Energy Status and Functioning of Phosphorus-Deficient Soybean Nodules

Tong-Min Sa and Daniel W. Israel*

Department of Soil Science, Plant Physiology Program (T.-M.S.) and U.S. Department of Agriculture, Agricultural Research Service, Department of Soil Science, Plant Physiology Program (D.W.I.), Box 7619 North Carolina State University, Raleigh, North Carolina 27695

ABSTRACT

Characterization of the effects of long-term P deficiency and of onset and recovery from P deficiency on bacteroid mass and number per unit nodule mass and energy status of soybean (Glycine max L. Merr.) nodules was used to investigate the mechanisms by which P deficiency decreases symbiotic N2 fixation. The continuous P deficiency treatment (0.05 millimolar P) significantly decreased the whole plant dry mass, P, and N by 62, 90, and 78%, respectively, relative to the P-sufficient control (1.0 millimolar) at 44 days after transplanting. Specific nitrogenase activity was decreased an average of 28% over a 16-day experimental period by P deficiency. Whole nodules of P-deficient controls contained 70 to 75% lower ATP concentrations than nodules of P-sufficient controls. Energy charge and ATP concentrations in the bacteroid fraction of nodules were not significantly affected by P treatment. However, ATP and total adenylate concentrations and energy charge in the plant cell fraction of nodules were significantly decreased 91, 62, and 50%, respectively, by the P deficiency treatment. Specific nitrogenase activity, energy charge, and ATP concentration in the plant cell fraction increased to the levels of nonstressed controls within 2, 2, and 4 days, respectively, after alleviation of external P limitation, whereas bacteroid mass per unit nodule mass and bacteroid N concentration did not increase to the level of nonstressed controls until 7 days after alleviation of external P limitation. All of these parameters except bacteroid mass per unit nodule mass decreased to the levels of the P-deficient controls by 11 days after onset of external P limitation. Concentration of ATP in the bacteroid fraction was not significantly affected by alleviation in the external P supply. Energy charge in the bacteroid fraction from plants recovering from P deficiency was decreased to a small (10%) but significant extent (P < 0.05) at two sampling dates relative to P-sufficient controls. These ATP concentration and energy charge measurements indicate that P deficiency impaired oxidative phosphorylation in the plant cell fraction of nodules to a much greater extent than in the bacteroids. The concurrence of significant changes in specific nitrogenase activity (2 days) and in the energy charge (2 days) and ATP concentration (4 days) in the plant cell fraction during recovery from external P limitation is consistent with the conclusion that P deficiency decreases the specific nitrogenase activity by inhibiting an energy-dependent reaction(s) in the plant cell fraction of the nodules.

An improvement in P status of several legumes solely dependent on symbiotic N2 fixation has been reported to increase tissue N concentrations as well as overall host plant growth (1, 14, 27). This response results from symbiotic N2 fixation being stimulated to a greater degree by improvement in P nutrition than by host plant growth. Greater stimulation of symbiotic N2 fixation than of host plant growth with improvement in P nutrition is associated with an enhancement in the specific nitrogenase activity of the nodules (2, 14, 16). The mechanism(s) that account for the increased activity have not been elucidated.

Fixation of N2 by nitrogenase requires an energy supply (ATP) and reductant (NADPH) (8, 9). A minimum requirement of 12 ATP for each mol of N2 reduced has been deduced from kinetic studies with purified nitrogenase and from physiological studies with several N2-fixing bacteria (26). Energy charge ([ATP] + 1/2[ADP])/([ATP] + [ADP] + [AMP]) and the ATP to ADP ratio have been shown to be positively correlated with nitrogenase activity in cell-free preparations (7, 8, 10).

The relationship between energy status and nitrogenase activity of soybean nodules has been examined (5, 6). Extending the dark period for 1 d decreased the ATP concentration by 70%, the sucrose concentration by 60%, the total adenylate concentration by 60%, the energy charge by 15%, and the specific nitrogenase activity by 50% in soybean nodules (5). Enriching the atmosphere around nodules to 40% oxygen stimulated nitrogenase activity 2.5-fold within 4 min with concomitant 40 and 14% increases in ATP concentration and energy charge, respectively (6). The positive correlations between specific nitrogenase activity and energy charge led Ching (6) to conclude that ATP and energy charge regulate the activity of nitrogenase in vivo and that active adenylate kinase must be operating in the nodules to maintain an energy supply for basal metabolism and for nitrogenase function under the imposed stress condition (extended darkness).

