AMINO ACID SEQUENCE HOMOLOGY IN TWO 1,3;1,4-β-GLUCAN ENDOHYDROLASES FROM GERMINATING BARLEY (*HORDEUM VULGARE*)

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1. Introduction

Two 1,3;1,4-β-glucan endohydrolases (EC 3.2.1.73) have been purified from extracts of germinating barley [1]. Enzyme I, which contains traces of associated carbohydrate, has M_r 28 000 and an isoelectric point of 8.5. Enzyme II has M_r 33 000, an isoelectric point >10, and is a glycoprotein containing 3.6% carbohydrate. The enzymes are very similar with respect to their substrate specificities, enzymic properties [2] and amino acid composition [1]. Furthermore, antibodies raised against the purified enzymes are cross-reactive [1]. On the basis of these similarities, we suggested that enzyme I might be derived from enzyme II by proteolytic removal of part of the protein molecule [1].

Here, we compare the NH_2-terminal amino acid sequences of the two 1,3;1,4-β-glucan endohydrolases and conclude that, although a high degree of homology exists, the enzymes in fact represent the products of separate genes.

2. Materials and methods

2.1. *Enzyme purification*

Barley (*Hordeum vulgare* cv. Clipper) was supplied by Mr K. Mander, Victorian Department of Agriculture. The 1,3;1,4-β-glucan hydrolases were purified from germinating barley by ammonium sulphate precipitation, ion-exchange and gel-filtration chromatography as in [1].

2.2. *Amino acid sequence analysis*

Automated amino acid sequence analysis of the native enzymes was performed using a Beckman model 890 C sequencer (Beckman Instr., Palo Alto CA) with a modified controller [3] and a 0.1 M Quadrol program. A sample of ~10 nmol was degraded with 5 mg Polybrene as carrier [4] and the phenylthiohydantoin derivatives were identified and quantified by high-performance liquid chromatography [5].

3. Results and discussion

Automated sequence analysis of the unmodified barley 1,3;1,4-β-glucan endohydrolases allowed the unambigous determination of the first 40 residues (fig.1). No phenylthiohydantoin derivative was detectable at cycle 4 of either enzyme, possibly due to the presence of a cysteine or cystine residue in this position, since the phenylthiohydantoin derivatives of these amino acids are unstable, or to glycosylation of the amino acid. The amino acid sequences are remarkably similar; the only differences in the 40 NH_2-terminal amino acids occur at positions 20 (asparagine/serine), 28 (asparagine/lysine) and 36 (aspartic acid/asparagine). Each of these can be attributed to commonly observed, single base substitutions in the DNA sequence [6]. Comparison of the 1,3;1,4-β-glucan hydrolase sequences with other amino acid sequences by computer search of a data collection [7] failed to reveal sequence homologies with other proteins.

The homology in amino acid sequence observed in the 40 NH_2-terminal residues (fig.1) is likely to extend along the molecules and presumably accounts for the cross-reactivity of antibodies raised against the enzymes [1]. However, in view of the differences in amino acid sequences (fig.1), in molecular size and in degree of glycosylation [1], we conclude that the two barley 1,3;1,4-β-glucan endohydrolases are derived

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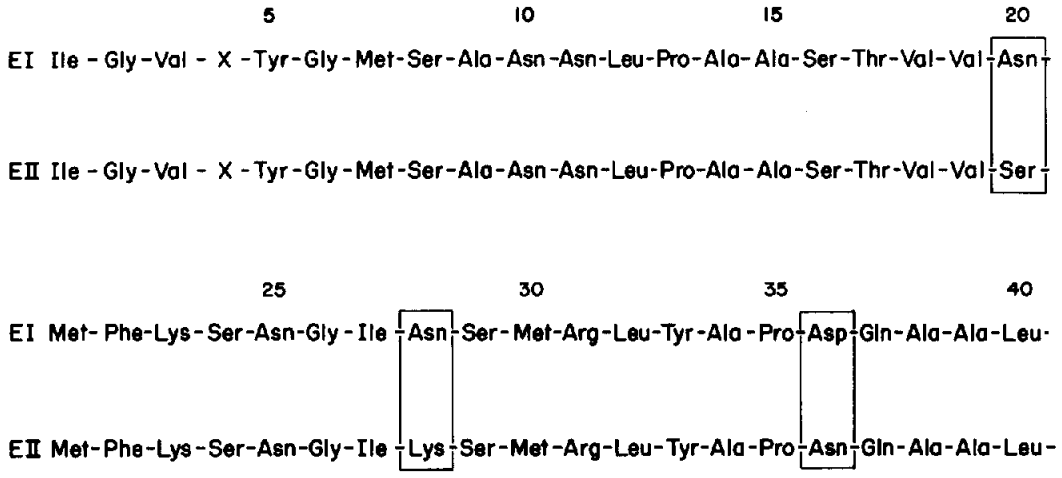

Fig.1. NH_2-Terminal amino acid sequences of barley 1,3;1,4-β-glucan endohydrolases I (EI) and II (EII). Residues which differ are shown in boxes.

from different genes. It is unlikely that the enzymes originate from multiple alleles at a single locus, since barley is predominantly self-fertilizing and plants of established varieties or cultivars are essentially homozygous [8]. Furthermore, the possibility that the enzymes isolated from the cultivar Clipper originate from a mixture of two separate lines or biotypes [9] in the grain sample used is effectively ruled out by our observation (unpublished) that two 1,3;1,4-β-glucan endohydrolases can also be extracted, in similar proportions, from another barley cultivar (Black Hull-less).

If it is assumed that the rate of evolution of the NH_2-terminal regions of the enzyme is equivalent to that of the fibrinopeptides, which at 9 amino acid substitutions per amino acid site per 10^9 year are the fastest evolving peptides so far characterized [10], it may be calculated [11] that gene duplication occurred at least 8×10^6 years ago.

The functional significance for two 1,3;1,4-β-glucan endohydrolase isoenzymes of apparently identical specificity is unknown. The 1,3;1,4-β-glucans are major components of barley endosperm cell walls [12] and their depolymerization is believed to be an important prerequisite for endosperm mobilization during germination [13]. While it is possible that both enzymes are secreted from the aleurone and are involved in endosperm cell wall degradation, they might also originate in different cell types or tissues and participate in quite different metabolic processes. Enzymes which degrade the non-cellulosic polysaccharides of primary cell walls have been implicated in cell expansion [13], and one of the barley 1,3;1,4-β-glucan endohydrolases could thus be involved in the growth of the embryo.

Barley 1,3;1,4-β-glucans need to be extensively degraded during the malting step of the brewing process [14,15]; thus 1,3;1,4-β-glucan endohydrolase activity may represent an important selection criterion in breeding programmes [16] and the apparent presence in barley of at least 2 genetic loci coding for this enzyme is potentially important information for plant breeders.

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