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Mapping of the ADP-glucose pyrophosphorylase genes in barley

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Abstract. cDNA probes encoding the barley endosperm ADP-glucose pyrophosphorylase (AGP) small subunit (bepsF2), large subunit (bepl10), and leaf AGP large subunit (blpl) were hybridized with barley genomic DNA blots to determine copy number and polymorphism. Probes showing polymorphism were mapped on a barley RFLP map. Probes that were not polymorphic were assigned to chromosome arms using wheat-barley telosomic addition lines. The data suggested the presence of a single-copy gene corresponding to each of the cDNA probes. In addition to the major bands, several weaker cross-hybridizing bands indicated the presence of other, related sequences. The weaker bands were specific to each probe and were not due to cross-hybridization with the other probes examined here. The endosperm AGP small subunit (bepsF2) major-band locus was associated with chromosome 1P and designated *Aga1*. The endosperm AGP large subunit (bepl10) major-band locus was mapped to chromosome 5M and designated *Aga7*. The endosperm AGP large-subunit minor bands were not mapped. The leaf AGP large-subunit major band was associated with chromosome 7M and designated *Aga5*. One of the leaf AGP large-subunit minor bands was mapped to chromosome 5P and designated *Aga6*. A clone for the wheat endosperm AGP large-subunit (pAga7) hybridized to the same barley genomic DNA bands as the corresponding barley probe indicating a high degree of identity between the two probes.

Key words: ADP-glucose pyrophosphorylase – *Hordeum vulgare* – RFLP-mapping – Wheat/barley ditelosomic addition lines

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Introduction

ADP-glucose pyrophosphorlase (AGP) (EC 2.7.7.27) is the key regulatory enzyme in the biosynthesis of starch in all plant tissues. A fully-active AGP is believed to be composed of two subunit-types, each encoded by distinct genes (Lin et al. 1988; Kleczkowski et al. 1991). Different genes of AGP are expressed in a tissue-specific manner (Krishnan et al. 1986; Olive et al. 1989; Villand et al. 1992a), suggesting the presence of multiple genes encoding AGP in plants. In barley, two cDNAs encoding the small and large subunits of AGP have been isolated from the endosperm of immature seeds, and one cDNA corresponding to the large subunit was obtained from leaves (Villand et al. 1992a, b). For wheat plants, two cDNAs encoding the large subunit of AGP have been isolated from seeds, and one cDNA, also corresponding to the large subunit, was isolated from leaf tissue (Olive et al. 1989). In *Arabidopsis*, evidence for the presence of at least three genes for the large subunit and one gene for the small subunit of AGP has been obtained (P. Villand, unpublished). At least two genes for AGP have also been demonstrated for other plants (Krishnan et al. 1986; Bae et al. 1990; Müller-Röber et al. 1990; Smith-White and Preiss 1992). The genes may encode proteins of distinct regulatory and physical properties, as recently found for the AGP enzymes from the endosperm and leaves of barley (Kleczkowski et al. 1993a, b).

Despite a great deal of interest in the mechanisms underlying starch formation in plants in general, and in the molecular and enzymatic properties of AGP in particular, very little is known about the organization and localization of AGP genes in plant genomes. To our knowledge, only the gene for the large subunit of maize seed AGP has been mapped (Shaw and Hannah 1992). Here

we report the results of Southern analyses and the mapping of genes encoding the endosperm small and large subunits of AGP and the leaf AGP large subunit in barley.

Materials and methods

Plant material

Polymorphisms were determined for six restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Xba*I) with the four parents (Steptoe, Morex, Harrington, TR306) of the mapping populations used by the North American Barley Genome Mapping Project (NABGMP). Polymorphic bands were mapped using 150 doubled haploid lines of the Steptoe × Morex cross (Kleinhofs et al. 1993). Cytologically-verified ditelosomic additions of barley cv Betzes in a wheat cv Chinese Spring background (Islam 1983) were obtained from Dr. B. Gill, Kansas State University, Manhattan, Kansas.

Probes

The probes used in this study are described in Table 1. The barley endosperm AGP small subunit full-length cDNA clone (bepsF2; approximately 1800 bp) was obtained from barley endosperm RNA by the RACE (rapid amplification of cDNA ends) technique and cloned into a plasmid vector (P. Villand, unpublished). Oligonucleotide primers were designed based on published sequence information (Villand et al. 1992a). The barley endosperm AGP large subunit (bepl10) (Villand et al. 1992b) and leaf AGP large subunit (blpl) cDNA clones (Villand et al. 1992a) have already been described. The wheat endosperm AGP large subunit cDNA clone was provided for mapping purposes by Olive et al. (1989).

Methods

Mapping techniques are described in Kleinhofs et al. (1993). The hybridization and washing stringency was adjusted to permit a maximum of 15% mismatch.

