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The precursor of barley plastocyanin

Sequence of cDNA clones and gene expression in different tissues

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Full length cDNA clones encoding the precursor of plastocyanin have been isolated from a leaf cDNA library from barley (a monocotyledon). The deduced amino acid sequence comprises an N-terminal transit peptide of 58 amino acids and a mature plastocyanin sequence of 97 amino acids (M_r 10185) which is two amino acids shorter than the sequence of plastocyanin from dicotyledons. Northern hybridization experiments show that the level of plastocyanin messenger increases greatly in older leaf cells and that the level is 8–20-fold higher in green leaves than in etiolated leaves. Plastocyanin messenger could not be detected in coleoptiles and roots.

Plastocyanin; cDNA clone; Precursor sequence; Light regulated gene expression; (Barley)

1. INTRODUCTION

Plastocyanin, a member of the photosynthetic electron transport chain, is a small copper protein (10.4–11.2 kDa) located in the chloroplast thylakoid lumen [1]. The nuclear-encoded plastocyanin is synthesized as a precursor in the cytoplasm and the precursor is processed in two steps during the posttranslational transport, first into the chloroplast and subsequently into the thylakoid lumen [2,3].

Early studies on the control of plastocyanin synthesis suggested that plastocyanin activity is unaffected by light [4], stimulated by light [5], and perhaps controlled by phytochrome [5]. More recent immunochemical studies showed that plastocyanin accumulation is significantly stimulated by light (about 30-fold) in greening pea, wheat and barley [6].

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The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession number Y00704

2. MATERIALS AND METHODS

2.1. Plant material

Hordeum vulgare c.v. Bomi was grown for 7 days with day periods of 16 h at 22°C and night periods at 18°C. Etiolated leaves were grown for 7 days in darkness and harvested under green safelight.

2.2. *The barley leaf cDNA library*

The library was constructed from total poly(A) RNA from the basal 4 cm of 7 day old leaves. The cDNA was inserted into the *Pst*I site in pBR327 us-

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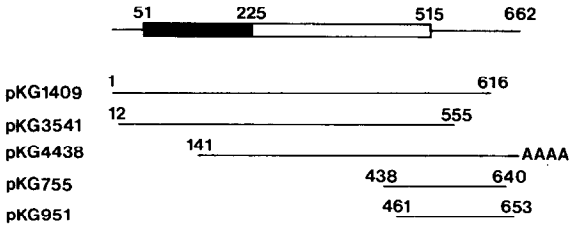

Fig.1. Diagram of inserts from five barley plastocyanin cDNA clones. In the top line, the black bar represents the region coding for the transit peptide and the white bar the region coding for mature plastocyanin. The numbering of nucleotides is the same as in fig.2.

ing oligo(dG-dC) tailing and 4800 recombinant clones were collected and stored at −80°C [7,8].

2.3. *Hybridization experiments*

Non-stringent colony hybridization of a nick-translated insert from the white campion plastocyanin clone, pPC2 [9] to the barley leaf cDNA library was as in [7] except that the salt concentration was increased to 6 × NaCl/Cit (1 × NaCl/Cit is 0.15 M NaCl, 0.015 M Na-citrate) during the hybridization and to 4 × NaCl/Cit during the washing of filters. The extraction of total RNA

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                                10        20        30        40        50
                    AACAATTGGCTAGGCTAGCTCTCCTCCTCTGCAGTACGTAGCAAGCAGCC

          60        70        80        90        100       110
ATGGCCGCCCTCTCCTCTGCAGCCGTGTCCGTCCCTTCCTTCGCCGCCGCCACCCCCATG
 M  A  A  L  S  S  A  A  V  S  V  P  S  F  A  A  A  T  P  M

         120       130       140       150       160       170
CGCAGCAGCAGAAGCAGCAGGATGGTTGTGCGCGCTTCCCTGGGCAAGAAGGCCGCCAGC
 R  S  S  R  S  S  R  M  V  V  R  A  S  L  G  K  K  A  A  S

         180       190       200       210       220       230
GCGGCCGTGGCCATGGCCGCCGGCGCCATGCTGCTGGGCGGGTCCGCGATGGCACAGGAC
 A  A  V  A  M  A  A  G  A  M  L  L  G  G  S  A  M  A  Q  D

         240       250       260       270       280       290
GTGCTCCTGGGTGCCAACGGCGGCGTGCTGGTGTTCGAGCCCAACGACTTCAGCGTCAAG
 V  L  L  G  A  N  G  G  V  L  V  F  E  P  N  D  F  S  V  K

