Plant Physiol. (1976) 57, 542-546

Relation between Glutamine Synthetase and Nitrogenase Activities in the Symbiotic Association between *Rhizobium japonicum* and *Glycine max*[1]

Received for publication August 19, 1975 and in revised form December 5, 1975

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ABSTRACT

The activity and extent of adenylylation of glutamine synthetase was examined in both free-living and bacteroid forms of *Rhizobium japonicum* in the presence of excess ammonia. Ammonia caused an apparent repression of glutamine synthetase in free-living *R. japonicum* and adenylylation of the enzyme was also increased. In contrast, neither the activity nor the extent of adenylylation of the bacteroid enzyme was consistently affected by ammonium treatment of bacteroid suspensions. Similar results were obtained after ammonium treatment of soybean plants even though nitrogenase activity was reduced markedly. We have been unable to demonstrate ammonium repression of nitrogenase activity in *R. japonicum-Glycine max* symbiotic association that is mediated through bacteroid glutamine synthetase. This result is in contrast to the situation in nitrogen-fixing strains of *Klebsiella* where a role of glutamine synthetase in the regulation of nitrogenase has been reported.

The inhibitory effects of fixed nitrogen on nodulation of and N_2 fixation by legumes have been well documented (1, 12, 16) but details of how nitrogenase synthesis is regulated in legume-*Rhizobium* associations are unknown. The mechanism of regulation of nitrogenase synthesis in free-living bacteria is beginning to be understood. Evidence by Tubb (22) and by Streicher *et al.* (20) indicates that catalytically active glutamine synthetase is necessary for nitrogenase synthesis by *Klebsiella pneumoniae*. They have also demonstrated that strains of *K. aerogenes*, and *K. pneumoniae* that exhibit the Gln C$^-$ mutant phenotype (constitutive synthesis of glutamine synthetase) and carry the *nif* operon(s) were partially derepressed for nitrogenase synthesis when cultured in the presence of NH_4^+. These results suggest that glutamine synthetase acts as a positive control element for nitrogenase synthesis in a fashion similar to that proposed for histidase synthesis in *K. aerogenes* (17). The *in vitro* transcription of the correct strand of DNA coding for the histidine utilization operon from *Salmonella typhimurium* was shown by Tyler *et al.* (23) to be activated by unadenylylated glutamine synthetase and not by the adenylylated form. Activation of transcription of the *nif* operon also has been inferred to take place in an analogous way (22). This information and the reported (7) NH_4^+ repression of nitrogenase synthesis in hybrid strains of *K. aerogenes* carrying *nif* genes from *R. trifolii* has led Streicher *et al.* (20) to speculate that the regulatory system for nitrogen fixation by the *Rhizobium*-legume system may be similar to that in *Klebsiella*.

We have investigated the effects of NH_4^+ on glutamine synthetase in both free-living *R. japonicum* and the bacteroid forms of the microorganisms in root nodules. Our experiments have led to the unexpected conclusion that neither activity nor the extent of adenylylation of bacteroid glutamine synthetase is consistently influenced by NH_4^+ even under conditions where nitrogenase activity was inhibited markedly. Glutamine synthetase in free-living *R. japonicum*, on the other hand, behaves like the enzyme in *Escherichia coli* (13, 24) showing repression and adenylylation when excessive NH_4^+ is supplied.

MATERIALS AND METHODS

Culture of Free-living Bacteria. *Rhizobium japonicum* strain OSR-2 was cultured in 1-liter flasks containing 500 ml of medium containing the following components dissolved in 1 liter of water: K_2HPO_4, 0.23 g; $MgSO_4 \cdot 7H_2O$, 0.10 g; sodium glutamate, 1.10 g; glycerol, 4 g; $CaCl_2$, 5 mg; H_3BO_3, 145 μg; $FeSO_4 \cdot 7H_2O$, 125 μg; $CoSO_4 \cdot 7H_2O$, 70 μg; $CuSO_4 \cdot 7H_2O$, 5 μg; $MnCl_2 \cdot 4H_2O$, 4.3 μg; $ZnSO_4 \cdot 7H_2O$, 108 μg; Na_2MoO_4, 125 μg; nitrilo-triacetate, 7 mg; riboflavin, 20 μg; *p*-aminobenzoic acid, 20 μg; nicotinic acid, 20 μg; biotin, 20 μg; thiamine-HCl, 20 μg; pyridoxine-HCl, 20 μg; calcium pantothenate, 20 μg; inositol, 120 μg. The cultures were incubated on a rotary shaker at 30 C.

