Exploring symbiotic nitrogen fixation and assimilation in pea root nodules by in vivo $^{15}$N nuclear magnetic resonance spectroscopy and liquid chromatography-mass spectrometry - DTU Orbit (19/06/2017)

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Nitrogen (N) fixation and assimilation in pea (Pisum sativum) root nodules were studied by in vivo N-15 nuclear magnetic resonance (NMR) by exposing detached nodules to N-15, via a perfusion medium, while recording a time course of spectra. In vivo P-31 NMR spectroscopy was used to monitor the physiological state of the metabolically active nodules. The nodules were extracted after the NMR studies and analyzed for total soluble amino acid pools and N-15 labeling of individual amino acids by liquid chromatography-mass spectrometry. A substantial pool of free ammonium was observed by N-15 NMR to be present in metabolically active, intact nodules. The ammonium ions were located in an intracellular environment that caused a remarkable change in the in vivo N-15 chemical shift. Alkalinity of the ammonium-containing compartment may explain the unusual chemical shift; thus, the observations could indicate that ammonium is located in the bacteroids. The observed N-15-labeled amino acids, glutamine/glutamate and asparagine (Asn), apparently reside in a different compartment, presumably the plant cytoplasm, because no changes in the expected in vivo N-15 chemical shifts were observed. Extensive N-15 labeling of Asn was observed by liquid chromatography-mass spectrometry, which is consistent with the generally accepted role of Asn as the end product of primary N assimilation in pea nodules. However, the Asn N-15 amino signal was absent in in vivo N-15 NMR spectra, which could be because of an unfavorable nuclear Overhauser effect. gamma-Aminobutyric acid accumulated in the nodules during incubation, but newly synthesized N-15 gamma-aminobutyric acid seemed to be immobilized in metabolically active pea nodules, which made it NMR invisible.

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