Plant Physiol. (1978) 61, 980–983

Initial Organic Products of Fixation of [^{13}N]Dinitrogen by Root Nodules of Soybean (*Glycine max*)[1]

Received for publication January 9, 1978 and in revised form February 20, 1978

JOHN C. MEEKS,[2] C. PETER WOLK,[3] NORBERT SCHILLING, AND PAUL W. SHAFFER
MSU-ERDA Plant Research Laboratory

YAEL AVISSAR
Department of Botany and Plant Pathology

WAN-SHEN CHIEN
Cyclotron Laboratory, Michigan State University, East Lansing, Michigan 48824

ABSTRACT

When detached soybean *Glycine max* (L.) Merr. cv. Hark, nodules assimilate [^{13}N]N_2, the initial organic product of fixation is glutamine; glutamate becomes more highly radioactive than glutamine within 1 minute; ^{13}N in alanine becomes detectable at 1 minute of fixation and increases rapidly between 1 and 2 minutes. After 15 minutes of fixation, the major ^{13}N-labeled organic products in both detached and attached nodules are glutamate and alanine, plus, in the case of attached nodules, an unidentified substance, whereas [^{13}N]glutamine comprises only a small fraction of organic ^{13}N, and very little ^{13}N is detected in asparagine. The fixation of [^{13}N]N_2 into organic products was inhibited more than 99% by C_2H_2 (10%, v/v). The results support the idea that the glutamine synthetase-glutamate synthase pathway is the primary route for assimilation of fixed nitrogen in soybean nodules.

Ammonium is the initial product of dinitrogen fixation by symbiotic (1) and free-living (19, 21) prokaryotes and by nitrogenase *in vitro* (16). Activities of the ammonium-assimilating enzymes glutamic acid dehydrogenase and glutamine synthetase, as well as glutamate synthase, have been detected in bacteroid fractions isolated from root nodules of soybean (2, 3) and lupin (2, 9). Nevertheless, free-living N_2-fixing *Rhizobium japonicum* and bacteroids of *R. japonicum* isolated from soybean release 90 to 94% of the products of fixation of $^{15}N_2$ into the medium as ammonium (1, 13). Dilworth and Brown (2) suggested that ammonium produced by bacteroids is assimilated in nodules through either the glutamine synthetase-glutamate synthase or glutamic acid dehydrogenase pathways, and favored the latter. In serradella, the major amino acids formed from $^{15}N_2$ were glutamate and glutamine, but the initial organic product, and thereby the primary assimilatory route, was not determined (8). The form in which nitrogen is translocated from the nodules to the root and shoot of the host plant has not been directly identified, but is assumed to be asparagine, based on analysis of compounds in the bleeding sap (4, 14, 17, 23).

In this study we identify glutamine as the initial organic product of fixation of [^{13}N]N_2 ($t_{1/2}$ [^{13}N] = 10 min) by detached soybean nodules. However, the major organic products that accumulate after 15 min of fixation of [^{13}N]N_2 are glutamic acid and alanine, with minimal amounts of glutamine. After 6 or 15 min of fixation of [^{13}N]N_2 by nodules attached to seedlings, these two amino acids and, in addition, an unidentified substance are highly labeled.

MATERIALS AND METHODS

Plant and Bacterial Strains, and Growth Conditions. Yeast extract-mannitol medium (YM) contains, in g/l: mannitol, 10; yeast extract (Difco), 1; K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; NaCl, 0.1; and $CaSO_4 \cdot 2H_2O$, 0.1. *Rhizobium japonicum* strain 3I1b-110, kindly supplied by K. D. Nadler, Department of Botany and Plant Pathology, Michigan State University, was maintained on YM agar (1.5%) slants and transferred every 3 months. Liquid cultures, started from slants in 20 ml of YM medium, were transferred to 500 ml of YM medium in 1-liter flasks, and were aerated by agitation on a reciprocating shaker. Cells from 2-day-old cultures were harvested by centrifugation at 6,000*g* for 10 min, washed and resuspended in ("N-free") legume nutrient medium free of combined nitrogen (6), and used as inoculum.