Preparation of Bacteroids. Soybean root nodules (7-9 g) from plant cultured by methods previously described (10) were mixed with 24 ml of 0.1 M K-phosphate (pH 7.6) buffer containing 0.2 M sucrose (sucrose-phosphate buffer) and 3 g of insoluble PVP and were macerated with a mortar and pestle at 4 C. The macerate was filtered through four layers of cheesecloth and then centrifuged at 40,000*g* for 15 min. The supernatant fraction (hereafter referred to as cytosol) was stored in an ice bath until used. The sedimented bacteroid fraction was washed by resuspension in 12 ml of sucrose-phosphate buffer and collected by centrifugation at 6,000*g* for 15 min. When large preparations of bacteroids were required, the relative proportions of sucrose-phosphate buffer and PVP were the same as described above, but a blender was used for the maceration process in place of a mortar and pestle (Table I), and the nodules were surface-sterilized by the sodium hypochlorite method utilized by Evans *et al.* (9). Bacteroids were prepared as described above and were resuspended in 100 ml of sterile sucrose-phosphate buffer. Fifty ml portions of the bacteroid suspension were placed in each of two sterile flasks (125 ml) and then appropriate amounts of Na_2SO_4 (control) or $(NH_4)_2SO_4$ (NH_4^+-treated) were added. In some experiments (Table I), penicillin G (Sigma) was added at a final concentration of 5000 units/ml in order to control bacterial contamination. Incubation of the bacteroid suspensions was con-

[1] This research was supported by the Oregon Agricultural Experiment Station (Technical Paper No. 4079) and by Grant BMS 74-17812 from the National Science Foundation.

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ducted on a rotary shaker at 30 C, and 10 ml aliquots were withdrawn at each sampling time. Contamination was monitored by plating 0.1-ml aliquots of the suspension on nutrient agar. Bacteroids are incapable of growth under these conditions.

Preparation of Crude Extracts. Crude extracts of both bacteroid and free-living *R. japonicum* cells were prepared by suspending about 1 cm^3 of packed cells in 1 ml of 0.1 M TES buffer (pH 8) followed by exposure at 0 to 4 C to three 30-sec periods of sonication. One ml of 0.1 M TES (pH 7.3) was added to the disrupted cells followed by centrifugation at 40,000*g* for 30 min. The protein content of the extracts was determined by the biuret method of Gornall *et al.* (14).

Assay for Glutamine Synthetase Activity. Glutamine synthethase activity was measured by the γ-glutamyl transferase assay in a medium containing 0.3 mM Mn^{2+} using the procedure outlined by Shapiro and Stadtman (18).

