59. *Assimilation and Transport of Fixed Nitrogen in Soybean Nodule as Revealed with* ^{15}N

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Using ^{15}N tracer technique assimilation and transport of fixed nitrogen in the soybean nodule were studied in detail.

Materials and methods. Nodules of the soybeans inoculated with *Rhizobium japonicums* and supplied with $^{15}N_2$, were macerated and separated into various nitrogenous fractions, and subjected to the determination of total nitrogen or heavy nitrogen. In some experiments nodules were separated into the bacteroid and the cytosol fractions.

Results and discussion. 1) Distribution of the fixed nitrogen in the nodule.[1] Table I shows the abundance and distribution of ^{15}N in 80% ethanol-soluble nitrogen of bacteroid and cytosol fractions separated immediately after exposure of $^{15}N_2$ gas to intact soybean nodules for 5 and 10 min. ^{15}N abundance of both fractions at 10 min was approximately twice as high as those at 5 min,

Table I. The abundance and distribution of ^{15}N in 80% EtOH-soluble fraction of bacteroid and cytosol separated from intact soybean nodules exposed to $^{15}N_2$

$^{15}N_2$ exposure time (min)		Atom% excess	μg-N per plant	μg-fixed N per plant	% ^{15}N distribution
5	Bacteroid	0.11	172	0.31	3
	Cytosol	0.25	2,330	9.55	97
10	Bacteroid	0.25	287	1.18	4
	Cytosol	0.44	1,796	26.21	96

$^{15}N_2$ (61.0 atom%): O_2: He=1:2:7.

indicating that newly fixed nitrogen was incorporated steadily into these fractions. ^{15}N abundance of cytosol fraction was remarkably higher than that of bacteroid in 5 and 10 min $^{15}N_2$ exposure. Distribution of fixed nitrogen in cytosol amounted to 97% and 96% of the total nitrogen in 80% ethanol-soluble fraction after 5 min and 10 min $^{15}N_2$ supply, respectively. These results indicate that the nitrogenase is situated in the surface membrane of bacteroid, and most of the newly fixed nitrogen is transferred to cytosol soon after the fixation.

2) Incorporation of the fixed nitrogen into free amino acids, amides and ureids.[2] Fig. 1 shows the time course of ^{15}N incorporation into various soluble components in the nodules. At the beginning ^{15}N abundance of the ammonia increased rapidly and soon reached the maximum value showing a hyperbolic

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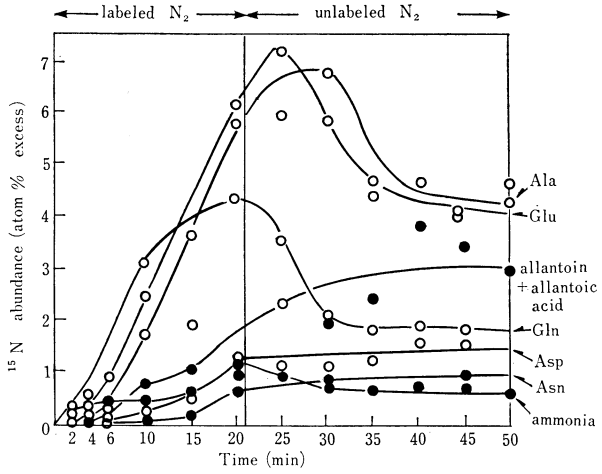

Fig. 1. The incorporation of ^{15}N into various nitrogenous compounds.

curve. This suggests that there are two or more compartments of ammonia in nodules and one of which has limited size and intimately connected with N_2 fixation process. Among amino acids, glutamine gave the highest ^{15}N abundance until 10 min of ^{15}N supply. The amido nitrogen of glutamine showed much higher ^{15}N content than its amino nitrogen in the early stage. From the data of glutamine, it appears that a portion of glutamine seems to be synthesized near the site of N_2 fixation, and located in plural compartments like ammonia. Following to glutamine, glutamic acid, and alanine showed high ^{15}N abundance. The time course of them gave relative short lag-time and continuous increase of ^{15}N during $^{15}N_2$ supply. It was noteworthy that allantoin showed higher ^{15}N level than the serine, aspartic acid, and asparagine. These amino acids and allantoin showed about 4–10 min lag-time in the ^{15}N incorporation into them. ^{15}N abundance in nitrate increased in the same way as in ammonia, but at lower level, suggesting the possibility of the formation of nitrate from ammonia.

