohol-soluble storage protein fraction from barley (*Hordeum vulgare* L.), occurs in protein bodies within the endosperm (Miflin and Shewry 1979b). Barley's malting quality and nutritional value are genetically influenced by hordein quantity and quality (Baxter and Wainwright 1979, Miflin and Shewry 1979a), and electrophoresis of hordein permits varietal identification (McCausland and Wrigley 1977, Shewry et al 1978d). The biosynthesis of hordein has been studied extensively (Brandt and Ingversen 1976, Fox et al 1976). For these reasons, much interest in the structures of hordein polypeptides is apparent.

Starch gel electrophoresis, sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), and isoelectric focusing show that hordein contains at least 15-20 distinct polypeptides (Laurière and Mossé 1977, Shewry et al 1978a), which may be classified on the basis of increasing molecular weight into A, B, and C fractions. B and C hordeins differ in amino-terminal amino acids (Shewry et al 1980b) and in C-terminal sequences (Schmitt 1979) as well as in molecular weight, and are controlled by two separate loci, each consisting of families of closely-linked genes (Shewry et al 1978c). Some individual hordeins may be structurally related (Holder and Ingversen 1978, Miflin and Shewry 1979b).

Considerable intra-species sequence homology has been demonstrated among prolamins and prolaminlike glutelin subunits from wheat, related *Triticum* and *Aegilops* species, rye, and corn (Autran et al 1979; Bietz and Wall 1980; Bietz et al 1977, 1979). In addition, antibodies to hordein cross-react with wheat gliadin, suggesting that prolamins from different cereals are structurally similar (Baker 1975). To determine if hordein polypeptides are homologous, to obtain basic information on their structures, and to further test whether prolamin sequences can demonstrate genetic relationships among cereals, a study of the amino-terminal amino acid sequence of whole hordein was undertaken.

MATERIALS AND METHODS

Protein Samples

Hordein was isolated from the barley variety Hiproly by a modified Osborne procedure. Hiproly is characterized by a mutation for suppressed hordein synthesis and increased lysine; qualitatively, however, hordeins from Hiproly and other varieties are similar. Ground meal was extracted twice with 1 *M* NaCl, once with water, and once with 70% ethanol. The protein dissolved in 70% ethanol was considered to be hordein. A ratio of 6:1 solvent to solid was used for each extraction, and each extraction was carried out for 6 min at low speed in a Waring Blendor. Supernatant solutions were separated by centrifugation at 13,200 × *g* for 20 min, concentrated under reduced pressure, and lyophilized. Hordein was then reduced with β-mercaptoethanol and converted to its *S*-pyridylethyl (PE) derivative (Friedman et al 1970).

Extraction of hordein with alcohol plus reducing agent (Miflin and Shewry 1979b) was avoided, even though prolamins and alcohol-soluble reduced glutelins from barley may be identical (Miflin and Shewry 1979b, Shewry et al 1978b, Shewry et al 1980b), because these protein fractions clearly differ in wheat and in corn (Bietz and Wall 1980; Bietz et al 1977; Paulis and Wall 1971, 1977).

Electrophoresis

PE-hordein was examined by PAGE in 6% gels with 5% cross-linking, using pH 3.2 aluminum lactate buffer (Mecham et al 1978) containing 1 *M* urea, and by SDS-PAGE on 5% gels at pH 8.9 (Bietz and Wall 1972).

Sequence Determination

PE-hordein was subjected to amino-terminal amino acid sequence analysis by automated Edman degradation using a Beckman 890C sequencer. Released phenylthiohydantoin (PTH) amino acids were identified as described previously (Bietz and Wall 1980; Bietz et al 1977, 1979). Because partial deamidation occurs during the acid-catalyzed conversion step of the Edman degradation, PTH-glutamine and PTH-asparagine were identified by the presence of both the amide and the corresponding dicarboxylic amino acid in the same fraction. Six separate degradations were performed, each using 11-16 mg of protein. Sequence analyses were terminated when cleaved products could no longer be identified with certainty (maximum, 40 cycles); no identifications were possible at positions 36-38, but amino acids could be assigned for residues 39 and 40. Results from replicate degradations were very similar.

