PARTIAL AMINO ACID SEQUENCE FROM HORDEIN POLYPEPTIDE B1

by

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Hordein polypeptide B1 has been cleaved with CNBr, *Staphylococcus* V8 protease and trypsin under denaturing conditions. Fragments have been isolated and examined by amino acid analysis and sequence determination. Amino acid residues were distributed irregularly along the B1 polypeptide chain with no indication of large Glx/Pro clusters. The single sequences presented account for about one fourth of the molecule. Two homologous but different tryptic peptides indicate either the presence of different B1 molecules or internal sequence repetition.

1. INTRODUCTION

The prolamins of barley – hordeins – serve as seed storage proteins in the endosperm. This group of polypeptides is characterized by its solubility in aqueous alcohol and can be separated into several components using electrophoretic techniques (for recent reviews see 6, 13, 16, 26).

N-terminal amino acid sequences have recently been obtained from total hordein (2, 20), partially purified hordein (22) and electrophoretically pure C hordein polypeptides (20, 24).

An evaluation of the N-terminal sequences obtained from total hordein has led to the proposal, that at least the amino termini of the C hordein group are under evolutionary constraint

(20). This has been corroborated by the demonstration, that γ -gliadin from wheat (*T. monococcum*) and C hordein from barley are homologous (22).

B1 hordein, which is different from C hordein in amino acid composition and carboxy-terminal structure (19, 20) was blocked to N-terminal analysis by dansylation (23) and Edman degradation (20). B1 hordein is of lower apparent molecular weight than C hordein but shares some chymotryptic peptides with C hordein (12), as judged by the »fingerprinting« technique.

In the present contribution we report on the amino acid sequences of some CNBr – and proteolytic fragments of B1 hordein. These data improve our understanding of the genetic relationship between B and C hordein as well as between prolamins of barley and other cereal species. Furthermore they will be used for comparison with the nucleotide sequences of a cloned reverse transcribed hordein messenger RNA molecule (5).

2. MATERIALS AND METHODS

2.1. Isolation of hordein

Hordein was isolated from barley seeds (*Hordeum vulgare* L.) cv. Bomi as described previously (20).

2.2. Isolation of hordein polypeptide B1

Hordein polypeptide B1 was isolated by ion exchange chromatography as described (19). In the scaled-up procedure used in the present study it was found that the fractions at the leading as well as at the trailing edge of the B1-peak contained minor impurities and only the center fractions were pooled. The purity of the isolated polypeptide B1 was checked by gel electrophoresis in the presence of sodium dodecyl sulfate as described (19). For all calculations a molecular weight of 27,000 (12) was used.

2.3. Alkylation of SH-groups

The fractions from the ion exchange column containing pure B1 polypeptide (cf. 2.2) were dried in vacuo. The samples were then redissolved to about 10 mg · ml⁻¹ in a buffer containing 0.13 M-Tris, 2.7 mM-EDTA and 6 M-guanidine

hydrochloride, adjusted to pH 7.5, followed by reduction and alkylation as described in (9) and used for chemical or enzymatic cleavage.

2.4. Fragmentation with cyanogen bromide

Cyanogen bromide cleavage was carried out on 700 nmoles of B1 polypeptide as described by SEBALD et al. (21). The reaction was stopped by addition of 5 volumes of water and subsequent freeze-drying.

2.5. Cleavage with *Staphylococcus protease* V8

600 nmoles of B1 polypeptide were dissolved in 24 ml of a solution containing 50 mM-ammonium bicarbonate and 0.2 % SDS, 0.8 mg *Staphylococcus* V8 protease (Miles Biochemicals) was added (7). Incubation time was 18 hours at 37 °C. The reaction was stopped by heating to 90 °C for 5 min. The sample was then lyophilized.

2.6. Cleavage with trypsin

600 nmoles of B1 polypeptide were dissolved in 40 ml of a solution containing 6 M-urea and 125 mM-sodium borate pH 9. 7 mg of cyclohexanedione was then added and the mixture incubated for 2 hours at 37 °C to block the arginine residues (18). The reaction was stopped by addition of 4 ml acetic acid and the sample was dialyzed against 1 % acetic acid and freeze-dried.

The sample was then dissolved in 2 ml of 8 M-urea and diluted with 5 ml borate buffer to give final concentrations of 2 M-urea, 0.1 M-borate pH 8 (WITTMAN-LIEBOLD, personal communication). To the turbid suspension 160 µg trypsin was added at $t = 0$ and $t = 4$ hours 20 min. The digestion at 37 °C was monitored by following the appearance of new amino termini with fluorescamine (10). After 18 hours of incubation the mixture was lyophilized.

2.7. Purification of proteolytic fragments

The peptides from the SV8 digest were not completely soluble in the eluant. The amino acid composition of the insoluble residue is given in Table I. Following centrifugation the soluble

Table I

Amino acid composition of hordein polypeptide B1 and of proteolytic fragments pooled from Sephadex LH60 separations (see Figure 2). Values are given in mole %.

