Final Project Report to Protein Research Trust

In situ localization of nodule lectin mRNA and analysis of lectin genes

Executive Summary

I.J. Law

ARC-Plant Protection Research Institute, Private Bag X134, Pretoria 0001

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Introduction and objectives

Peanut (*Arachis hypogaea* L.) contains two closely related forms of galactose-binding lectin (GL) respectively found in nodules (NGL) and seeds (SGL) of this plant. Both are generally believed to function as storage proteins and the nodule lectin may provide a nitrogen sink for excess nitrogen fixed in nodules by *Bradyrhizobium* bacteria. Previous cDNA analysis showed that NGL and SGL originate from members of a multigene family within which tissue-specific expression occurs. Two genes for NGL (NGLa and NGLb) were identified that differed in level and pattern of expression. Their respective open reading frames did not, however, correspond in N-terminal amino acid sequence to that obtained for purified NGL protein.

The aim of the present study was to (a) detect genes for nodule GL that might correspond more closely to purified NGL, (b) isolate genes for NGL and SGL from genomic DNA and compare putative promoter regions that control expression, and (c) use *in situ* hybridization to compare the site of expression of NGLa and NGLb in nodule tissues.

Results and discussion

New cDNA Libraries were prepared from peanut nodule mRNA and 29 NGL cDNA clones were isolated. As all corresponded exactly either to NGLa or NGLb, these are presumed to be the major genes for NGL expressed in nodules. It is not known whether analysis of mixed polypeptides derived from NGLa and NGLb could have been responsible for the sequence obtained for purified NGL.

Previous work used genetic material from peanut cv. Sellie. Attempts to generate a genomic DNA library from this cultivar were unsuccessful and instead a library from cv. NC4 was obtained. This library yielded genes that corresponded closely to those for SGL and NGLb from cv. Sellie, but a gene for NGLa was not detected because the library is probably incomplete. DNA sequence analysis showed that the putative promoter regions of two NGL genes, *NGLb-1* and *NGLb-2*, had regions of high and low homology whereas those of SGL genes, *SGL-1* and *SGL-2*, were almost identical. Nucleotide motifs typical of promoter elements were detected. Sequence similarity between *NGLb* and *SGL* promoters was less than 40%, compared to 78-92% for lectin-encoding regions. Several promoter elements were identified that might be involved in determining specificity of expression in nodules or seeds. Homology between the NGL and SGL promoters occurred mainly in the region of the TATA box and putative transcription start sites.

Nodules were harvested after inoculation at 28 d when nitrogen-fixing activity was high and at 42 d when activity was low. *In situ* hybridization showed that expression of NGLa and NGLb occurred at identical sites, mainly vascular and central *Bradyrhizobium*-infected tissues, in both 28-d and 42-d nodules. An immunolocalization method showed, however, that distribution of the lectin antigen changed markedly. In the 28-d-old nodule, NGL was mainly detected in parenchyma cells surrounding vascular and central infected tissues, whereas the 42-d-old nodule contained this lectin only in cells of the central infected zone. These observations agree with the proposed role of NGL as a transient storage protein able to respond to changes in nitrogen supply and demand in various nodule tissues at different times. They also suggest that translational and post-translational control of NGL accumulation in tissues is important.

Further study of nitrogen source/sink effects on the level and distribution of NGL in nodules should provide a greater understanding of the role of this lectin. The present work paves the way for the identification of key promoter elements and regulatory proteins that determine tissue-specific expression of NGL and SGL and will facilitate manipulation of these genes in peanut and other plants.

Aspects of this work will be presented at the 8th Conference of the African Association for Biological Nitrogen Fixation, 23-27 November 1998, Cape Town. An article will be prepared for submission to Plant Science.

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