Determination of Relative Adenylylation of Glutamine Synthetase. Relative adenylylation of glutamine synthetase in crude extracts was estimated by a modification of the snake venom phosphodiesterase method described by Tronick *et al.* (21), and also from absorbances obtained from the transferase assay conducted in the presence and absence of 60 mM Mg^{2+} (19). For this procedure, an equal volume of 1% streptomycin sulfate in 10 mM imidazole-HCl buffer (pH 7) containing 1 mM $MnCl_2$ (imidazole-Mn^{2+} buffer) and crude extract was mixed together. After 30 min of incubation in an ice bath, the streptomycin precipitate was removed by centrifugation at 10,000*g* for 10 min. The supernatant fraction was dialyzed for 15 hr at 0 to 4 C against 100 volumes of imidazole-Mn^{2+} buffer and then centrifuged at 10,000*g* for 10 min. The dialyzed extract was diluted with imidazole-Mn^{2+} buffer so that addition of an aliquot of 0.05 ml to the glutamine synthetase transferase assay (containing 60 mM Mg^{2+} and 0.3 mM Mn^{2+}) resulted in an absorbance of about 0.15 at 540 nm. Relative adenylylation was determined by adding an aliquot (0.05 ml) of the diluted extract to each of four tubes. Two of these received 0.1 ml of tris-acetate buffer (pH 8.8) containing 15 mM magnesium acetate, and the other two tubes received 0.1 ml of the tris acetate-magnesium acetate buffer containing 30 μg of SVD[4] (Sigma). Nontreated samples and those treated with SVD were removed after incubation at 37 C for periods of 60 and 120 min, and glutamine synthetase transferase activity was determined in the presence of 60 mM Mg^{2+} and 0.3 mM Mn^{2+}. The absorbance at 540 nm resulting from the transferase assay of an SVD-treated sample divided by the absorbance of a reaction containing a nontreated sample is reported as the relative adenylylation value (21). A value of 1.00 or less indicates the absence of adenylylated enzyme, and a value greater than 1.00 indicates the presence of adenylylated enzyme. Relative adenylylation also was determined by the ratio obtained from the absorbance of a transferase assay conducted with 0.3 mM Mn^{2+} divided by the absorbance of an assay reaction containing 0.3 mM Mn^{2+} and 60 mM Mg^{2+}.

Assay for Nitrogenase Activity. Nitrogenase activities of nodulated plants were measured by a modification of the procedure described by Fishbeck *et al.* (11). Stems of four plants from each culture were cut about 1 cm above the crown, and each group of four nodulated roots was placed in a 250-ml flask. The flasks were closed with rubber stoppers, each of which was fitted with a serum stopper to allow gas injection and removal. Acetylene (25 cm^3) was injected into each flask and incubation was conducted at 20 C. Samples (0.5 cm^3) were withdrawn after 30 min, and ethylene contents determined as described by Fishbeck *et al.* (11).

Determination of NH_4^+ in Nodules. The ammonium content of nodules was estimated by Nesslerization after microdiffusion (6). In each determination, a 0.3-g sample of thoroughly rinsed nodules (which were stored until used at −80 C after freezing in liquid N_2) was macerated with 1 ml of H_2O in a mortar and pestle. The nodule macerate was transferred with the addition of 1 ml of H_2O into a 21-ml serum vial. From this point, distillation and microdiffusion were conducted at 38 C for 2 hr using a procedure similar to that described by Burris (6). The only modification was the substitution of 2 ml of Archibald's borate buffer (2) for 1 ml of saturated K_2CO_3. In this procedure, the production of NH_4^+ by enzymic hydrolysis of endogenous amides is a potential source of error but tests in which exogenous glutamine was added indicated that the contribution of NH_4^+ from amide hydrolysis was negligible.

RESULTS

Effects of NH_4^+ on Glutamine Synthetase in Free-living *R. japonicum*. It was of interest to determine the response to NH_4^+ of glutamine synthetase from free-living *R. japonicum*. This seemed especially pertinent since Tronick *et al.* (21) found that the enzyme from *R. japonicum* was adenylylated when the organism was cultured in complex media. Figure 1 shows that incubation of a nitrogen-starved culture of *R. japonicum* for 180 min in the presence of 15 mM $(NH_4)_2SO_4$ resulted in a 58% reduction in glutamine synthetase activity. This suggests that NH_4^+ represses glutamine synthetase in *R. japonicum*, although the possibility exists that the decrease was caused by a shift in the pH optimum of the enzyme which could occur upon adenylylation. The relative adenylylation of glutamine synthetase in free-living *R. japonicum* (Fig. 2) increased in the NH_4^+-treated culture, reaching a maximum at 90 min. After this, relative adenylylation decreased and remained fairly constant throughout the remainder of the experiment. Relative adenylylation of the enzyme from control cultures remained fairly constant except for a peak at 90 min which we cannot explain. These results indicate that regulation of glutamine synthetase in free-living *R. japonicum* occurs in a manner essentially the same as that described for the enzyme in *E. coli*.