Glycine max (L.) Merr. cv. Hark seeds were surface-disinfected for 20 sec with 1% NaOCl, washed in running tap water, and planted in a Vermiculite-Perlite bed. The bed was inoculated with the *Rhizobium* suspension. Aerated N-free medium was circulated through the bed of germinating seeds for 1 week. The beds were illuminated with two 40-w Gro-lux fluorescent lamps (Westinghouse Electric Corp., Bloomfield, N.J.) at a distance of 60 cm. Inoculated seedlings were transferred to pots (five/pot) containing a mixture of Vermiculite and Perlite and grown in a greenhouse with supplementary illumination from four 40-w Gro-lux fluorescent lamps at a distance of 35 to 45 cm. Seedlings were watered with N-free medium. Under these inoculation conditions, nodules form predominantly at the crown of the root (12). Seedlings 2.5 to 5 weeks old, and nodules from such seedlings, were used in these experiments.

Acetylene Reduction. Detached nodules were placed in 5-ml vials which were then sealed with a serum stopper. The vials were evacuated three times and filled to atmospheric pressure with $Ar/O_2/CO_2$ (80:19:1, v/v/v). Acetylene was injected to a final concentration of 0.006% (v/v) (60 μl/l), or 6% (v/v) (60 ml/l). When 6% C_2H_2 was used, 40 μl of the atmosphere was analyzed for C_2H_4 with a Varian Aerograph model 1200 gas chromatograph

[1] This research was supported by the United States Energy Research and Development Administration under Contract EY-76-C-02-1338, and by the National Science Foundation. Y. A. was supported by National Science Foundation Grant PCM-76-22793 to K. D. Nadler. N. S. received partial support from the Deutsche Forschungsgemeinschaft.

[2] Present address: Department of Bacteriology, University of California, Davis, California 95616.

[3] To whom requests for reprints should be sent.

(Varian Instrument Division, Palo Alto, Calif.) (22) after 10 to 30 min of incubation at room temperature (about 22 C). When the lower concentration of C_2H_2 was used, 1 ml of atm. from the vials was analyzed with a Varian Aerograph series 2400 gas chromatograph after 3 to 4 min (in certain cases, also after 15 and 30 min) of incubation at room temperature. This instrument was equipped with a column (45 × 0.32 cm) containing Al_2O_3, and was operated at 80 C with N_2 as the carrier gas (7).

Detached nodules to be used for fixation of [^{13}N]N_2 were selected on the basis of weight (fresh wt ≤ 20 mg) and of activity for reduction of 60 μl/l of C_2H_2. The selected nodules were transferred to 1-ml reaction vials, which were evacuated three times and refilled each time to atmospheric pressure with $Ar/O_2/CO_2$ (80:19:1, v/v/v). Nodules were used for fixation of [^{13}N]N_2 within 40 min after detachment.

Fixation of [^{13}N]N_2. ^{13}N was generated by irradiation of 18.6 mg of ^{13}C with protons, and [^{13}N]N_2 was formed by Dumas combustion of the target (20, 21). Detached nodules were exposed to [^{13}N]N_2 in 1-ml Reactivials (Pierce Chemical Co., Rockford, Ill.) fitted with a serum stopper, whereas nodules attached to seedlings were exposed to [^{13}N]N_2 in 3-ml reaction chambers. A reaction chamber consisted of a 1.5-cm length of 7-mm glass tubing with a side arm fitted with a serum stopper, and with a rubber nipple inserted over one end of the glass tubing. The secondary roots were trimmed from the seedlings; the primary root truncated to about 2 cm, under water; and a 0.25-ml glass reservoir filled with tap water inserted over the cut end of the primary root (but not over the nodules) and sealed with silicon tubing. The seedling was inserted into the reaction chamber with the attached reservoir extending into the nipple, and the stem held by a split rubber stopper and sealed with Plasteline (Jolly King modeling material, Sculpture House, New York). Reaction vials and chambers were briefly evacuated, [^{13}N]N_2 (approximately 0.02 atmospheric cc) introduced, and $Ar/O_2/CO_2$ (80:19:1, v/v/v) then added to 1 atm. In certain experiments, acetylene was then immediately added to a final concentration of 10% (v/v).

