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BRIEF COMMUNICATION

Phosphoenolpyruvate carboxylase activity, fixation of ^{14}C in amino acids and nitrogen transport in stem nodules of *Sesbania rostrata*

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Abstract

The *in vivo* $^{14}CO_2$ fixation assay and xylem sap analysis showed that in *Sesbania rostrata* the transport of fixed nitrogen from stem nodules was in the amide form. The majority of nitrogen was transported as asparagine. The close relationship between nodule phosphoenolpyruvate carboxylase and nitrogenase activities suggested that nodule CO_2 fixation contributed directly to nitrogen assimilation in stem nodules of *S. rostrata*.

In annual legumes 30 % of the carbon gained through photosynthesis is used for nodule function and maintenance, and approximately 60 % of the carbon partitioned to the nodule is lost as CO_2 through respiration (Vance *et al.* 1983). The nonphotosynthetic CO_2 fixation occurs *via* phosphoenolpyruvate carboxylase (PEPC) and acts as a mechanism for recovering some of this respired CO_2, thus increasing nodule efficiency (Layzell *et al.* 1979) and maintaining pools of tricarboxylic cycle intermediates required for ammonia assimilation and amino acid biosynthesis (Coker and Schubert 1981).

Several investigations on the role of PEPC in nodule metabolism have involved exposure of either excised or attached nodules to $^{14}CO_2$. According to Maxwell *et al.* (1984), aspartate, asparagine, alanine, glutamate and glutamine are the most heavily labelled compounds in the amino acid fraction of both alfalfa and birdsfoot trefoil nodules exposed to $^{14}CO_2$. Rosendahl *et al.* (1990) have reported that in pea root nodules 72 % of radioactivity in the basic fraction of the cytosol is in aspartic acid.

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Lawrie and Wheeler (1975) demonstrated in excised nodules of *Vicia faba* a high radioactivity initially in glutamate and aspartate and later in asparagine.

Among several types of leguminous green manure, *S. rostrata* is an outstanding nitrogen fixer that has nitrogen fixing nodules both on its root and stem and has 5-10 times more nodules than most root nodulated legumes (Ventura *et al.* 1987). Manguiat *et al.* (1987) have reported that *S. rostrata* may accumulate 27.5 mg m^{-2} (275 kg ha^{-1}) of nitrogen. But the recycling of CO_2 in stem nodules and the form of N transport have not been studied in this plant species. Therefore we measured the PEPC activity of stem nodules of *S. rostrata* along with nodule nitrogenase and determined ^{14}C labelled nitrogen compounds in stem nodules exposed to $^{14}CO_2$.

Seeds of *S. rostrata* were inoculated with *Azorhizobium caulinodans* ORS 571 and sown into pots. After 25 d *Azorhizobium* was again sprayed on the plants. Stem nodules of 40, 50, 60, 70, 80 and 90 d old plants were assayed for PEPC activity according to Ting and Osmond (1973) and protein was estimated by the method of Bradford (1976). The nitrogenase activity of intact stem nodules on excised stem from these plants was estimated by the acetylene reduction technique (Turner and Gibson 1980). *In vivo* CO_2 fixation was analysed as described by Vance *et al.* (1983). Stem nodules (100 mg) from 65 d old plants were placed on moist filter paper at the bottom of a sealed 10 cm^3 reaction flask. The assay was initiated by injection of 4 M lactic acid into center well containing 50 mm^3 of aqueous $NaH^{14}CO_3$ (18 kBg). After incubation for one hour at 23 °C, the reaction was terminated by injection of 1.5 cm^3 of hot 50 % ethanol (70 °C) to the nodules and the flask was opened to release any unreacted $^{14}CO_2$. The nodule sample was homogenized in 50 % ethanol followed by extraction in a 45 °C water bath for 20 min, and centrifuged for 15 min. Radioactivity of aliquots of the supernatant was determined using a proportional counter. Aliquots of the supernatant were then subjected to ascending paper chromatography using phenol : water (4:1, v/v). The paper was then developed with 0.2% ninhydrin in isobutanol. The spots were eluted using ethanol and counted. The amino acids were identified by co-chromatography with standards. The paper chromatogram was then exposed to X-ray film (*Sigma*) and placed in -70 °C for 75 d prior to development. Xylem sap was collected at two different stages before and after stem nodulation by cutting the shoot and drawing the sap by inserting a tefflon capillary tube into the xylem vessel through a syringe. Amino nitrogen content in the sap was estimated by the method of Misra *et al.* (1975) and ureide nitrogen content by the method of Thomas and Schroder (1981). The amino acids in xylem sap were identified by the paper chromatography described above.

Activities of both the PEPC and nitrogenase in stem nodules showed a similar trend (Table 1). Hence the *S. rostrata* nodules actively fixed CO_2 *via* PEPC which was closely associated with nodule N_2 fixation capacity. The transport form of fixed nitrogen in legume species may determine the nature of the relationship between CO_2 fixation and N_2 fixation activity; they are positively correlated in species that transport nitrogen primarily as amide such as lupin (Christeller *et al.* 1977). We confirmed this for *S. rostrata.*

When stem nodules were exposed to $^{14}CO_2$ the ethanol extract showed 349.4 kBq kg^{-1}(nodule f.m.) s^{-1}. Its paper chromatography revealed four spots corresponding to asparagine, aspartic acid, glutamine and glutamic acid (Table 2). Most of the nitrogen transported was in the form of asparagine which was confirmed by autoradiography of the $^{14}CO_2$ labelled compounds. The large quantity of labelled asparagine indicated a direct link between nodule CO_2 and N_2 assimilation, possibly through oxaloacetic acid, the initial product of nodule CO_2 fixation by PEPC (Deroche and Carrayol 1988).

Table 1. Phosphoenolpyruvate carboxylase and nitrogenase activity in stem and nodules of *Sesbania rostrata*.

Plant age [d]	PEPC [mmol(NADH oxid.) kg^{-1}(protein) s^{-1}]	Nitrogenase [nmol(ethylene) kg^{-1}(stem nodules) s^{-1}]
40	375.0	358.3
50	425.7	630.6
60	481.0	709.2
70	742.8	654.7
80	621.7	645.0
90	85.3	143.3

Table 2. Distribution of assimilated ^{14}C in amino acids of stem nodules.

Amino acid	Radioactivity [% of total]	counts [s^{-1}]
Asparagine	36.6	4.07
Asparatic acid	8.6	0.97
Glutamine	5.6	0.65
Glutamic acid	7.7	0.87
Residual count	41.5	4.65

Xylem sap analysis showed that the content of amino nitrogen was higher than that of ureide nitrogen prior to stem nodulation (46.7 and 5.8 g m^{-3}, respectively) and, moreover, the relative amino nitrogen content increased after the formation of stem nodules (159.0 and 47.0 g m^{-3}, respectively). This increase indicated that the fixed nitrogen from stem nodules was assimilated and transported in the form of amino nitrogen.

The chromatography of xylem sap showed again that nitrogen was transported as the four amino acids glutamine, glutamic acid, asparagine and aspartic acid. This agrees with the findings of Maxwell *et al.* (1984) that in xylem sap of effectively nodulated alfalfa and birdsfoot trefoil, asparagine is the major amino acid, followed by aspartate and glutamine.

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