Effect of NH_4^+ on Glutamine Synthetase in Bacteroid Suspensions. Since the bacteroid tissue of legume root nodules is the site

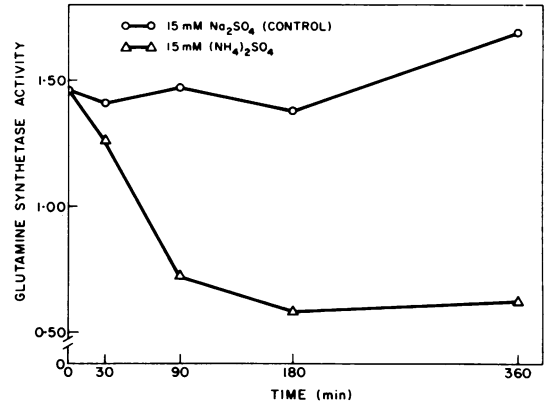

FIG. 1. Effect of NH_4^+ on glutamine synthetase activity in free-living *R. japonicum*. Nine 1-liter flasks each containing 450 ml of medium inoculated with 50 ml from a 48-hr culture were incubated for 36 hr with vigorous shaking. Cells from each flask were harvested aceptically and resuspended in an equal volume of medium lacking Na glutamate followed by a 4-hr incubation period with vigorous shaking. The time course was initiated by adding Na_2SO_4 to four flasks (final concentration 15 mM) and $(NH_4)_2SO_4$ to four other flasks (final concentration 15 mM). The remaining flask served as the zero-time sample. Cell extracts were prepared from separate flasks as described under "Materials and Methods". Each experimental point represents the mean of triplicate determinations of specific activity (μmoles of γ-glutamyl hydroxamate produced/mg protein·min at 25 C). Standard errors of means ranged from 2 to 11% of values reported.

[4] Abbreviation: SVD: snake venom phosphodiesterase.

for N_2 fixation (3) and since crude extracts of bacteroids exhibit relatively low glutamine synthetase activity (5, 8), it seemed logical to investigate the response of the bacteroid enzyme to NH_4^+. One major difficulty in experiments of this type is the control of contamination of bacteroid suspensions from large populations of bacteria on nodule surfaces (9). Contamination was monitored in the experiment described in Table I. Assuming a value of 10^{12} bacterial cells/g wet weight of cell mass, bacterial contamination was estimated to be more than 0.05% of the cell mass.

Data presented in Table I show that incubation of bacteroid suspensions with 30 mM $(NH_4)_2SO_4$ has no consistent effect on either the activity or relative adenylylation of bacteroid glutamine synthetase. Relative adenylylation values for the enzyme in both control and NH_4^+-treated suspensions show that glutamine synthetase was partially adenylylated. A repeat of this experiment, in which no precautions were exercised in the control of bacterial contamination, gave essentially the same results after a 4-hr period of incubation (results not shown). From these data it appears that bacteroid glutamine synthetase is less responsive to NH_4^+ than the enzyme in free-living *R. japonicum*.

NH_4^+-Treatment of Soybean Plants and Its Effect on Activities of Nitrogenase and Glutamine Synthetase. Treatment of soybean plants with 5 mM $(NH_4)_2SO_4$ for 4 days resulted in some NH_4^+ uptake by the nodules and 65% reduction in nitrogenase activity (Table II, experiment I). No striking effect on either the activity or extent of adenylylation of the bacteroid glutamine synthetase was observed. This treatment, however, did cause a marked decrease in nitrogenase activity of nodules.

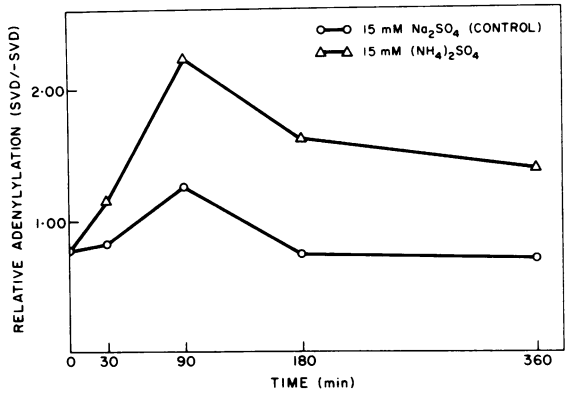

FIG. 2. Effect of NH_4^+ on adenylylation of glutamine synthetase in free-living *R. japonicum*. All conditions were as described in the legend for Fig. 1. Relative adenylylation values are means of duplicate determinations.