RESULTS

Electrophoresis

PAGE of PE-hordein revealed a heterogeneous banding pattern very similar to those previously published by Laurière and Mossé (1977) and McCausland and Wrigley (1977) and by Shewry et al (1978a) using starch gel electrophoresis. Compared to wheat gliadin, PE-hordein has three intense bands with mobilities corresponding to ω-gliadins, two to four faint bands in the γ-gliadin region, and four to five medium bands corresponding in mobility to β-gliadins. SDS-PAGE revealed five major bands in PE-hordein, having apparent molecular weights of 32,000, 38,000, 47,400, 53,200, and 65,600. Bands with molecular weights of 38,000 and 53,200 stained intensely with Coomassie brilliant blue R250, and the other three bands stained lightly. The SDS-PAGE pattern was quite similar to those shown by Holder and Ingversen (1978), Shewry et al (1977, 1978d), and Miflin and Shewry (1979a) for various varieties and included both C and B hordeins.

[1] Mention of firm names or trade products does not imply endorsement or recommendation by the USDA over other firms or similar products not mentioned.

Sequence Analysis

Table I summarizes the analytical data obtained by sequence analysis of whole (70% ethanol-soluble) PE-hordein, and presents the amino-terminal amino acid sequence deduced from these data. Primarily, a single sequence resulted for this heterogeneous mixture, suggesting significant homology among many hordein polypeptides. A few minor residues (10–20% of the total reactive protein) could also be identified, suggesting that mutations have changed the amino acids present at these positions in one or more homologous hordein polypeptides.

The observed amino-terminal residue, arginine, agrees with that found for C hordein by Shewry et al (1980b) but, as these authors point out, does not agree with other amino-terminal analyses of hordein. The differences may be related both to inherent difficulties in identifying PTH-arginine and to the low yield obtained (less than one-third that of the subsequent residue, glutamine). PTH-arginine cannot be determined by gas-liquid chromatography and exhibits a broad, somewhat variable peak upon high performance liquid chromatography. Because it is insoluble in a solvent containing 35% CH_3CN (Bietz and Wall 1980), its low yield probably results from marginal solubility in the similar 32% CH_3CN solvent used (Gates et al 1979).

During the course of this research, communications were maintained with other researchers interested in hordein isolation and characterization.[2] Following initial submission of this article, we learned that independent sequence analyses of hordein from the varieties Bomi and Julia had been performed (Schmitt and Svendsen 1980; Shewry et al 1980a, 1980c). These independently determined sequences almost totally agree, confirming the present data and showing that most hordeins from different varieties of barley are similar or identical. The few reported differences in N-terminal amino acid sequences may be varietal characteristics.

Shewry et al (1980b) were unable to determine amino-terminal amino acids for B hordein. Subsequently purified B1 hordein and mixtures of B hordeins have been found to have blocked N-termini (Schmitt and Svendsen 1980, Shewry et al 1980c). Thus, the determined sequence (Table I) apparently represents C hordein components of Hiproly barley but not B hordein components. This agrees with our PAGE analysis of whole PE-hordein, which suggested that more than 50% of the material was C hordein, and also with the SDS-PAGE results and recovery data (Table I). The actual yield at cycle 1 in the Edman degradation was approximately 8.17 nmol/mg, based on a repetitive yield of 96.1% (determined from leucine recoveries at cycles 3, 10, 18, and 28). An average

[2] J. Schmitt, P. R. Shewry, and D. D. Kasarda. Personal communications.

TABLE I
Sequence Analysis[a] of PE-Hordein[b]