         300       310       320       330       340       350
GCCGGCGAGACCATCACCTTCAAGAACAACGCCGGGTACCCGCACAACGTGGTGTTCGAC
 A  G  E  T  I  T  F  K  N  N  A  G  Y  P  H  N  V  V  F  D

         360       370       380       390       400       410
GAGGACGCCGTGCCCTCCGGCGTCGACGTCTCCAAGATCTCCCAGGAGGAGTACCTCACA
 E  D  A  V  P  S  G  V  D  V  S  K  I  S  Q  E  E  Y  L  T

         420       430       440       450       460       470
GCCCCCGGCGAGACCTTCTCCGTCACCCTCACCGTCCCCGGCACCTACGGCTTCTACTGC
 A  P  G  E  T  F  S  V  T  L  T  V  P  G  T  Y  G  F  Y  C

         480       490       500       510       520       530
GAGCCCCACGCTGGGGCCGGCATGGTCGGAAAGGTCACCGTCAACTAATGAAGCTACCAA
 E  P  H  A  G  A  G  M  V  G  K  V  T  V  N

         540       550       560       570       580       590
TTTGGGTCGATTGATCGCTCAAATTGGACGTGTCGTGCATGCATCATATTGATTAATTGT

         600       610       620       630       640       650
ACGCTATATGTATGTATATGTTTTCTACTCTACTCTACTCTACTCTATAAGATCGATGAG

         660
GCTCTTCGTGAT(A)11
```

Fig.2. DNA sequence of the messenger-like strand and the deduced amino acid sequence of the barley plastocyanin cDNA clones. The probable processing site between the transit peptide and mature plastocyanin is indicated by an arrowhead.

```
                    10
B.    Q D V L L G A N G G V L V F E P N D F
W.    A E . . . . S S D . G . A . V . S . L
C.  D V T . K . . . D S . A . . . . . S S V

  20                30
B.  S V K A G E T I T F K N N A G Y P H N V
W.  . I A S . . K . . . . . . . F . . . .
C.  T I . . . . . V . W V . . . . F . . . I

  40                50
B.  V F D E D A V P S G V D V S K I S - - Q
W.  . . . . . E . . A . . . . T . . . M P E
C.  . . . . . E . . . . A N A E A L . - - H

  60                70
B.  E E Y L T A P G E T F S V T L T V P G T
W.  . D L . N . . . . E Y . . . . . E K . .
C.  . D . . N . . . . S Y . A K F D T A . .

