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Brief report

Isolation and characterization of a cDNA coding for cytoplasmic glutamine synthetase of barley

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Glutamine synthetase (GS; EC 6.3.1.2) is a key enzyme in the nitrogen metabolism of plants since it catalyzes the assimilation of ammonia into glutamine. In the glutamine synthetase–glutamate synthase cycle, glutamine is then used to form glutamate, the nitrogen donor in the main biosynthetic pathways of nitrogenous compounds (MIFLIN and LEA 1980).

Glutamine synthetase in plants can exist as distinct isoforms which are associated with specific organs and cellular compartments (MC NALLY et al. 1983). Most commonly, plants have two isoforms, one in the cytosol (GS1) and the other in the chloroplasts (GS2), while a nodule-specific form (GSn) can be induced during nodulation in symbiosis-forming legumes (FORDE and CULLIMORE 1989). In barley two isoforms were found by MANN et al. (1979) and the cDNA encoding the chloroplastic isoenzyme has been characterized (BAIMA et al. 1989; FREEMAN et al. 1990; STRØMAN et al. 1990).

Here we report the characterization of a full-length cDNA which codes for cytoplasmic glutamine synthetase from barley (*Hordeum vulgare* L.). The clone was obtained from a λgt11 cDNA library given to us by K. Apel (Botanisches Institut, Christian-Albrechts Universität, Kiel, Germany). The screening was effected using the labelled 1.2 kb Bgl II–Stu I fragment of the pGS100 plasmid (DASSARMA et al. 1986), a gift of H. M. Goodman (Dept. of Molecular Biology and Dept. of Genetics, Harvard Medical School, Massachusetts, USA). After subcloning into pUC18, the resulting pGS3 plasmid was used for further characterization.

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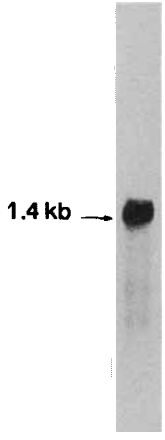

Fig. 1. Northern analysis of Poly A^+ obtained from light-grown barley leaves. 15 μg/lane were fractionated by size in a 1.4 % agarose/formaldehyde gel. The probe was the EcoRI insert from pGS3.

In order to verify whether the insert was a full-length GS1 cDNA or an incomplete GS2 cDNA, after labelling, it was used in a Northern blot analysis of poly A^+ RNA obtained from light-grown barley leaves. Under high stringency conditions it gave a hybridization band of about 1.4 kb (Fig. 1), as expected for barley cytoplasmic GS (BAIMA et al. 1989).

The insert is 1.456 bp long and contains an open reading frame encoding 356 amino acid residues (Fig. 2). The sequence is relatively GC-rich (58 %) with a high frequency (72 %) of either G or C in third position. This pattern of bias in the nucleotide composition is consistent with that observed in other monocotyledon sequences (FORDE and CULLIMORE 1989). Like with other plant species, also in barley the similarity between the cytoplasmic and the chloroplastic sequences is

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          GAATTCCCTCCCTCCCTGCCCTCAGTCGTCCAGCCGGGTTCCTCCATCCCTCCCGCC -57
ATG GCG CTC CTC ACC GAT CTC CTC AAC CTC GAC CTC TCC GGC TCC ACG GAG  51