Results and discussion

Endosperm ADP-glucose pyrophosphorylase – small subunit

The bepsF2 probe hybridized to the genomic DNA of barley cultivars Steptoe, Morex, Harrington and TR306 revealed one band with *Bam*HI-digested DNA, two equal intensity bands with *Dra*I, *Eco*RI and *Eco*RV, and three bands with *Hin*dIII and *Xba*I. These results suggested either a single gene with several internal restriction sites or several gene copies. A preliminary restriction map of an approximately 15-kb barley AGP endosperm small subunit genomic clone (T. Thorbjørnsen, unpublished data) confirmed the presence of internal restriction sites yielding the fragment sizes observed with the Southern-blot analysis. These results indicate a single copy of the endosperm AGP small subunit gene in barley. Hybridization of the same probe with hexaploid wheat DNA (cv Chinese Spring) revealed six bands with each restriction enzyme, suggesting the presence of one or two genes per wheat genome. We cannot differentiate between these two possibilities at this time. The observation of a single endosperm AGP small subunit gene per barley genome would suggest that the same may be true for the wheat genome. On the other hand, the NADH nitrate reductase gene exists as a single copy in the barley genome but is present in two copies per genome in wheat (Kleinhofs et al. 1988). Analysis of a wheat genomic clone(s) is needed to resolve this issue.

Polymorphisms were not detected among the four parents by the six enzymes used by NABGMP or 30 other barley genotypes tested including Bomi, the source of the genomic clone. This represents a remarkable lack of polymorphism for this genome region in a species with moderate levels of polymorphism overall. In order to associate the barley endosperm AGP small subunit gene with a chromosome arm, the probe was hybridized to wheat/barley ditelosomic addition line DNA. All bands corresponding to Betzes barley DNA were localized to the addition line with chromosome 1 M (minus arm, also referred to as the "long" or "alpha" arm). This locus was designated *Aga1*.

Endosperm ADP-glucose phyrophosphorylase – large subunit

The endosperm AGP large subunit cDNA clones from barley (bepl10) and wheat (pAga7) were hybridized as above. The hybridization patterns were identical with the two probes, indicating that they recognize the same sequences. This reflects the very high level of DNA sequence conservation between the barley and wheat genes (Villand et al. 1992a, b). One major and one-to-three minor bands were detected with all enzymes except *Eco*RI which showed two approximately-equal intensity bands and one minor band. This suggests the presence of a single copy of this gene in the barley genome and the existence of other related sequences (the minor bands did not cross-hybridize with any of the other probes characterized in this report). There was substantial polymorphism among the barley cultivars tested. The major band was mapped to the distal region of chromosome 5 M (minus or long arm) and the locus designated *Aga7* (Kleinhofs et al. 1993). The minor bands have not been mapped.

Leaf ADP-glucose pyrophosphorylase – large subunit

The leaf AGP large subunit cDNA clone blpl hybridized to one-to-two major and two-to-three minor bands. This suggests the presence of one or two copies of this gene and other related sequences. The minor bands were unique to this probe and did not cross hybridize with other probes characterized in this report. They may represent related, but diverged se-

Table 1. Organization and mapping of AGP genes in barley

Probe	Function[a]	Band	Locus	Chr[c]	Copy no.
bepsF2	Endosperm, SS	Major	*Aga1*	1P	1
bepl10	Endosperm, LS	Major	*Aga7*	5M	1
	Endosperm, LS	Minor	ND[b]	ND	1–2
blpl	Leaf, LS	Major	*Aga5*	7M	1
	Leaf, LS	Minor	*Aga6*	5P	1–2

[a] SS, small subunit; LS, large subunit
[b] ND, not determined
[c] Chromosome: P, plus or shot arm; M, minus or long arm

quences, possibly pseudogenes. In wheat, three bands were detected with several restriction enzymes suggesting one copy per genome. Polymorphism was not detected for the major bands with the standard NABGMP blot using four parents and six enzymes. Polymorphism for a minor band detected with the *Eco*RI digest was mapped to the most telomeric location of chromosome 5 P. This locus was designated *Aga6* (Kleinhofs et al. 1993). A major band was associated with chromosome 7 M and designated *Aga5*.

Number of AGP genes in barley

The organization and mapping of AGP genes in barley is summarized in Table 1. A conservative estimate indicates the presence of three single-copy genes corresponding to each of the barley cDNA clones used as probes in these experiments, and of several related DNA sequences (Table 1). The hybridizations in these experiments were conducted at high stringency requiring approximately 85% identity, indicating that the minor bands represent closely-related sequences. How many of these sequences are actually active genes, encoding a functional subunit of AGP protein, is unknown. The presence of multiple genes for AGP may reflect the need of different tissues to express AGP proteins which respond differentially to regulation by effectors, as found for the enzyme from barley seed endosperm and leaves (Kleczkowski 1993a, b).

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