Because N2 fixation is an energy intensive process, and because P has a key role in the energy metabolism of all cells, P deficiency is predicted to have a negative impact on the energy status of legume nodules. Dinitrogen fixation, which occurs in bacteroids, and ammonium assimilation into amino acids and ureides, which occurs in the plant cell fraction of nodules, are both energy-consuming processes. Therefore, how P deficiency affects the energy status of these compartments has relevance for nodule function. The objectives of this study were: (a) to determine the effects of P deficiency on

nodule bacteroid content (mass and number per unit nodule mass), the adenylate concentrations and energy charge of whole nodules and isolated bacteroids, and the distribution of ATP within nodules, and (b) to examine the relationship between these parameters and the specific nitrogenase activity of nodules. These parameters were monitored in long-term P-deficiency treatments and during onset and recovery from P deficiency.

MATERIALS AND METHODS

Plant Culture and Inoculation

Soybean (Glycine max L. Merr.) plants were grown in outdoor pot culture from mid-June to the end of July 1988. Nutrient solutions were prepared in tap water as described by McClure and Israel (20) except that KH₂PO₄ was the sole source of P. The P-deficiency treatment solution contained 0.05 mM P, and the control treatment solution contained 1.0 mM P. At 28 DAT,² one-half of the P-sufficient and P-deficient control plants was switched to the opposite P concentration after exhaustive flushing of pots with tap water. The other half of the plants in each treatment remained at the same P concentration and served as continuous P-deficient and P-sufficient controls. The potassium concentration in the deficiency treatment solution was adjusted to the same concentration as that of the control treatment solution by addition of K₂SO₄. The initial pH of both treatment solutions was adjusted to 6.0.

Seeds of “Ransom” soybean were germinated in 0.5 mM CaSO₄ for 72 h at 30°C and 95% RH. Roots of seedlings were dipped in an inoculum of Bradyrhizobium japonicum strain MN 110 (19) just before transplanting into 6-L pots filled with Perlite³ amended with 300 g of calcium carbonate in the form of crushed oyster shells to control acidification of the rhizosphere (15). Immediately after transplanting, 0.5 mL of inoculum was applied to the Perlite at the base of each seeding. Inoculum was grown to stationary phase (about 10⁶ colony-forming units/mL) in galactose-arabinose-glutamate medium (3). Two and three seedlings were transplanted into each pot for the control and P-deficiency treatment, respectively.

From 1 to 4 DAT, each pot was irrigated with 500 mL of tap water at 0800 and 1400 h; from 5 to 12 DAT, pots were supplied 250 mL of nutrient solution after each irrigation with 500 mL of tap water at 0800 and 1400 h; and from 13 DAT to the last sampling date, each pot was irrigated with 1.5 to 2 L of tap water at 0800 and 1400 h, and 500 mL of appropriate nutrient solution was supplied after the 1400 h irrigation.

Plant material was harvested at 0, 2, 4, 7, 11, and 16 d after changing the external P concentrations. Plants were separated into leaflets, stem plus petioles, root, and nodule fractions. Nodules were detached from roots as rapidly as possible (15–30 min), frozen in liquid nitrogen, and stored at −80°C. The remaining plant material was dried at 65°C for 72 h, weighed, and ground to pass through a 1-mm screen.

