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### Molecular Cloning, Functional Characterization, and Subcellular Localization of Soybean Nodule Dihydrolipoamide Reductase

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## Molecular Cloning, Functional Characterization, and Subcellular Localization of Soybean Nodule Dihydrolipoamide Reductase<sup>1,2</sup>

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Nodule ferric leghemoglobin reductase (FLbR) and leaf dihydrolipoamide reductase (DLDH) belong to the same family of pyridine nucleotide-disulfide oxidoreductases. We report here the cloning, expression, and characterization of a second protein with FLbR activity, FLbR-2, from soybean (*Glycine max*) nodules. The cDNA is 1,779 bp in length and codes for a precursor protein comprising a 30-residue mitochondrial transit peptide and a 470-residue mature protein of 50 kD. The derived protein has considerable homology with soybean nodule FLbR-1 (93% identity) and pea (*Pisum sativum*) leaf mitochondria DLDH (89% identity). The cDNA encoding the mature protein was overexpressed in *Escherichia coli*. The recombinant enzyme showed  $K_m$  and  $k_{cat}$  values for ferric leghemoglobin that were very similar to those of DLDH. The transcripts of FLbR-2 were more abundant in stems and roots than in nodules and leaves. Immunoblots of nodule fractions revealed that an antibody raised against pea leaf DLDH cross-reacted with recombinant FLbR-2, native FLbR-2 of soybean nodule mitochondria, DLDH from bacteroids, and an unknown protein of approximately 70 kD localized in the nodule cytosol. Immunogold labeling was also observed in the mitochondria, cytosol, and bacteroids of soybean nodules. The similar biochemical, kinetic, and immunological properties, as well as the high amino acid sequence identity and mitochondrial localization, draw us to conclude that FLbR-2 is soybean DLDH.

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<http://www.plantphysiol.org/cgi/content/abstract/128/1/300>