Analysis of Products of Fixation of [^{13}N]N_2. Fixation by detached nodules was terminated by evacuating the [^{13}N]N_2 (about 5 sec), injecting 0.1 ml of cold 80% methanol, and grinding the nodules in the Reactivial with a tapered stainless steel pestle (about 7 sec). The contents of the Reactivials were washed into a 10-ml centrifuge tube with 1.4 ml of 80% methanol. Fixation by seedlings was terminated by evacuating the [^{13}N]N_2, removing the seedling from the reaction chamber, excising the nodules, and grinding them in 1.5 ml of 80% methanol in a 10-ml centrifuge tube (about 20 sec). Extracts of the nodules were, in both instances, clarified by centrifugation at 20,000*g* for 1 min. The supernatant solutions were concentrated by evacuation, and subjected to high voltage (3 kv) electrophoresis on thin layers of cellulose with 70 mM borate buffer (pH 9.2) (21). Radioactive constituents were localized and quantified by one-dimensional radioelectrophoretogram scanning, and identified as described previously (10, 21). In certain experiments, electrophoresis was followed by chromatography in an orthogonal direction in phenol-water (3:1, v/v) equilibrated with 3% aqueous NH_4OH, and the thin layer plates then scanned in two dimensions (10). In other experiments, the methanolic extract of detached nodules was subjected sequentially to vacuum distillation at pH 10 to recover $^{13}NH_3$ and to steam distillation in strong alkali to recover [^{13}N]amide nitrogen (21). Radioactivity in the original extract and in the distillates was determined by scintillation spectrometry (21).

In certain experiments, 5 μl of water containing [^{14}C]glutamine (about 2 × 10^5 dpm, 230 μCi/μmol, New England Nuclear) or [^{14}C]asparagine (about 2 × 10^5 dpm, 150 μCi/μmol, New England Nuclear) were placed on nodules, and the nodules then ground and extracted as described above. The extracts were concentrated, and subjected to electrophoresis at pH 9.2 and to radioscanning as described above.

RESULTS

Acetylene Reduction. Preliminary experiments indicated that both the rate of fixation of [^{13}N]N_2 and the rate of reduction of C_2H_2 by single detached soybean root nodules, per mg fresh wt, varied extensively, up to 20-fold variation being found with nodules of approximately equal weight obtained from a single plant. It was therefore necessary to assess the N_2-fixing capacity of individual nodules prior to fixation of [^{13}N]N_2. However, after nodules had been assayed for nitrogenase activity at 6% C_2H_2 (v/v) and evacuated three times to about 100 μm Hg, they failed to fix [^{13}N]N_2, and continued to release C_2H_2, *e.g.* 13 nmol of C_2H_2 after 5 min increasing linearly to 22 nmol after 25 min. Acetylene, an alternate substrate for nitrogenase, is also an inhibitor of N_2 fixation. Apparently, sufficient C_2H_2 was retained by the nodules to inhibit fixation of [^{13}N]N_2. We therefore developed an assay or reduction of C_2H_2 by detached nodules using a low concentration of C_2H_2 (0.006%). The rates of C_2H_2 reduction with a low concentration of C_2H_2 are, as expected, lower than, but appear to increase correlatively with, the rates at a higher concentration of C_2H_2 (Table I). Reduction of 0.006% C_2H_2 was linear with time for at least 30 min. Determination of the relative activities of the nodules using a low concentration of C_2H_2 enabled us to choose nodules with the highest nitrogenase activities without a subsequent, detrimental effect on the fixation of [^{13}N]N_2 by residual C_2H_2.

Products of Fixation of [^{13}N]N_2 by Detached and Attached Nodules. Incubation of detached and attached nodules with [^{13}N]-N_2 for up to 15 min resulted in incorporation of ^{13}N into three organic constituents; in attached nodules, a fourth compound was found in addition (Table II). The three compounds formed in attached and detached nodules were identified as glutamate, glutamine, and alanine on the basis of their co-migration with stable amino acids during electrophoresis at pH 9.2 and during subsequent chromatography in phenol-water (3:1, v/v) equilibrated with 3% aqueous NH_4OH. After 20 sec of fixation of [^{13}N]N_2 by detached nodules, glutamine was the most highly radioactive organic compound, accounting for about 82% of the total organic ^{13}N recovered (Fig. 1). The fraction of ^{13}N in glutamine decreased and that in glutamate increased until, after times of fixation greater than 1 min, glutamate was more highly radioactive than glutamine. The fraction of ^{13}N in glutamate reached a maximum after 6 min of fixation whereas the fraction of ^{13}N in glutamine continued to decline slowly for up to 15 min of fixation. Alanine became detectably labeled between 0.5 and 1 min of