  80                90
B.  Y G F Y C E P H A G A G M V G K V T V N
W.  . K . . . A . . . . . . . . . . . . . .
C.  . . Y F . . . . Q . . . . K . T I . . Q
```

Fig.3. Comparison of plastocyanin amino acid sequences. (B) Barley, (W) white campion [9], (C) *Chlorella* [15]. Amino acids that are identical to the barley sequence are indicated by dots, a dash indicates a lacking amino acid. Residues 39–40 in the white campion sequence have been corrected after publication ([9], Smeekens, S., personal communication).

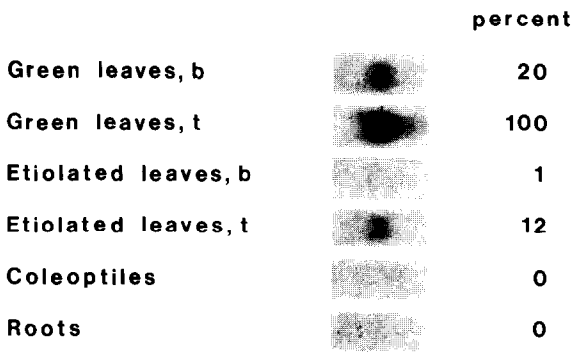

Fig.5. Expression of plastocyanin gene(s) in leaves, coleoptiles and roots of barley. Total RNA (7.5 and 15 μg samples) from the basal (b) one third and top (t) two thirds of green and etiolated leaves, from coleoptiles and roots were denatured with glyoxal, fractionated on agarose gels, and transferred to GeneScreen nitrocellulose filters. Filters were hybridized with a nick-translated insert from the plastocyanin cDNA clone, pKG4438, washed and autoradiographed. The figure shows an autoradiogram of filters with 15 μg RNA samples. The percentage values are the results of densitometric measurements of a series of autoradiograms.

and Northern hybridization of the RNA samples has been described [7,8].

2.4. *DNA sequence analysis*

Selected restriction fragments from the recombinant plasmids were subcloned in M13 mp9 [10] and sequenced by the dideoxynucleotide chain termination method [11].

3. RESULTS AND DISCUSSION

A barley leaf cDNA library [7,8] was screened by colony hybridization at reduced stringency with a nick-translated plastocyanin probe from white campion [9]. The library (4800 clones) contained five plastocyanin clones, shown in fig.1. They were indistinguishable by restriction enzyme site map-

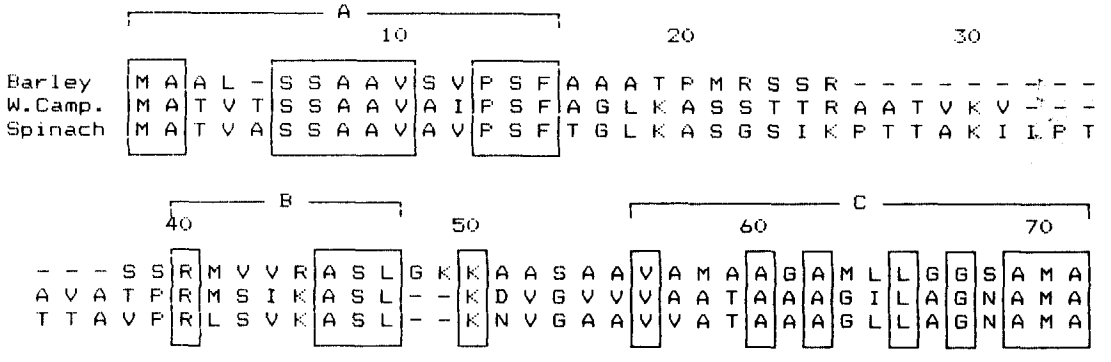

Fig.4. Comparison of transit peptides of plastocyanin precursors from barley, white campion, and spinach. Identical amino acids are boxed and gaps introduced to maximize homology are indicated by dashes. The domains, A, B and C are described in the text.

ping in the regions where they overlap and the nucleotide sequences (determined for 85% of the combined inserts) were identical. Thus it is likely that the cDNA clones are derived from the same gene and Southern hybridization of barley DNA (not shown) suggests that it could be the only plastocyanin gene. In spinach there is similarly no evidence for more than one plastocyanin gene [12].

The nucleotide sequence of the plastocyanin cDNA clones is shown in fig.2. The long open reading frame that encodes the precursor of plastocyanin is preceded by a 5′-untranslated region containing three stop codons, the last of which is in frame with the first methionine codon. Alignment of the precursor sequence from barley, white campion [9], and spinach [12] suggests that the mature barley plastocyanin starts with glutamine (figs 3 and 4), an amino acid not hitherto found in the N-terminus of plastocyanin. In fig.3 the sequence of mature barley plastocyanin is compared with the sequence from white campion (representing 14 different dicotyledon plastocyanin sequences) and *Chlorella* (representing two different green algae sequences). The most notable feature is the deletion of amino acids 57–58 shared by barley and green algae plastocyanin. This deletion, however, is not a specific monocotyledon/algae feature since one dicotyledon sequence (from parsley [13]) curiously also lacks amino acids 57–58.

Three regions with distinct similarity are discernible in the transit peptides from different plastocyanin precursors (fig.4) and functional studies have implicated these regions in separate steps of the transport process [2]. Region A through to B is functional during import into the chloroplast with intermediate processing near residue 43. The remaining part of the transit peptide is functional during thylakoid membrane transfer and region C includes part of a signal-peptide-like domain and the final processing site [2].

3.1. *Expression of plastocyanin gene(s)*

The relative content of plastocyanin messenger was determined by Northern hybridizations of total RNA from different parts of 7 day old barley seedlings. Leaves from green and etiolated plants were divided into two parts, the basal one third, containing cells up to the age of about 36 h, and the top two thirds where most cells are between 1.5 and 5 days old [14]. Coleoptiles and roots were from green plants. The results (fig.5) indicate that the expression of the probable single active barley gene is stimulated by light and is specific for leaves (and other organs with photosynthetic capability). Previously it was found that the accumulation of plastocyanin increases 30-fold during greening [6] and although not directly comparable, the 5–20-fold difference in plastocyanin messenger levels in green and etiolated leaves, suggests that plastocyanin synthesis is principally regulated at the level of gene expression.

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