Thionin genes specifically expressed in barley leaves

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Abstract. Complementary-DNA (cDNA) clones encoding thionin were identified as one of the most frequent types of clones in a cDNA library constructed from total polyadenylated RNA from young barley leaf cells. One full-length clone codes for a precursor protein that starts with a signal peptide (28 amino acids) followed by the mature thionin (46 amino acids) and terminated by a long acidic extension (63 amino acids). The amino-acid sequence of the leaf thionin is 52% homologous to thionins from barley endosperm and in the C-terminal extension the homology decreases to 41%. In contrast, the leaf thionin is 72% homologous to viscotoxin from mistletoe leaves. Leaf thionin is coded by a multigene family with an estimated nine to eleven genes and analysis of the cDNA clones showed that at least two extremely homologous genes are expressed. Northern hybridization experiments indicate that the leaf thionin genes are not expressed in endosperm and roots. In leaves, the expression of the thionin genes is strongly repressed by light.

Key words: Hordeum (thionin) – Multigene family (thionin) – Thionin (barley leaf).

Introduction

Thionins from wheat and barley endosperm and viscotoxins from mistletoe are homologous, basic proteins with 45-46 amino acids and a high cysteine content. The proteins cause skin and skeletal muscle contraction and are toxic to animals (when injected), yeast and bacteria. Their toxicity may be related to their affinity for membranes since they inhibit sugar uptake in yeast and lyse erythrocytes and other mammalian cells in culture (reviewed by Ramshaw 1982). The crystal structure of a closely related hydrophobic protein, crambin (from Abyssinian cabbage) has been resolved at 0.15 nm (Hendrickson and Teeter 1981) and biophysical studies indicate that the membrane-bound crambin and thionins have identical structures (Williams and Teeter 1984). As for other toxic plant proteins like the lectins and protease inhibitors, the functions of thionins and viscotoxins in plants are not known. The finding that thionin can substitute for a chlorplast thioredoxin in redox regulation of enzyme activity indicates that thionins may have similar regulatory roles elsewhere in the cell (Wada and Buchanan 1981).

Thionin in barley endosperm is synthesized as a precursor including both a signal peptide and a C-terminal extension which is longer than the mature protein itself (Ponz et al. 1983; Ponz et al. 1986). The fate and possible function of the C-terminal extension is not known. Clones of cDNA encoding barley endosperm thionin have recently been isolated (Ponz et al. 1986; Hernández-Lucas et al. 1986) and the derived amino-acid sequences agree with the tripartite structure of thionin precursors. Here it is shown that different thionin genes are highly expressed in barley leaves. The derived amino-acid sequence of the leaf thionin precursor has a structure similar to the endosperm precursor but the sequence of the mature leaf protein is more closely related to viscotoxin and phoratoxin from mistletoe.

Material and methods

Plant material. Seeds of Hordeum vulgare cv. Bomi were germinated in darkness and grown for 7 d with day periods of 16 h at 22°C and night periods at 18°C. Etiolated leaves were grown for 7 d in darkness and harvested under green safelight. For the preparation of RNA the leaves were cut close to the seeds, the coleoptiles and second leaves were removed, and the basal one-third and top two-thirds of the leaves and the coleoptiles were frozen in liquid N2 and stored at -80°C until use. Roots were harvested from seedlings grown in liquid supported by a metal grid. Endosperms were obtained from spikes harvested...
Hybridization experiments. The preparation of radioactive cDNA, plasmid DNA and PstI fragments from the plasmids and their labeling by nick-translation as well as procedures for hybridizations with bacterial colonies, RNA, and DNA have been described by Gausing and Barkardottir (1986). Hybridization experiments with endosperm RNA at reduced and normal stringency conditions were carried out as follows. Nitrocellulose filters (GeneScreen; New England Nuclear, Boston, Mass., USA) containing size-fractionated leaf and endosperm total RNA were pre-incubated for 3 h at 42°C in hybridization buffer (0.45 M NaCl; 0.045 M Na-citrate; 0.1% sodium dodecyl sulphate; 10-fold-concentrated Denhardt solution, Denhardt 1966; 50 µg·ml⁻¹ denatured, sonicated salmon sperm DNA; 35 or 50% formamide). Hybridization with nick-translated PstI inserts from pKG1348 (5 ng·ml⁻¹, 5·10⁷ cpm·µg⁻¹ DNA) were at 42°C for 40 h and the filters were washed three times at 42°C with hybridization buffer without carrier DNA and once at room temperature with 0.45 M NaCl, 0.045 M Na-citrate, 0.1% sodium dodecyl sulphate.

The barley leaf cDNA library. The library was constructed from total poly(A)RNA from the basal 4 cm of 7-d-old green leaves (total length 12.5 cm). Double-stranded DNA synthesized from the poly(A)RNA was inserted into the PstI site in the vector pBR327 using oligo (dG-dC) tailing and 4800 recombinant clones were collected and stored in numbered snap-cap tubes and in 96-well microtiter plates at -80°C (Gausing and Barkardottir 1986). All clones in the library were analysed by differential hybridizations with radioactive cDNA prepared from total poly(A)RNA from the basal one-third of green leaves, etiolated leaves, and roots. Twenty five percent of the clones hybridized detectably with cDNA from one or more of the three types of tissue and 31 clones hybridized very strongly to cDNA from green leaves. Nick-translated PstI inserts from individual recombinant plasmid DNAs from this strongly hybridizing group were hybridized to all other clones in the library - they hybridized to other strongly hybridizing clones and to clones that had given medium-strong hybridization in the first screening with leaf cDNA. In this way the 31 strongly hybridizing clones were accounted for; they correspond to five different types of genes, one of which is shown here to code for thionin. The differential hybridization experiments further indicated that the thionin genes also are highly expressed in etiolated leaves but not in roots.

Analyses of DNA sequences. Suitable restriction-enzyme fragments from the four recombinant plasmids sequenced were cloned in M13mp9 (Messing and Vieira 1982). Isolation of single-stranded DNA and sequencing by the method of di-deoxy-nucleotide chain-termination was essentially as described by Sanger et al. (1980).

Results

Isolation and initial characterization of thionin-cDNA clones from barley leaves

The isolation of cDNA clones encoding the precursor of thionin is the result of a general survey of a cDNA library (4800 clones) constructed from total poly(A)RNA from the basal (young) one-third of 7-d-old barley leaves. The library was screened for clones coding for abundant messengers and represented by a correspondingly large number of clones in the library. Five groups of clones that strongly hybridized to all other clones in the library - they hybridized to other strongly hybridizing clones and to clones that had given medium-strong hybridization in the first screening with leaf cDNA. In this way the 31 strongly hybridizing clones were accounted for; they correspond to five different types of genes, one of which is shown here to code for thionin. The differential hybridization experiments further indicated that the thionin genes also are highly expressed in etiolated leaves but not in roots.

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