Table I. Reduction of C_2H_2 by Intact Nodules at Low and High Concentrations of C_2H_2

Fresh wt. of nodule (mg)	pmoles C_2H_4 formed per min 0.006% C_2H_2	6% C_2H_2
13.4	2.4	21.6
18.9	2.6	30.0
16.5	2.8	43.2
15.4	3.5	97.7
12.7	3.7	110.5
9.6	5.0	184.6
13.2	9.2	450.1
20.0	10.0	318.8
8.9	13.4	499.6

Nodules from 3- to 5-week old plants from the same pot were weighed, and placed into 5-ml vials which were stoppered and then evacuated and refilled with $Ar:O_2:CO_2$ (80:19:1, v/v/v). The vials were then injected with C_2H_2 to a final concentration of 60 ppm, and assayed for formation of C_2H_4 after 3 min of incubation. After these assays, additional C_2H_2 was added to a final concentration of 6 x 10^4 ppm and the vials assayed for formation of C_2H_4 after an additional 30 min of incubation.

Table II. Principal products observed after fixation of [^{13}N]N_2 for 6 and 15 min by detached and attached nodules of soybean. Methanolic extracts were subjected to electrophoresis at pH 9.2 on thin layers of cellulose, the thin layer plates scanned, and the peaks in the scans integrated and corrected for decay. The values are reported as the fraction of total extractable ^{13}N ± standard deviation of the mean.

Nodules	Incubation time, min	^{13}N found in compound, as fraction of total ^{13}N extracted[a] Glu	Unidentified	Gln	Ala
Detached	6	0.67 ± 0.04	-	0.11 ± 0.03	0.16 ± 0.03
Detached	15	0.67 ± 0.03	-	0.08 ± 0.01	0.25 ± 0.02
Attached	6	0.57 ± 0.03	0.29 ± 0.03	0.04 ± 0.01	0.11 ± 0.01
Attached	15	0.51 ± 0.04	0.30 ± 0.05	0.014± 0.002	0.16 ± 0.02

[a]The small difference between the sum of these values, and unity, corresponds to minor peaks not consistently seen.

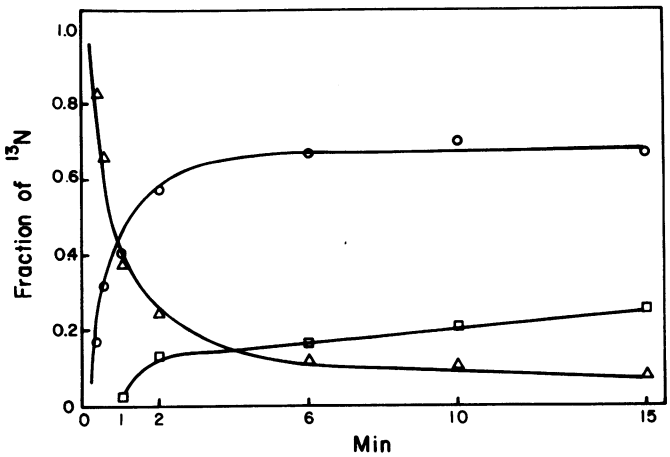

FIG. 1. Distribution of ^{13}N in organic products extracted with 80% methanol after fixation of $[^{13}N]N_2$ for 0.33, 0.5, 1, 2, 6, 10, and 15 min by detached soybean nodules. The radioactivity in the constituents of extracts subjected to electrophoresis at pH 9.2 was quantified by integration of peaks in radioscans, with corrections applied for decay. Values presented are means, from 2 or 3 experiments, of the fraction of organic ^{13}N migrating with stable glutamine (△), glutamate (○), and alanine (□).

