Identification of γ-type hordeins in barley

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Received 30 July 1985

The barley mutant Risφ 56 has a deletion of at least 85 kb of DNA at the *Hor 2* locus, resulting in the absence from the seeds of almost all of the major B hordein group of storage proteins. We have purified two fractions containing the three remaining 'B hordein-like' bands and determined their N-terminal amino acid sequences. One fraction, containing the two bands with higher M_r, gave a single major N-terminal sequence which was closely related to those reported for γ-type prolamins of wheat and rye. The sequence of the third band was also homologous with those of the γ-type prolamins, but less closely so. We consider that the γ-type prolamins are most closely related to the ancestral S-rich prolamins of the Triticeae, and this is the first report of their presence in barley.

Barley *Seed protein* *Amino acid sequence* *Homology*

1. INTRODUCTION

B hordeins are the major storage proteins in barley grain, accounting for 80–90% of the total hordein and 30–40% of the total seed nitrogen [1]. They have broadly similar amino acid compositions to the α, β and γ-gliadins and low molecular mass subunits of wheat glutenin and the γ-secalins of rye, and these proteins have been grouped together as S-rich prolamins by Shewry et al. [2]. Although N-terminal amino acid sequences of S-rich prolamins of wheat and rye have been reported [3–5], attempts to determine the N-terminal amino acid sequence of B hordeins have failed [6,7]. The recent demonstration of glutamine as the putative N-terminal amino acid of a B hordein polypeptide sequence deduced from genomic DNA [8] indicates that N-terminal blocking could result from cyclization to pyrrolidone carboxylic acid [9].

Risφ 56 is a γ-ray induced mutant line of barley which is characterized by a reduced content of B hordein. The mutation maps at or close to the B hordein structural locus *(Hor 2)* [10]. Detailed analysis has shown that the major B hordein polypeptides present in the parental cultivar (Carlsberg II) have been lost, but that several minor polypeptides which give 3 bands on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) remain [11]. This is associated with the loss of all but two of the fragments of genomic DNA which hybridize to a B hordein-related cDNA clone, the total loss of DNA being at least 85 kb [11].

We report here the N-terminal amino acid sequence of fractions containing the 3 'B-hordein-like' bands from Risφ 56. The sequences are homologous with those of γ-type gliadins of wheat and γ-secalins of rye, indicating that they are not typical B hordeins. We consider that the γ-type prolamins correspond most closely to the ancestral S-rich prolamins present in the ancestor of the Triticeae, and their demonstration in barley is important new evidence in support of this hypothesis.

2. MATERIALS AND METHODS

B hordein fractions containing bands 1+2 and band 3 were purified from Risφ 56 using a combination of ion exchange chromatography and gel filtration [11]. N-terminal amino acid sequences were determined by automated Edman degrada-

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00145793/85/$3.30

tion using a Beckmann 890B amino acid sequencer and 1M Quadrol programme 112078. PTH amino acids were identified by HPLC [12] and TLC [13]. SDS-PAGE was as described [14].

3. RESULTS AND DISCUSSION

SDS-PAGE analyses of the 2 B hordein fractions and total hordeins from Risø 56 and Carlsberg II (the parental cultivar) are shown in fig.1. One fraction contained bands 1 and 2 (fig.1, track c) which could not be separated further by ion exchange chromatography or gel filtration. The second fraction contained only band 3 (fig.1, track d). The N-terminal amino acid sequences determined by automated Edman degradation of these fractions are given in fig.2a. Bands 1 + 2 were analysed only once, with a yield at position 2 of 30.9 nmol methionine from about 3 mg protein.

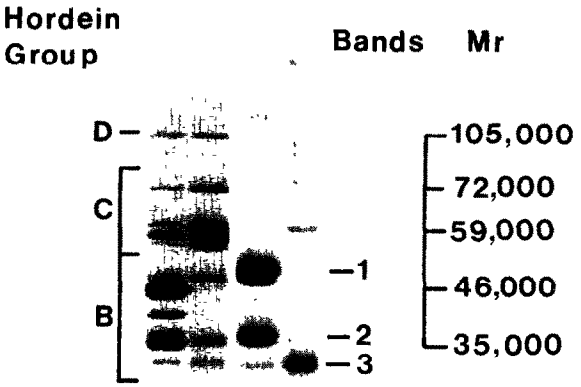

Fig.1. SDS-PAGE of total hordein from Carlsberg II (a) and Risø 56 (b) and purified B hordein fractions from Risø 56 containing bands 1 and 2 (c) and band 3 (d). M_r values were determined in a previous study [23].

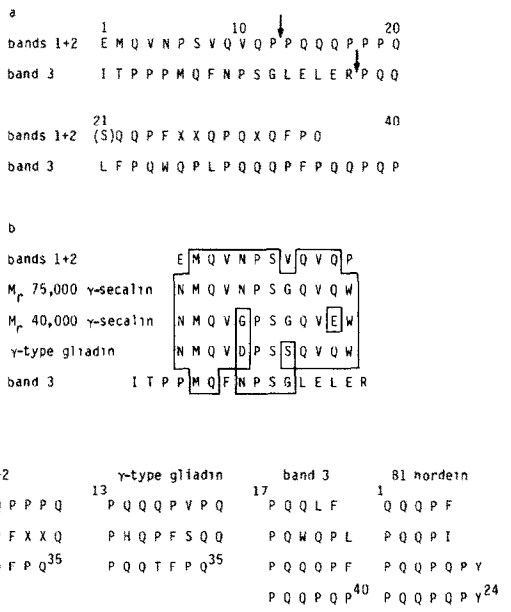

Fig.2. (a) The N-terminal amino acid sequences determined by automated Edman degradation of bands 1 + 2 and band 3 from Risø 56. The arrows indicate the junctions between the N-terminal and repetitive domains. (b) Alignment of the N-terminal domains of bands 1 + 2 and band 3 with those of 75 kDa γ-secalins [5], 40 kDa γ-secalins [5] and a γ-type gliadin [15]. (c) Possible alignments of the repetitive sequences of bands 1 + 2, band 3, a γ-type gliadin [15] and B1 hordein [8]. The alignments are based on Kreis et al. [16]. Standard single letter abbreviations are used: D, Asp; E, Glu; F, Phe; G, Gly; I, Ile, L, Leu; M, Met; N, Asn; P, Pro, Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp.