Adenylate Extraction and Quantitation

Adenylates were extracted from the frozen nodules by the methanol/TCA method of Khym (18). Nodules were weighed (0.8–1.2 g) and placed into a 30-mL centrifuge tube containing a cold (4°C) solution of 30% (v/v) methanol and 8% (w/v) TCA. The tissue was homogenized for 30 s with a Polytron homogenizer. The initial grinding was followed by a 10-s rinse with 5 mL of methanol/TCA solution. Samples were kept on ice throughout extraction to minimize acidic and enzymatic hydrolysis of nucleotides. The homogenate was centrifuged at 12,000g for 10 min at 4°C. TCA was removed from the resulting supernatants by partitioning into 0.5 M tri-n-octylamine in trichlorotrifluoroethane. Aliquots (4 mL) of the supernatant were transferred into screw-cap test tubes, and equal volumes of 0.5 M tri-n-octylamine trichlorotrifluoroethane were added. The mixtures were then mixed vigorously for 30 s. The top aqueous layers that contained nucleotides free of TCA were transferred into vials. Samples were frozen at −80°C until analyzed for adenylate. After appropriate dilutions, ATP was determined by the luciferin-luciferase assay system. The intensity of light produced in the luciferase assay was quantified with an Amino Chem-Glow photometer. The luciferin-luciferase assay was also used to measure ADP and AMP in the extract after enzymatic conversion to ATP. Pyruvate kinase was used to catalyze the phosphoenolpyruvate-dependent conversion of ADP to ATP. Myokinase, which utilizes endogenous ATP to convert AMP to ADP, and pyruvate kinase were used to convert AMP to ATP. Samples subjected to enzymatic conversion reactions were incubated at 35°C for 30 min. Reactions were terminated by transferring the samples onto ice. The recovery of standards by the enzymatic conversion method was 91.8 ± 8.1% for ADP and 96.5 ± 5.1% for AMP. Recovery of ATP after exposure to the conditions of the conversion reactions was 94.7 ± 5%. The recovery of internal standards with nodule extracts was 93.5 ± 4.8% for ATP, 92.5 ± 5.9% for ADP, and 89.5 ± 8.7% for AMP. Energy charge (EC) was calculated as EC = ([ATP] + 1/2[ADP])/([ATP] + [ADP] + [AMP]).

Preliminary stability tests showed that adenylate concentrations in extracts of nodules from P-deficient and P-sufficient control plants did not change during 24 h of storage at −80°C (data not shown). After 7 d of storage at −80°C, ATP and ADP concentrations in the extracts decreased 20 to 30% and AMP concentrations increased approximately 20%. All extracts were assayed within 24 h of preparation.

Bacteroid Isolation

Nodules were surface-sterilized in 80% ethanol for 15 s, rinsed with cold redistilled water, and homogenized in a Polytron for 30 s in 25 mL of 20 mM Hepes buffer (pH 6.9) containing 5% (w/v) PVP, 10 mM sodium ascorbate, 10 mM glucose, and 100 μg/mL chloramphenicol. This suspension was filtered through four layers of cheesecloth to remove nodule cortex tissue, and the filtrate was centrifuged at 12,000g for 10 min at 4°C. The pellet of bacteroids was

² Abbreviation: DAT, days after transplanting.
³ Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.
resuspended and centrifuged again in the same buffer (10 mL) lacking the PVP. The bacteroid suspension (1 mL) was diluted, and bacteroid number was counted directly with a hemacytometer. Aliquots (2.0 mL) of the bacteroid suspension were mixed with 20 mL of the methanol/TCA solution to extract adenylates. All other steps were the same as those described for measurement of adenylate in whole nodules. The remainder of the suspensions (5 mL) was dried at 70°C for 72 h to determine bacteroid dry mass yield.

Adenylate concentration in the plant cell fraction of nodules was estimated by measuring adenylates in a subsample of whole nodules and in bacteroids isolated from a second subsample of nodules from the same plant. The adenylate concentrations in bacteroid and plant cell fractions of nodules were expressed on a gram dry weight of nodule basis as follows. Bacteroid adenylate concentration on a nodule dry weight basis was calculated as the product of the bacteroid to whole nodule dry weight ratio and the bacteroid adenylate concentration, expressed on a bacteroid dry weight basis. Adenylate concentrations in the plant cell fraction were calculated by subtracting bacteroid adenylate concentration from whole nodule adenylate concentration, all expressed on a gram nodule dry weight basis. The plant cell dry mass per unit nodule dry mass was obtained by subtracting the bacteroid dry mass per unit nodule dry mass from 1.0 and used to calculate adenylate concentrations on a plant cell dry weight basis. The dry-weight to fresh-weight ratio of subsamples of nodules from each plant was measured and used to convert whole nodule fresh weight extracted for adenylates to whole nodule dry weight.