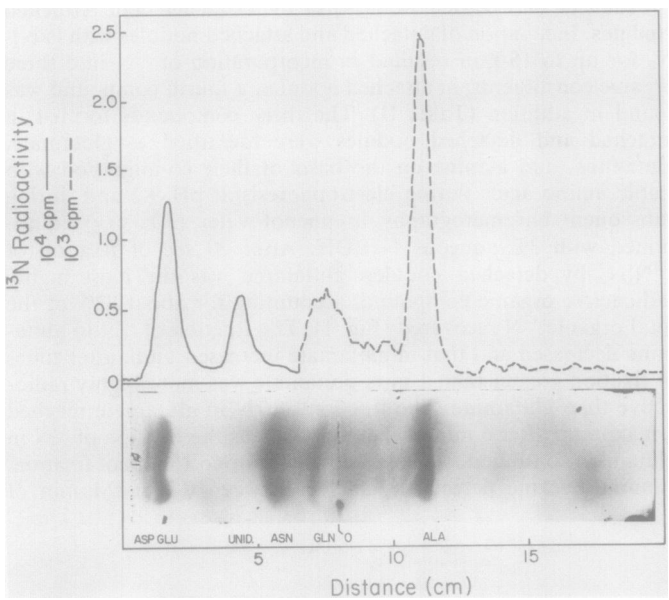

FIG. 2. Scan of radioactivity from ^{13}N in an electrophoretogram of organic compounds extracted from a soybean nodule with 1.5 ml 80% methanol after 6 min of fixation of ^{13}N-labeled N_2 by a seedling. The extract, supplemented with stable aspartate, glutamate, asparagine, glutamine, and alanine as markers, was applied to a cellulose thin layer plate in a thin strip (origin = 0). After displacement of lipids from the origin by chromatography (21), the extract was subjected to electrophoresis at 3 kv for 11 min in 70 mM sodium borate buffer (pH 9.2). The plate was scanned at 2 cm/min (τ = 3 sec) from + (at left) to −, and was then dried and sprayed with a solution of ninhydrin to localize the marker amino acids. The ^{13}N-containing substances detected which migrated with glutamate, an unidentified compound (unid.), glutamine and alanine during electrophoresis were present in ratio (time-corrected) 1.00:0.44:0.06:0.19. Less than 3% of the radioactivity was attributable to asparagine.

fixation and continued to accumulate ^{13}N for at least 15 min.

When half of the methanolic extract of three detached nodules (average fresh wt, 14.1 mg) which had fixed $[^{13}N]N_2$ for 15 min was subjected to vacuum distillation at pH 10, no ^{13}N (*i.e.* < 0.07% of the ^{13}N in organic products) was detected in the ammonia distillate. Approximately 5% of the ^{13}N distilled as amide nitrogen. Six per cent of the organic ^{13}N in the remaining extract migrated with glutamine during electrophoresis.

The fourth ^{13}N-labeled compound which was formed after 6 or 15 min of incubation by nodules attached to seedlings, and which migrated between glutamic acid and asparagine during electrophoresis at pH 9.2 (Fig. 2), has not been identified. As of 6 or 15 min of assimilation by attached nodules, up to about 30% of the fixed ^{13}N was present in this substance, with most of the residual extracted radioactivity distributed between glutamic acid and alanine; glutamine accounted for a smaller fraction of the fixed ^{13}N in attached than in detached nodules (Table II). In extracts of nodules, no more than traces of ^{13}N-labeled asparagine were observed (Fig. 2).

As of 15 min of fixation of $[^{13}N]N_2$, at most 10% as much ^{13}N could be extracted from the remainder of the seedling as could be extracted from the nodules. Fixation of $[^{13}N]N_2$ into organic products in attached and detached nodules was inhibited about 99.5%, or more, after 15 min of incubation in the presence of 10 C_2H_2 (v/v). When nodules were ground and extracted in the presence of [^{14}C]asparagine or [^{14}C]glutamine, and the extracts subjected to electrophoresis at pH 9.2, ^{14}C was detected only in the regions to which asparagine or glutamine had migrated.

DISCUSSION

After 20 sec of fixation of $[^{13}N]N_2$ by detached soybean nodules, glutamine accounts for the great majority of ^{13}N-labeled metabolites (Fig. 1). After 1 min, glutamic acid exceeds glutamine in radioactivity. The relative proportion of ^{13}N in glutamic acid continues to increase, and the fraction in glutamine continues to decline, for an additional 5 min. The kinetics of incorporation of ^{13}N into glutamine and glutamic acid in soybean nodules is similar to the kinetics observed in cyanobacteria (10, 21), the only other organisms heretofore subjected to a similar study. Based in part on inhibitor studies and pulse-chase experiments, it was concluded that the glutamine synthetase-glutamate synthase pathway is the primary route by which free-living cyanobacteria assimilate N_2-derived and exogenously supplied NH_4^+ (10, 21). Because of the similarity in kinetics, we suggest that the major route for assimilation of N_2-derived NH_4^+ in soybean nodules also consists of the glutamine synthetase-glutamate synthase pathway. Our results are consistent with the hypothesis, recently advanced by Miflin and Lea (11), that the assimilation of NH_4^+ by plants occurs in general by the glutamine synthetase-glutamate synthase pathway, rather than by the glutamic acid dehydrogenase pathway.