Hordein B1*	CNBr I	CNBr II	CNBr III	CNBr IV	CNBr V	CNBr VI	CNBr VII	CNBr VIII	SV8 I	SV8 II	SV8 III	SV8 IV	SV8 P***	T I	T III	T IV	T V	T VI	T VII
Cys	2.8	—	—	—	—	—	—	—	—	—	—	—	—	—	0.5	—	1.1	0.5	—
Asx	1.5	0.6	0.9	2.0	5.5	5.6	12.8	7.4	1.3	4.8	5.8	4.8	19.4	1.4	1.2	1.3	2.9	4.1	—
Thr	1.8	2.0	1.8	4.5	0.5	1.9	—	2.1	2.1	3.0	5.6	9.3	5.9	2.1	2.4	1.6	2.4	3.4	11.1
Ser	2.9	3.0	2.5	3.8	4.6	8.5	2.2	5.8	4.7	8.0	8.9	9.0	5.2	3.5	5.6	5.1	7.5	7.7	9.9
Glx	34.2	39.7	37.1	39.5	28.0	4.7	26.8	16.7	34.6	29.0	26.6	5.4	14.1	38.5	39.7	41.0	30.6	17.0	12.2
Pro	16.8	21.3	25.5	12.9	9.7	10.4	7.5	3.8	2.1	22.0	11.6	10.9	10.4	24.6	15.6	18.9	11.0	7.8	—
Gly	3.8	2.3	2.2	3.7	6.3	15.5	12.2	32.9	40.4	3.3	7.7	10.3	9.5	2.5	3.8	3.2	4.8	9.2	14.6
Ala	3.0	2.6	1.6	5.6	10.0	1.0	4.1	1.4	5.0	2.5	5.0	6.3	5.5	1.6	3.8	2.9	3.3	6.3	18.1
Val	6.1	4.8	3.7	5.7	6.0	23.5	10.1	—	4.7	7.9	7.4	13.4	5.3	3.5	6.2	5.3	8.3	11.5	—
Met	1.1	0.2	0.8	—	0.3	0.4	0.7	—	1.2	1.8	—	0.8	0.7	0.5	0.8	0.8	2.2	1.6	—
Ile	4.1	4.3	3.7	5.5	4.2	4.9	3.7	1.2	1.0	4.2	4.4	6.7	4.5	3.3	3.6	3.6	4.6	7.2	10.8
Leu	8.8	8.8	7.7	8.8	10.8	11.1	4.5	2.0	7.5	7.3	9.2	11.9	4.9	7.1	7.8	6.6	10.6	9.8	8.3
Tyr	2.5	2.5	2.9	1.5	1.1	4.4	—	—	2.6	1.2	—	1.3	2.6	2.6	1.2	2.0	1.4	2.4	8.3
Phe	4.7	5.5	5.8	4.6	3.5	—	1.3	1.1	5.5	3.3	—	—	3.7	5.8	4.6	4.7	2.8	1.6	4.1
His	1.6	1.5	1.1	2.2	2.1	0.9	2.8	23.1	19.0	1.0	1.4	—	—	0.7	1.2	1.2	1.8	2.0	—
Lys**	1.0	0.7	1.3	0.3	0.9	0.4	1.6	0.9	0.7	0.9	1.6	0.9	4.2	1.0	0.6	0.5	1.0	1.9	2.3
Arg	2.8	2.0	1.3	2.6	4.0	9.7	N.D.	—	1.9	2.7	3.8	8.0	1.9	0.7	1.3	1.1	2.4	5.6	—

* data taken from SCHMITT (19).

** the value for lysine includes pyridylethylcysteine for all fragments.

*** composition of peptide material insoluble in column eluant.

The composition of the trypsin II fraction has not been determined.

Figure 1. Hydrolysis of hordein B1 with trypsin in 0.1 M-borate buffer pH 8, containing 2 M-urea. ↑ denotes new addition of trypsin.

proteolytic fragments were separated on columns of Sephadex LH60 as described by GERBER *et al.* (11). After separation, the fractions were dried in vacuo and then taken up in 1 ml of 0.1 M-ammonium hydroxide for absorbance measurements. Some fractions were only partially soluble under these conditions.

2.8. Amino acid analysis

Samples for amino acid analysis were hydrolyzed for 24 hours as described previously (19) and applied on a Durrum D-500 amino acid analyzer. In the chromatographic system used pyridylethylcysteine coeluted with lysine. No performic acid oxidation was carried out and the values are given without corrections.

2.9. Sequence determination

Automatic sequence determinations were performed with a Beckman model 890C liquid phase sequencer according to the method of EDMAN and BEGG (8). The procedure used has been described in details by JOHANSEN *et al.* (14). The samples were applied to the cup, dissolved in 58 per cent isopropanol or in a mixture of ethanol and formic acid (7 to 3 v/v) and dried using the Beckman sample application subroutine programme 02772. Identification of PTH-amino acids was made by HPLC using a Hewlett Packard chromatograph, model 1084B as described by SVENDSEN *et al.* (25). Additional information was obtained from thin layer chromato-

graphy on polyamide sheets according to KULBE (15).