This corresponds to about 30% of the maximum calculated on the basis of M_r of about 30 000. The repetitive yield was good, with 20.8 nmol valine recovered from position 10 and about 10 nmol phenylalanine from position 25. The low initial yield is not surprising since the N-terminal glutamic acid could cyclize to pyrrolidonecarboxylic acid under the acidic conditions used for purification [9]. Taking this into account the yield is sufficiently high to assume that the sequence derives from both bands. Band 3 was analysed twice, with yields at position 1 of 60 and 46% of the maximum calculated for an M_r of 30 000. The repetitive yields were again good; for example the recoveries of isoleucine from position 1 and leucine from positions 13, 21 and 28 were 26.7, 12.8, 6.7 and 5.2 nmol respectively in one run.

Both N-terminal sequences have clear domain structures, with non-repetitive N-terminal domains (residues 1–12 in bands 1 + 2, 1–17 in band 3) (fig. 2a, arrows) followed by repetitive sequences rich in proline and glutamine. The unique N-terminal domains are homologous with those reported for γ-type prolamins of wheat and rye (fig.2b). Bands 1 and 2 are most closely homologous, with 9 residues in common with the 75 kDa γ-secalin, 7 with the 40 kDa γ-secalin and 8 with the γ-type gliadin of Scheets et al. [15]. Band 3 is less closely related, with an N-terminal extension of 4 residues, 5 residues in common with bands 1 + 2 and 6 in common with the 75 kDa γ-secalin. In contrast to the γ-type prolamins, the major B1 hordein of the cultivar Sundance has no unique N-terminal domain, with repetitive sequences extending to the N-terminus (fig.2c) [8].

The alignment and interpretation of the repetitive sequences of S-rich prolamins is at best highly subjective [16]. Possible alignments of these regions of bands 1 + 2 and 3 are shown in fig.2c. No clear consensus repeat motifs can be recognized; although most of the blocks start with the tripeptide PQQ, they vary in length and precise sequence. It is not possible to precisely align the repetitive sequences of bands 1 + 2 and band 3 with each other, or with the repeats present at the N-terminus of B1 hordein. There is, however, clear homology between the repetitive sequences of bands 1 + 2 and the γ-type gliadin of Scheets et al. [15]. These have 16 out of the 19 identified residues in common.

We also have evidence for small amounts of γ-type hordeins in normal cultivars of barley. Automated Edman degradation of 2.5 mg total B hordein from the cultivar Julia released isoleucine (11 nmol), threonine (4 nmol), glutamine (2 nmol), valine (3 nmol) and asparagine (1 nmol) from positions 1–5, respectively. These residues are present at positions 1 and 2 of band 1 and 3–5 of band 3, and the yields correspond to less than 10% of the theoretical maximum.

Although bands 1–3 all appear to be γ-type hordeins, they differ in their aggregation behaviour. Whereas band 3 is monomeric (see [10]), bands 1 and 2 appear to be present as aggregates stabilized by disulphide bonds (P.R. Shewry and S. Parmar, unpublished). The γ-secalins of rye show similar diversity, with the 40 kDa group present as monomers and the 75 kDa group (which are most closely related to bands 1 + 2 in their N-termini) as aggregates [17,18]. Although the γ-type gliadins of wheat are generally considered to be monomeric, polypeptides with homologous N-termini are present in aggregate fractions prepared by gel filtration of total alcohol-soluble prolamins [19]. It therefore appears that the γ-type prolamins of wheat, barley and rye occur in monomeric and aggregative forms.

The identification of γ-type prolamins in barley has important implications for prolamin evolution. Kreis et al. [16] made a detailed comparison of all the available amino acid sequences of S-rich prolamins and concluded that B hordein was a derived type, most closely related to the low molecular mass subunits of wheat glutenin. The ancestral type of S-rich prolamin represented by the γ-type gliadins and γ-secalins did not appear to be present in barley. This study demonstrates that they are present, although as a small proportion of the total hordein in normal cultivars.

In wheat the γ-type gliadins and S-poor ω-gliadins are encoded by single multigenic loci, designated *Gli-1*, located on the short arms of the group 1 chromosomes [20]. A similar situation may also be present in rye [21]. The linkage relationships of the γ-type hordeins are not known. Although the major B hordeins are encoded by a locus *(Hor 2)* which is separate but linked to that encoding the S-poor C hordeins *(Hor 1)* (see [22]), it is possible that the γ-type hordeins are also encoded by *Hor 1*, as in wheat. This would explain why these proteins have not been affected by the deletion at the *Hor 2* locus in Risφ 56, which is currently being investigated.

ACKNOWLEDGEMENTS

We are grateful to Dr H. Doll for providing seed of Risφ 56 and to Dr B.J. Miflin for discussions. The study was supported by NATO grant no. 324/82.

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