Glutamic acid and alanine are prominent among the radioactive products in both detached and attached nodules after 6 and 15 min of fixation of $[^{13}N]N_2$. [^{13}N]Alanine, because it appears after [^{13}N]glutamic acid (Fig. 1), and because the corresponding transaminase is active in nodules (15), may be formed by transamination from glutamic acid. However, [^{13}N]glutamine constitutes a much smaller fraction of the ^{13}N-labeled metabolites extracted from attached than from detached nodules after 6 and 15 min of fixation of $[^{13}N]N_2$ (Table II). In addition, up to 30% of the total ^{13}N extracted from attached nodules found in an unidentified compound (Fig. 2 and Table II) which is conspicuously labeled only in attached nodules. One possible explanation for the differences in the amount of ^{13}N in glutamine and in the unknown is that root tissue supplies attached nodules with a substrate or cofactor for a reaction in which nitrogen is transferred from glutamine, and that the substance supplied becomes limiting in detached nodules.

We have not yet identified the interactions between the nodules and the root tissue, including which ^{13}N-labeled compounds are translocated. Glutamic acid is found in the bleeding sap of broad bean and soybean nodules (14, 23). Alanine, a major amino acid in the transport stream of soybean leaves fed $^{14}CO_2$ (5), is a minor constituent of the bleeding sap of broad bean nodules (14). Both amino acids could function either as translocation compounds directly or, by transamination, as nitrogen donors to other com-

pounds which are translocated. Asparagine, the predominant amino acid in the bleeding sap of broad bean and soybean nodules, and therefore presumably the major vehicle for transport of nitrogen (14, 23), was not appreciably labeled in nodules in our experiments. Conceivably, [^{13}N]asparagine was not formed in detached nodules because a required substance could not be provided from the detached root system (see above), and was not observed in attached nodules because it had been exported. Alternatively, 15 min may have been insufficient time for [^{13}N]asparagine to accumulate.

Streeter (18) suggested that asparagine is rapidly degraded by asparaginase, even during extraction of nodule tissue with 75% ethanol. However, we determined that neither [^{14}C]asparagine nor [^{14}C]glutamine is degraded during extraction of nodule tissue by our procedures. It therefore appears that degradation cannot account for the virtual absence of [^{13}N]asparagine from our electrophoretograms, for the low level of [^{13}N]glutamine in nodules after relatively long periods of fixation, or for the appearance of the unidentified, ^{13}N-labeled compound observed after fixation of [^{13}N]N_2 by attached nodules.

The electrophoretic mobility of the unidentified compound differs from the mobilities of aspartate, glutamate, and cystine, but could correspond to it being a different dicarboxylic acid. This compound was not detected after 15 min of exposure to [^{13}N]N_2 in the presence of 10% C_2H_2 (v/v). It therefore does not appear to be an artifact due to nonbiological complexing of ^{13}N.

Acknowledgments—We thank A. Hanson for helpful discussions, B. Uechi for technical assistance with the two-dimensional scanner and with computations, and P. Miller and D. Gossman for generous cooperation in use of the cyclotron.