The addition of 30 mM $(NH_4)_2SO_4$ for a period of 2 days to 20-day-old soybean plants had no marked effect on the nitrogenase activity of nodules (Table II, experiment II). The $(NH_4)_2SO_4$ treatment, when compared to the Na_2SO_4 control, also had no consistent effect on glutamine synthetase activity or on the relative adenylylation of glutamine synthetase in bacteroids. This treatment, however, did result in a relatively small increase in the NH_4^+ content of nodules. This experiment was repeated using 30-day-old soybean plants, and the trends of results were essentially the same as those presented in Table II, experiment II.

Since exposure of soybean plants to high concentrations of $(NH_4)_2SO_4$ over an extended period of time is likely to cause damage to the plants, a time-course experiment was conducted in order to establish the minimum treatment period that would definitely inhibit nitrogenase activity in nodules. This experiment (Fig. 3) provided evidence that a 6-day treatment with 30 mM $(NH_4)_2SO_4$ caused an appreciable inhibition of nitrogenase activity (55% inhibition based on the control). The results in Table II, experiment III, show that activity and adenylylation of bacteroid glutamine synthetase failed to respond to NH_4^+-treatment after 6 days, even though the NH_4^+ content of nodules was increased from 8.4 to 14.9 μeq/g. During the 6-day $(NH_4)_2SO_4$ treatment the nitrogenase activity of nodules was reduced to only 9% of that observed in control cultures.

In addition, cytosol glutamine synthetase activity was unaltered by NH_4^+ treatment, and the enzyme also appeared to be unadenylated under all conditions tested (results not shown).

Effects of Nitrogen Deprivation on Adenylylation of Bacteroid Glutamine Synthetase in Nodulated Roots and Effects of NH_4^+ on Adenylylation of Enzyme in Sliced Nodules. Since the addition of NH_4^+ to bacteroid suspensions or nodulated plants failed to consistently influence the adenylylation of glutamine synthetase, an effort was made to deprive bacteroids of nitrogen and induce deadenylylation of their glutamine synthetase. In these tests, a sample of nodulated soybean roots was incubated for 12 hr in a gas mixture of 80% A and 20% O_2. Another sample of nodulated roots was incubated in a comparable mixture to which was added 10% C_2H_2 (high concentrations of C_2H_2 noncompetitively inhibit N_2 reduction by nitrogenase). In comparison to control samples of nodules that were incubated in air, these treatments did not appreciably alter the relative adenylylation of bacteroid glutamine synthetase.

In those experiments where intact nodulated plants were exposed to nutrient solutions containing $(NH_4)_2SO_4$ at concentra-

Table I. *Effect of NH_4^+ on Activity and Adenylylation of Glutamine Synthetase in Bacteroid Suspensions*

Bacteroid suspensions were prepared from soybean root nodules as described under "Materials and Methods" and Na_2SO_4 or $(NH_4)_2SO_4$ was added as indicated. Suspensions were prepared from surface-disinfected nodules (9), 5000 units of penicillin G were added/ml of suspension, and bacterial contamination after 10 hr did not exceed 0.05% of the cell mass.

Duration of treatment (hrs)	Additions		Glutamine synthetase (Sp.act.)[1]	Relative adenylylation of glutamine synthetase[2]	
	Na_2SO_4 (mM)	$(NH_4)_2SO_4$ (mM)		$Mn^{2+}/Mn^{2+} + Mg^{2+}$	SVD/-SVD
0	none	none	0.15	1.29	1.21
5	30	none	0.10	1.69	1.70
5	none	30	0.10	1.25	1.26
10	30	none	0.14	1.55	1.55
10	none	30	0.14	1.51	1.58

[1] Means of four replicate determinations. Standard errors of mean were less than 8.5% of values reported. Specific activity is defined as μmoles of γ-glutamyl hydroxamate formed/mg of protein/minute (see "Materials and Methods").

