The isolation and characterization of a cDNA clone encoding *Lupinus angustifolius* root nodule glutamine synthetase

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Abstract

Glutamine synthetase, purified from *Lupinus angustifolius* legume nodules, was carboxymethylated and succinylated prior to chemical or enzymatic cleavage. Peptides were purified and sequenced. An oligonucleotide probe was constructed for the sequence MPGQW. This probe was used to identify a glutamine synthetase cDNA clone, pGS5, from a lupin nodule cDNA library constructed in pBR322. pGS5 was sequenced (1043 bp) and computer-assisted homology searching revealed a high degree of conservation between this lupin partial cDNA clone and other plant glutamine synthetases at both the amino acid (>90%) and nucleotide (>80%) level. Northern and Southern analyses using pGS5 supported the conclusion that a multigene glutamine synthetase family exists in lupin which is differentially expressed in both an organ-specific and temporal manner. Western and Northern blot analyses indicated the accumulation of a glutamine synthetase specific mRNA species during nodule development corresponded to the appearance of a novel glutamine synthetase polypeptide between 8 and 10 days after rhizobial inoculation.

Introduction

Glutamine synthetase has been proposed to have a major role in the assimilation of ammonia produced by nitrogen fixation in the bacteroids of legume nodules (see [40] for review). The activity of the enzyme in the nodules of 21-day-old lupin plants is 500 times higher (on a fresh weight basis) than in the roots of the same plants and the activity increases in parallel with nitrogenase and leghaemoglobin synthesis [41].

Interest in the synthesis of glutamine synthetase in plants has heightened since the report of the induction of a gene encoding a nodule-specific form of glutamine synthetase in *Phaseolus* root nodules [11]. Evidence for a glutamine synthetase multigene family came initially from the work of Cullimore *et al.* [10]. Glutamine synthetase cDNA clones have also been isolated from lucerne [50], pea [49], soyabean [22] and a genomic clone from lucerne has been sequenced [50]. Indeed, glutamine synthetase in higher

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X15578.
plants has become a model system for the study of the differential regulation of multigene families [3, 22, 48 and references, therein].

In addition, a putative ATP-binding domain, glutamate-binding site and active site have been predicted for glutamine synthetase from prokaryote and eukaryote enzymes by homology studies [24, 37, 50] and atomic modelling [1].

Here we report the isolation and sequence of a Lupinus angustifolius glutamine synthetase cDNA clone and examine the induction of glutamine synthetase during root nodule development.

Materials and methods

Plant material and bacterial cultures

Lupin (Lupinus angustifolius L.) seeds, imbided and germinated for 2 days under sterile conditions were inoculated with Rhizobium lupini (NZP2257) and grown in a controlled-environment cabinet (12 h day, 24 °C/21 °C day/night temperature) in stainless steel troughs containing sterile vermiculite or pumice. Plants were supplied with a sterile nitrogen-free nutrient solution (1/5 strength Jensen’s solution [25], supplemented with trace elements.

R. lupini were maintained and cultured as described [42].

Preparation of cell-free extracts

Rhizobial, bacteriod and plant cytosol fractions from the legume root, the early nodulation zone (infection zone) and nodules were prepared essentially as described [13].

Purification of glutamine synthetase from lupin nodules

The procedure used here was based on that of McCormack et al. [32] with the following modifications.

Glutamine synthetase activity was precipitated between 48% and 65% ammonium sulphate saturation at 4 °C, and the pellet resuspended in a minimum volume of 0.05 M potassium phosphate (pH 7.6). Insoluble material was pelleted by centrifuging at 10000 × g for 5 min, and the supernatant loaded onto a Sephacryl S-300 column (2.5 cm × 1.5 m).

Preparation and characterisation of antibodies to lupin glutamine synthetase

Antibodies were raised to purified glutamine synthetase in New Zealand White rabbits. The rabbit was subsequently exsanguinated and the 25–45% saturated ammonium sulphate fraction of the serum yielded a crude IgG fraction that was resuspended in phosphate-buffered saline containing 50% glycerol and stored at −20 °C until required.

Electrophoresis and immunoblotting

SDS-PAGE using 15% or 17.5% acrylamide resolving mini-gels and Coomassie Brilliant Blue R-250 staining was performed essentially as described [34].

Immunoprobing of SDS polyacrylamide gels was achieved by blotting to nitrocellulose and immunoreactive material was detected with horseradish peroxidase-conjugated goat anti-rabbit IgG (TAGO Inc., USA).

RNA preparation

Total RNA was prepared from frozen tissue ground to a fine powder under liquid nitrogen. This material was initially extracted in guanidinium isothiocyanate [31], followed by guanidinium hydrochloride, and phenol/chloroform extractions and a final differential precipitation with 3 M sodium acetate, pH 6.0 [7]. Poly (A)⁺ RNA was isolated with oligo(dT) cellulose [2].