Cycle	TLC[c]	GLC[d] (nmol/mg sample)	HPLC[e] (nmol/mg sample)	Assignment	
				Major	Minor
1	R		R(2.50)	R	
2	E, Q, M	Q(6.56), E(3.38), M(0.45)	Q(4.20), E(3.82), M(0.76)	Q	M
3		L/I(6.05)	L(7.90)	L	
4	D, N	N(2.99), D(0.57), V(1.08)	N(5.54), D(1.46), V(1.15)	N	V
5	P	P(3.69)	P(3.76)	P	
6	(S)	(S) (1.41)	S(2.26)	S	
7			S(4.04)	S	
8	E, Q, V,	E(1.91), Q(1.72), V(0.70)	E(2.99), Q(3.12), V(1.15)	Q	V
9	E	E(2.23)	E(4.27)	E	
10		L/I(4.65)	L(5.99)	L	
11	E, Q	E(3.01), Q(1.34)	E(3.63), Q(2.10)	Q	
12			S(1.81), P(0.64), G(0.68)	(S)	
13	P		P(2.48)	P	
14	E, Q	E(1.34), Q(1.15)	E(2.10), Q(1.15)	Q	
15	E, Q	E(1.78), Q(0.89)	E(2.48), Q(1.34)	Q	
16	(P), (N)	P(0.38)	P(0.64), D(0.64), N(0.37)	(P), (N)	
17	Y	Y(1.27)	Y(2.55)	Y	
18		L/I(1.46)	L(2.29)	L	
19	E, Q	E(0.32)	E(0.89), Q(0.75)	(Q)	
20	E, Q		E(1.40), Q(0.92)	Q	
21	P	P(0.70)	P(1.21)	P	
22	Y, V	Y(1.34), V(0.51)	Y(2.55), V(0.55)	Y	V
23	P	P(0.83)	P(1.91)	P	
24			W(1.78), E(0.88), Q(0.31)	(Q, W)	
25	D, N	N(0.89), D(0.19)	D(1.15), N(0.76), F(0.32)	N	F
26			P(0.58)	(P)	
27	Y	Y(0.57)	Y(1.27)	Y	
28		L/I(0.89)	L(1.40)	L	
29		P(0.89)	P(1.59)	P	
30		Q(0.51)	Q(0.45)	(Q)	
31		Q(0.96), E(0.64)	E(0.70), Q(0.32), I(0.38)	Q	I
32	P, A	P(0.89), A(0.51)	P(1.34), A(0.38)	P	A
33		F(0.38)	F(1.34)	F	
34			T(0.25)	(T)	
35	V	V(0.38)	V(0.76)	V	
36					
37					
38					
39	F		F(0.70)	F	
40			W(0.76)	(W)	

[a] Standard single-letter amino acid abbreviations: A, alanine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; I, isoleucine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; and Y, tyrosine.
[b] Only data necessary to make assignments are given; amino acids present in trace amounts are omitted. Assignments in parentheses are not totally certain.
[c] Thin-layer chromatography.
[d] Gas-liquid chromatography.
[e] High performance liquid chromatography.

molecular weight of 57,000 for C hordein (Shewry et al 1980a) corresponds to 17.54 nmol of possible free amino-terminal amino acid per milligram of protein. Our initial recovery data is quite consistent with this figure, considering that our sample weights included moisture and unreactive B hordeins and that initial yields of 50–70% were obtained for other cereal prolamins (Bietz et al 1977, 1979).

DISCUSSION

These results demonstrate marked homology among most C hordeins (although the possibility of some blocked C-hordein components of differing sequence cannot be ruled out) and tentatively confirm the absence of free N-terminal amino groups in B hordeins. Most electrophoresis studies indicate at least four or five C hordeins (Holder and Ingversen 1978, Shewry et al 1978a), supporting suggestions that C hordeins are coded by genes at a single locus (Shewry et al 1978c) that resulted from gene duplication, combined with mutation or different chain termination or initiation points (Holder and Ingversen 1978). Our observations that the amino-terminal sequences of most of these hordeins are highly homologous and that minor amino acids can be assigned with certainty at some positions (for example 2, 4, and 8) strongly suggest that gene duplication followed by mutation is the most likely explanation for the occurrence of homologous prolamins in barley, as it is in wheat (Bietz et al 1977) and corn (Bietz et al 1979).

Comparison of the hordein sequence determined in our study to the amino acid composition of a purified C2 hordein (Schmitt 1979) suggests a fairly uniform amino acid distribution throughout the polypeptide chains. The amino-terminal hordein sequence shows no more similarity to α, β, or γ gliadins (Bietz et al 1977) or to zein (Bietz et al 1979) than can be expected by chance, considering the high glutamine and proline contents of these prolamins. Most prolamins apparently function only as storage proteins and can tolerate increased mutation rates; this perhaps suggests that most prolamin sequences may not be good indicators of distant genetic relationships among cereals. Recent studies of more closely related cereal genera and species, however, do suggest that many genetic relationships can be demonstrated through prolamin sequence analysis (Autran et al 1979), and Shewry et al (1980a) have demonstrated homology between C-hordein and a purified ω-gliadin component from *Triticum monococcum*. Thus, some prolamin gene sequences can be conserved over a long period of time.

Homology has now been demonstrated within the prolamin mixtures of barley (our study), wheat (Bietz et al 1977), corn (Bietz et al 1979), rye and various *Triticum* and *Aegilops* species (Autran et al 1979), and among the alcohol-soluble glutenin subunits of wheat (Bietz and Wall 1980). This suggests that significant homology may occur among alcohol-soluble polypeptides within all cereals, perhaps including all polypeptides originating in protein bodies.

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