3. RESULTS AND DISCUSSION

Hordein polypeptides are soluble in dilute alcohol but insoluble in aqueous media (17). We therefore chose cleavage methods which would function under denaturing conditions to ensure at least partial solubility of the substrate. The degradations were performed in formic acid/water (CNBr), or in buffers containing SDS (SV8 protease) or urea (trypsin). In the case of the proteases, the denaturants used have also a potentially inhibiting effect on the enzyme. This can be demonstrated by following the time course of the tryptic digestion (Figure 1). The exposure of new amino termini reached a plateau after about two hours, but reinitiation of digestion could be observed upon addition of fresh enzyme. In view of this fact it is possible that the enzymatic digestions are incomplete.

Based on a molecular weight of 27,000 (12) for hordein B1 the molecules should contain three methionine residues and thus four peptides are to be expected upon cleavage with cyanogen bromide. One of these, the N-terminal peptide, is blocked to Edman degradation. A series of CNBr-peptides has been isolated (Figure 2 and Table I) which in part must represent either incomplete reaction products or peptides derived from non-specific cleavage at tryptophan residues. Identical N-terminal sequences were obtained with CNBr-peptides I, II and III emphasizing what is discussed above. The sequence of CNBr-III is shown in Figure 3. That the largest CNBr-fragments have a free N-terminal amino acid would indicate that one of the methionine residues must be located close to the N-terminus. CNBr-IV and CNBr-VI reacted negatively to Edman degradation as well as dansylation and may well represent blocked N-terminal peptides of different length.

One more peptide (CNBr-V) has been sequenced through 11 steps. Judged from the amino acid composition (Table I) this peptide probably consists of 19 amino acid residues.

Chromatography of the peptides obtained by cleavage with Staphylococcal protease gave rise to at least four peaks as shown in Figure 2 and Table I. The largest peak (SV8 I) did not respond

Figure 2. Separation on Sephadex LH60 (11) of peptides obtained by CNBr-cleavage, digestion with *Staphylococcus* V8 protease, and digestion with trypsin. The bars denote fractions pooled.

CNBr III	:H ₂ N-Leu-Gln-Gln-Ser-Ser- X -His-Val-Leu-Gln-Gln-Gln-Lys- X -Gln-Glu-Leu-Pro-Gln-	10
CNBr V	:H ₂ N- X -Ser-Val-Asn-Val-Pro-Leu-Tyr-Arg-Ile-(Leu)-	10
SV8 II	:H ₂ N-Gly-Val-Ser-Gln-Pro-Gln-Gln-Gln-Leu-Trp-Pro-Gln-Gln-(Val)-Gly-Gly- X -Leu-	10
SV8 IV	:H ₂ N-Ala-Thr- X -Ser-Ile-Ala-Leu-Arg-Thr-Leu-Pro-Val-	10
T III	:H ₂ N-Ser-Ile-Phe-Leu-Gln-Glu-Gln-Pro-Gln-Gln-Leu-Val-Gln-Gly-Val-Ser-Gln-Pro 20 -Gln-Glu-Gln-Leu-(Trp)	10
T IV	:H ₂ N-Val-Ile-Leu-Leu-Gln-Glu-Gln- X -Glx-Glx-Leu-Val-Glx-Gly-Val- X -Gln-Pro 20 - X - X -Gln-Leu-(Trp)	10

Figure 3. Partial amino acid sequences of B1 hordein peptides obtained by CNBr-cleavage and digestion with *Staphylococcus aureus* V8 protease or trypsin.

Unidentified residues are denoted by an X, residues whose identification is subjected to some uncertainty are given in brackets.

to Edman degradation and therefore represents either undigested hordein or a large N-terminal peptide. Two peptides, SV8 II and IV, respectively, were clean and partly sequenced (see Figure 3). Peptide SV8 III was a mixture of two peptides which were rapidly washed out of the sequencer cup.

Digestion with trypsin under conditions where the arginine residues are blocked was dictated by the low content of lysine in hordein B1 (Table I). At a molecular weight of 27.000 three lysine residues are to be expected, and thus four tryptic peptides. The elution pattern of the resulting peptides is shown in Figure 2. Peaks I, II and V contained mixtures of peptides the sequences of which could not be resolved. However, peptides T III and T IV were sequenced for 23 steps (Figure 3). These sequences are homologous, but deviate at least in two positions (1 and 3). It is uncertain whether this homology expresses internal repetition of the sequence or homology between two different sequences of hordein B1 with identical C-termini.

An overlap is noted between peptide SV8 II (residues 1 to 10) and tryptic peptide III (residues 14 to 23). This finding shows that the Gln found in position 13 in peptide trypsin III in part must be present as a glutamic acid.

No extensive sequence homologies between the B1 polypeptide and C hordein or prolamin sequences of other cereals could be detected (1, 2, 3, 4, 20, 24). The identity of the pentapeptide Gln-Pro-Gln-Gln-Gln (SV8 II, 4-8) with a pentapeptide from γ_3 -gliadin (18-22) (3) is thought to be of little significance if not backed by other evidence, since Glx and Pro are the most abundant amino acids in both proteins.

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