AAG ATC ATC GCC GAG TAC ATA TGG ATC GGC GGA TCT GGC ATG GAT CTC AGG  102

AGC AAG GCC AGG CAC CTC CCC GGC CCG GTC ACC CAC CCC AGC AAG CTG CCC  153

AAG TGG AAC TAC GAC GGC TCC AGC ACC GGC CAG GCC CCG GGC GAG GAC AGC  204

GAG GTC ATC CTG TAC CCA CAG GCC ATC CTC AAG GAC CCG TTC AGG GAG GGA  255

AAC AAC ATC CTT GTC ATG TGC GAT TGC TAC ACC CCA CGT GGA GAG CCA ATC  303

CCC ACC AAC AAG AGA TAC AAC GCT GCT AAG ATC CTT AGC AAC CCC GAT GTT  357

GCC AAG GAG GAG CCA TGG TAC GGT ATT GAG CAG GAG TAC ACC CTC CTA CAG  408

AAG GAC ATC AAC TGG CCT CTC GGC TGG CCT GTT GGT GGC TTC CCT GGT CCT  459

CAG GGT CCC TAC TAC TGT GGT ATT GGT GCT GAC AAG TCG TTT GGG CGT GAC  510

ATA GTT GAC TCC CAC TAC AAG GCT TGC CTC TTT GGC GGC GTC AAC ATC AGT  561

GGC ATC AAC GGC GAG GTC ATG CCC GGA CAG TGG GAG TTC CAA GTT GGC CCG  612

ACT GTT GGC ATT TCT GCT GGT GAC CAA GTG TGG GTC GCT CGC TAC ATT CTT  663

GAG AGG ATC ACC GAG ATC GCC GGA GTT GTC GTC ACG TTT GAC CCC AAG CCC  714

ATC CCA GGC GAC TGG AAC GGT GCT GGT GCT CAC ACG AAC TAC AGT ACC GAG  765

TCG ATG AGG AAT GAC GGT GGG TTC AAG GTC ATC GTG GAC GCG GTC GAG AAG  816

CTC AAG CTG AAG CAC AAG GAG CAC ATC GCG GCC TAC GGC GAG GGC AAC GAG  867

CGC CGT CTG ACC GGC AAG CAC GAG ACG GCC GAC ATC AAC ACC TCC AGC TGG  918

GGT GTG GCA AAC CGT GGC GCG TCG GTG CGC GTG GGC CGG GAG ACG GAG CAG  969

AAC GGC AAG GGC TAC TTC GAG GAC CGC CGG CCG GCG TCC AAC ATG GAC CCC  1020

TAC GTG GTC ACC TCC ATG ATC GCC CAG ACC ACC ATC CTG TGG AAG CCC TGA  1071

AGCTCCGATCGCCGTGTGATGGACCGTCGGTGATGGGGTCCGGTGGTGGCCATTGGAGGATTCGTGC
CTTGGGCGAAAATTCTTCCAGCATTTTCCTTTTACGTGTGGNTGNATACTACTCCTAGTCCGCTTAG
GTAGGTCACATCATCATGGTCATCTCATCAGGGTGTCTGGTCTCTCTTCTCGCTCTCGTCTNTGGGT
GGGTGGTGGGTGATGGGTGGCAAGGGGCGTGTCAAAGCAGATTGATATGGTAATAAAACAAGATTAC
TACAGTATNTGGGTGATTGTTAACCCTTGCCGTCTGGATGCTATGGTCTCGTGTAATCTC
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Fig. 2. Nucleotide sequence of the cDNA for the barley cytoplasmic glutamine synthetase (GS3).

lower (70.2 % at the nucleotide and 73 % at the aminoacid level, respectively) than that between cytoplasmic sequences of other plant species (similarity 75–87 % and 79–89 %, respectively). The deduced protein sequence (Fig. 3) has 4 cysteins, 3 of which (position 92, 159, 179) are common to all known plant cytoplasmic GS, the fourth (position 94) is only found in the monocotyledon sequences.