LITERATURE CITED

1. Bergersen FJ, GL Turner 1967 Nitrogen fixation by the bacteroid fraction of breis of soybean root nodules. Biochim Biophys Acta 141: 507–515
2. Dilworth MJ, CM Brown 1976 Chemostat studies of the control of ammonia incorporation in rhizobia. *In* WE Newton, CJ Nyman, eds, Proc First Int Symp Nitrogen Fixation. Wash State Univ Press, Pullman, pp 476–488
3. Dunn SP, RV Klucas 1973 Studies on possible routes of ammonium assimilation in soybean root nodule bacteroids. Can J Microbiol 19: 1493–1499
4. Gibson A 1976 Limitation to dinitrogen fixation by legumes. *In* WE Newton, CJ Myman, eds, Proc First Int Symp Nitrogen Fixation. Wash State Univ Press, Pullman, pp 400–428
5. Housley TL, DM Peterson, LE Schrader 1977 Long distance translocation of sucrose, serine, leucine, lysine, and CO_2 assimilates. I. Soybean. Plant Physiol 59: 217–220
6. Johnson GV, HJ Evans, TM Ching 1966 Enzymes of the glyoxylate cycle in rhizobia and nodules of legumes. Plant Physiol 41: 1330–1336
7. Kende H, AD Hanson 1976 Relationship between ethylene evolution and senescence in morning glory flower tissue. Plant Physiol 57: 523–527
8. Kennedy IR 1966 Primary products of symbiotic nitrogen fixation. II. Pulse-labelling of serradella nodules with $^{15}N_2$. Biochim Biophys Acta 130: 295–303
9. Kennedy IR 1973 Glutamine synthetase/glutamate synthetase for ammonia assimilation in symbiotic N_2 fixation. Proc Aust Biochem Soc 6: 33
10. Meeks JC, CP Wolk, J Thomas, W Lockau, PW Shaffer, SM Austin, W-S Chien, A Galonsky 1977 The pathways of assimilation of $^{13}NH_4^+$ by the cyanobacterium, *Anabaena cylindrica*. J Biol Chem 252: 7894–7900
11. Miflin BJ, PL Lea 1976 The pathway of nitrogen assimilation in plants. Phytochemistry 15: 873–885
12. Nadler KD, Y Avissar 1977 Heme synthesis in soybean root nodules. I. On the role of bacteroid δ-aminolevulinic acid synthase and δ-aminolevulinic acid dehydrase in the synthesis of the heme of leghemoglobin. Plant Physiol 60: 433–436
13. O'Gara F, KT Shanmugam 1976 Regulation of nitrogen fixation by rhizobia. Export of fixed N_2 as NH_4^+. Biochim Biophys Acta 437: 313–321
14. Pate JS, BES Gunning, LG Briarty 1969 Ultrastructure and functioning of the transport system of the leguminous root nodule. Planta 85: 11–34
15. Ryan E, PF Fottrell 1974 Subcellular localization of enzymes involved in the assimilation of ammonia by soybean root nodules. Phytochemistry 13: 2647–2652
16. Smith BE, RNF Thorneley, MG Yates, RR Eady, JR Postgate 1976 Structure and function of nitrogenase from *Klebsiella pneumoniae* and *Azotobacter chroococcum*. *In* WE Newton, CJ Nyman, eds, Proc First Int Symp Nitrogen Fixation. Wash State Univ Press, Pullman, pp 105–176
17. Streeter JG 1972 Nitrogen nutrition of field-grown soybean plants. I. Seasonal variations in soil nitrogen and nitrogen composition of stem exudate. Agron J 64: 311–314
18. Streeter JG 1977 Asparaginase and asparagine transaminase in soybean leaves and root nodules. Plant Physiol 60: 235–239
19. Thomas J, JC Meeks, CP Wolk, PW Shaffer, SM Austin, W-S Chien 1977 Formation of glutamine from [^{13}N]ammonia, [^{13}N]dinitrogen and [^{14}C]glutamate by heterocysts isolated from *Anabaena cylindrica*. J Bacteriol 129: 1545–1555
20. Wolk CP, SM Austin, J Bortins, A Galonsky 1974 Autoradiographic localization of ^{13}N after fixation of ^{13}N-labeled nitrogen gas by a heterocyst-forming blue-green alga. J Cell Biol 61: 440–453
21. Wolk CP, J Thomas, PW Shaffer, SM Austin, A Galonsky 1976 Pathway of nitrogen metabolism after fixation of ^{13}N-labeled nitrogen gas by the cyanobacterium, *Anabaena cylindrica*. J Biol Chem 251: 5027–5034
22. Wolk CP, E Wojciuch 1971 Photoreduction of acetylene by heterocysts. Planta 97: 126–134
23. Wong PP, HJ Evans 1971 Poly-β-hydroxybutyrate utilization by soybean (*Glycine max* Merr.) nodules and assessment of its role in maintenance of nitrogenase activity. Plant Physiol 47: 750–755