[2] Relative adenylylation is defined under "Materials and Methods". Values are means of duplicate determinations. (Standard errors of means were less than 8% of reported values.)

Table II. *Effect of $(NH_4)_2SO_4$ Treatment of Soybean Plants on Nitrogenase Activity of Nodules and on Activity and Adenylylation of Bacteroid Glutamine Synthetase*

Inoculated soybean plants were cultured for 20 days with a nitrogen-free nutrient solution in pots of perlite (8–13 plants per culture). At the initiation of treatments (0 days) nitrogenase activity of nodules and glutamine synthetase activity and relative adenylylation of bacteroid glutamine synthetase were measured. In experiment I, 80 cultures were treated for 4 days with 5 mM Na_2SO_4 and another 80 cultures were treated for an equal time with 5 mM $(NH_4)_2SO_4$. In experiment II, two groups of 10 cultures each were subirrigated for 2 days with 20 liters of nitrogen-free nutrient solution containing Na_2SO_4 or $(NH_4)_2SO_4$. In experiment III, two groups of 12 cultures each were treated with Na_2SO_4 or $(NH_4)_2SO_4$. Nutrient solutions were changed after use for 3 days.

Duration of Treatment	Source of Nitrogen	NH_4^+ Content	Nitrogenase Activity[2]	Bacteroid Glutamine Synthetase (specific activity)[3]	Relative Adenylylation of Glutamine Synthetase[4]	
					$Mn^{2+}/Mn^{2+} + Mg^{2+}$	SVD/-SVD
days		*μeq per g fresh nodule*[1]				
Experiment I						
0	N_2	ND	11.03	0.24	ND[5]	1.24
4	N_2 + Na_2SO_4 (5 mM)	6.9	8.84	0.23	ND	1.16
4	N_2 + $(NH_4)_2SO_4$ (5 mM)	8.5	3.06	0.30	ND	1.18
Experiment II						
0	N_2	ND	13.39	0.11	1.23	1.35
2	N_2 + Na_2SO_4 (30 mM)	7.7	15.07	0.07	1.35	1.29
2	N_2 + $(NH_4)_2SO_4$ (30 mM)	9.5	15.10	0.07	1.31	1.27
Experiment III						
0	N_2	ND	8.93	0.18	1.32	1.32
6	N_2 + Na_2SO_4 (30 mM)	8.4	12.88	0.15	1.26	1.13
6	N_2 + $(NH_4)_2SO_4$ (30 mM)	14.9	1.19	0.15	1.32	1.25

[1] Ammonium contents are means of duplicate samples. Standard errors of means as percentages of values reported were less than 3% (expt. I), 2% (expt. II), and 2 to 4% (expt. III).

[2] Means of determinations on five replicate cultures expressed as μmoles C_2H_2 reduced/hr·g fresh nodules. Standard errors of means as percentages of values reported ranged from 4 to 22% (expt. I), 4 to 10% (expt. II), and 4 to 6% for treatments without NH_4^+ and 44% for the treatment with $(NH_4)_2SO_4$ (expt. II).

[3] Means of four replicate determinations. Standard errors of means were no greater than 4.3% (expt. I), 1 to 5% (expt. II), and 6 to 13% (expt. III) of the values reported (see footnote 1 of Table I).

[4] Means of duplicate determinations. Standard errors of means were less than 3% (expt. I), 1 to 4% (expt. II), and 1 to 4% (expt. III) of the values reported.

[5] Not determined.

tions from 5 to 30 mM (Table II, experiment I–III), the maximum increase in free NH_4^+ in nodules was from 8.4 to 14.9 μeq/g (experiment III). In an effort to increase NH_4^+ uptake, an experiment was conducted in which one sample of soybean nodules was sliced then incubated with vigorous shaking for 2 hr with 15 mM NH_4Cl. A second sample, for comparison, was incubated for 2 hr with 15 mM KCl. The relative adenylylation of bacteroid glutamine synthetase was about the same (a value of 1.35) for both samples. Relative adenylylation values of cytosol glutamine synthetase were 0.95 and 1.03, respectively, for the NH_4Cl and KCl treatments in this experiment. These differences between values are within experimental error. All efforts to induce a marked change in the relative adenylylation of bacteroid glutamine synthetase by manipulating the nitrogen supply have produced negative results.