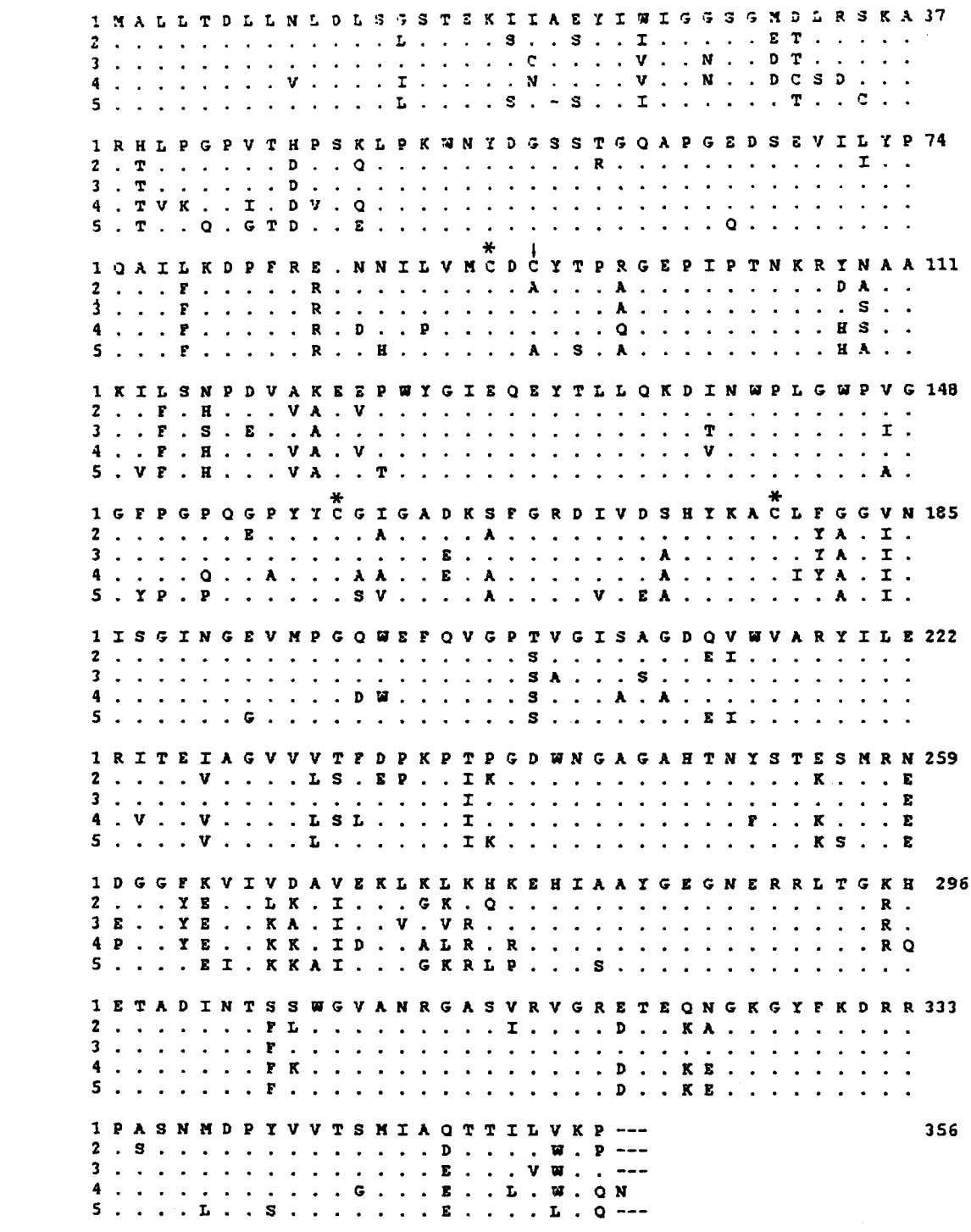

Fig. 3. Comparison of the deduced primary structure of higher plant cytoplasmic glutamine synthetases from 1. barley, 2. alfalfa (TISCHER et al. 1986), 3. maize (SNUSTAD et al. 1988), 4. rice (SAKAMOTO et al. 1989), 5. pea (TINGEY et al. 1987). Asterisks indicate cysteins common to all glutamine synthetases; arrow indicates a cystein residue present only in the monocotyledons.

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