These results seem to indicate that fixed ammonia is firstly incorporated into glutamine especially amido group nitrogen, and secondly into glutamic acid. This hypothesis was proved using various kind of inhibitors of nitrogen assimilation such as methionine sulfoximine, azaserine or aminooxyacetate.[1]

It was also shown that in the bacteroid fraction, glutamic acid showed the highest ^{15}N content, followed by alanine, and glutamine, but in the cytosol fraction, the ^{15}N content of glutamine was the highest, followed by glutamic acid, alanine, and allantoin plus allantoic acid in this sequence. The content of ^{15}N in asparagine in both fractions increased very slowly.[1] Most of ureides (allantoin and allantoic acid) were detected in cytosol and their ^{15}N contents higher than those in bacteroid. ^{15}N abundance of allantoin showed higher value than allantoic acid. These results show that ureides formation occurs in cytosol by the pathway of breakdown of purine.[2]

3) Comparison of asparagine formation between Hydrogen-uptake positive (*Hup*$^+$) and negative (*Hup*$^-$) *Rhizobium japonicum* strain. The hydrogenase system in legume nodule bacteroids was reported to participate in the recycling of H_2 produced by nitrogenase[3] and to increase N_2-fixation and plant growth.[4),5]

It is possible therefore that a greater amount of carbon skeleton is required for ammonia assimilation in the presence of the hydrogenase system. In this section, the $^{14}CO_2$ and $^{15}N_2$ incorporation into amino acids was compared between intact soybean nodules formed with *Hup*$^+$ and *Hup*$^-$ strains.

Table II. Distribution of ^{14}C and ^{15}N in aspartic acid and glutamic acid in the nodules after feeding of $^{14}CO_2$ and $^{15}N_2$ for 20 min

	$^{14}CO_2$ ($\times 10^3$ dpm)		^{15}N (atom% excess)	
Strain	A1017 (*Hup*$^+$)	J5033 (*Hup*$^-$)	A1017 (*Hup*$^+$)	J5033 (*Hup*$^-$)
Aspartic acid	550.8	299.0	2.32	1.51
Glutamic acid	285.0	270.4	5.50	5.86
Asp/Glu	1.93	1.11	0.407	0.258

Table III. Comparison of the apparent transport rate of various nitrogenous compounds from nodules formed with *Hup*$^+$ and *Hup*$^-$ strains***

Strain	A1014	A1017	J501	J5033
	H_2-uptake positive		H_2-uptake negative	
	ng/min/plant		ng/min/plant	
Asp	7.7	9.5	6.8	12.8
Asn	205.4	207.7	29.0	77.0
Glu	1.2	1.5	0.8	0.9
Gln	99.3	72.0	8.5	28.1
A*	397.0	446.0	122.7	190.0
AA**	1,189.0	1,304.3	473.0	777.0
A*+A**	1,586.0	1,750.3	596.0	967.0
Total-N	2.55×10^3	2.41×10^3	0.98×10^3	1.40×10^3

A*, allantoin; AA**, allantoic acid. *** Data represent the mean of three replicates.

Soybean nodules fed with $^{14}CO_2$ and $^{15}N_2$ were macerated and radioactivities and ^{15}N abundances of the amino acids and amides were determined.

As shown in Table II,[6] $^{14}CO_2$ was rapidly incorporated into amino acids, such as aspartic acid and glutamic acid. The carbon skeleton of aspartic acid has generally been considered to be formed from oxaloacetic acid and CO_2 by PEP carboxylase system.[7] ^{14}C radioactivity ratios of aspartic acid to glutamic acid in the nodules formed with *Hup*$^+$ strains were higher than with *Hup*$^-$, and suggested more active formation of aspartic acid in *Hup*$^+$ nodules.

^{15}N concentration ratios of aspartic acid to glutamic acid in the nodules formed with *Hup*$^+$ strains were higher than those formed with *Hup*$^-$ suggesting that more active transamination from glutamic acid to oxaloacetic acid.

Table III shows the apparent transport rate of various nitrogenous compounds in xylem sap from nodules formed with *Hup*$^+$ and *Hup*$^-$ strains.[8] Transported quantity of nitrogenous compounds is remarkably higher in the plants nodulated with *Hup*$^+$ strains than *Hup*$^-$. Ureides (allantoin and allantoic acid) are the predominant transported forms of nitrogen in all cases, and

asparagine plays a complementary role to transport nitrogen especially from *Hup*$^+$ nodules.

These results show that in *Hup*$^+$ nodules the increase of nitrogen fixation accompanies with the increase of CO_2 incorporation via oxaloacetic acid-aspartic acid pathway which can complementally supply the carbon skeleton to form ureides or asparagine, and make the transport of fixed nitrogen smooth.

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References

1) Ohyama, T., and Kumazawa, K.: Soil Sci. Plant Nutr., **26**, 205–213 (1980).
2) ——: ibid., **24**, 525–533 (1979).
3) Dixon, R. O. D.: Arch. Mikrobiol., **85**, 193–201 (1972).
4) Albrecht, S. L. *et al.*: Science, **203**, 23 (1979).
5) Minamisawa, K. *et al.*: Jpn. J. Soil Sci. Plant Nutri., **56**, 292–299 (1985) (in Japanese).
6) Minamisawa, K., Arima, Y., and Kumazawa, K.: Soil Sci. Plant Nutr., **27**, 387–391 (1981).
7) Minchin, F. R., and Pate, J. S.: J. Exp. Bot., **24**, 259–271 (1973).
8) Minamisawa, K., Arima, Y., and Kumazawa, K.: Soil Sci. Plant Nutr., **29**, 85–92 (1983).