DISCUSSION

The addition of excess NH_4^+ to a medium used for the culture of *R. japonicum* resulted in repression and adenylylation of glutamine synthetase. These observations are consistent with those of Tronick *et al.* (21) who reported that the relative adenylylation value of glutamine synthetase from *R. japonicum* cultured on a medium high in fixed nitrogen was 1.41. We found that neither activity nor the extent of adenylylation of glutamine synthetase from *R. japonicum* bacteroids was consistently influenced by the addition of NH_4^+ to nodulated soybean plants or to bacteroid preparations. Our experiments show that bacteroid glutamine synthetase remains partially adenylylated after bacte-

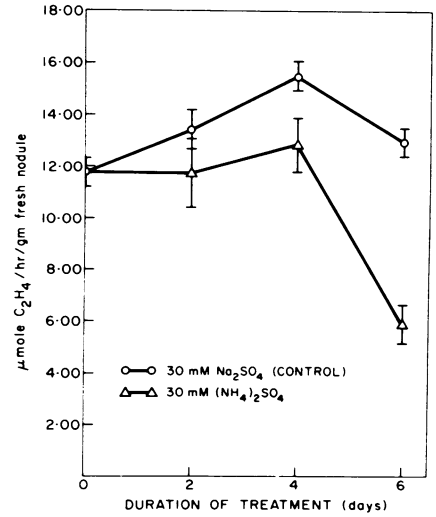

FIG. 3. Effect of NH_4^+ treatment of soybean plants on nitrogenase activity. Soybean cultures, twenty-four days old, were divided into two groups of 15 cultures each. The first group was subirrigated with 20 liters of nitrogen-free nutrient solution (21) containing 30 mM Na_2SO_4 (control), and the second group was subirrigated with 20 liters of nitrogen-free nutrient solution containing 30 mM $(NH_4)_2SO_4$ (NH_4^+-treated group). Each experimental point is the mean of determinations on five replicate cultures (μmoles C_2H_2 reduced/hr/g of fresh nodules); ± standard errors of the means are indicated.

roid suspensions and nodulated roots are incubated in the absence of a source of combined nitrogen. Under these conditions adenylylated enzyme would be expected to become unadenylylated. Perhaps bacterial contamination of the bacteroid preparations was responsible for the observed partial adenylylation but this seems unlikely since contamination accounted for less than 0.05% of the total cell mass in the experiment described in Table I.

In agreement with reports from some other laboratories (5, 8), levels of glutamine synthetase in legume bacteroids are relatively low ranging from 5 to 20% of those in nitrogen-starved free-living *R. japonicum*. According to Kurz *et al.* (15), the specific activity of glutamine synthetase in bacteroids from *Pisum sativum* is equivalent to that of nitrogenase. However, they concluded that the glutamine synthetase-glutamate synthase pathway in bacteroids will not account for the assimilation of NH_4^+ produced from N_2 fixation by *Pisum sativum* nodules. Brown and Dilworth (5) have investigated NH_4^+ assimilation routes in nodules of a series of legumes and concluded that the plant rather than the bacteroid is the most probable site for assimilation of the primary product of N_2 fixation. Since the amount of glutamine synthetase is reported (20, 22) to be an important factor in the regulation of nitrogenase in *K. pneumoniae* and *K. aerogenes*, the level of activity in *R. japonicum* bacteroids may be insufficient for participation in a control process.

It is possible that bacteroids have lost their capability to regulate glutamine synthetase via the adenylylation-deadenylylation cascade control system (13). The observed activities and state of adenylylation of the enzyme might result from residual enzyme formed during the prebacteroid developmental stage.

In a previous study (4), we reported a marked decrease in nodule weight after treatment of nodulated soybean plants with NH_4^+. Possibly, the regulation of nitrogenase activity in the symbiotic system is controlled indirectly by a plant gene(s) involved in nodule development.

Acknowledgment – We thank S. Russell for his assistance in various phases of this work.

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