Nitrogenase Activity

A set of plants was grown at the same time and under the same treatment conditions as plants used for adenylate measurements and used for assay of nitrogenase activity by the acetylene reduction technique. Acetylene reduction assays were performed on the excised root systems (28) at the same time that nodules were harvested from comparably grown plants for adenylate measurement. Nodulated root systems were incubated for 30 min at 25°C in 1-L jars containing 0.1 atm of acetylene. Ethylene and acetylene in the samples were separated and quantified with a Carle model 311 H gas chromatograph equipped with flame ionization detector and a column of Porapak N (182 x 0.32 cm).

Nitrogen and Phosphorus Determination

The total N concentration of tissue samples (100–200 mg) was determined by a Kjeldahl procedure that included a salicylic acid predigestion step (24) and employed a copper-zirconium catalyst (11). After alkalization of digests, ammonia was steam-distilled into boric acid and quantified by titration with potassium biocidate. Appropriate aliquots of diluted Kjeldahl digest were analyzed for total P by the ammonium molybdate method of Murphy and Riley (23). These procedures were also used to determine total N and P in whole nodules and isolated bacteroids. The N and P concentrations in the host plant cell fraction were then calculated in the same manner as described for adenylate concentration.

Experimental Design and Statistical Analysis

A randomized complete block design with three replications was used. All combinations of treatment and sampling date were assigned randomly within each block. The data were analyzed by the analysis of variance procedure of Statistical Analysis System (12).

RESULTS

Dry Matter, Phosphorus, and Nitrogen Accumulation

The continuous P-deficiency treatment decreased whole plant dry mass, P, and N at the final sampling date by 62, 90, and 78%, respectively, compared with the continuous P-sufficient control treatment (Fig. 1). Whole plant dry matter, P, and N increased significantly relative to the P-deficient control 16, 4, and 11 d, respectively, after increasing the external P concentration (Fig. 1). In contrast, whole plant P and N decreased significantly relative to the P-sufficient control 11 and 16 d, respectively, after decreasing the external P concentration, whereas a significant decrease in whole plant dry matter had not occurred by the 16-d sampling (Fig. 1).

Phosphorus Concentrations in Plant Organs

Phosphorus concentrations in leaf, stem, and nodule tissues increased significantly 2 d after increasing the external P concentration (Fig. 2). Phosphorus concentrations in leaf...
Values calculated in earlier publications indicate that P and N concentrations in bacteroids from P-sufficient controls (Fig. 3). Significant increases in bacteroid P and N concentrations occurred 2 and 7 d after increasing the external P concentration (Fig. 3). A significant decrease in bacteroid N concentration occurred 11 d after decreasing the external P concentration, but bacteroid P concentration was not significantly affected during the experimental period (Fig. 3).

The average P concentration (2.5 mg/g dry weight nodule) in the plant cell fraction of nodules from P-deficient plants was about half that of P-sufficient controls (Fig. 4). The P concentration increased to that of P-sufficient controls within 2 d of increasing the external P concentration (Fig. 4). In contrast, a significant decrease in the P concentration did not occur until 7 d after decreasing the external P concentration (Fig. 4). Nitrogen concentrations in the plant cell fraction of nodules were not affected in any consistent manner by P treatments (Fig. 4).

**Nitrogenase Activity**

Specific nitrogenase activity was decreased an average of 28% by continuous P deficiency during the 16-d experimental period (data not shown).

**Phosphorus and Nitrogen Concentrations in Nodule Components**

Phosphorus and nitrogen concentrations in bacteroids from P-deficient controls averaged 9 and 95 mg/g dry weight bacteroid, respectively, and were 25 and 17% lower, respectively, than P and N concentrations in bacteroids from P-sufficient controls (Fig. 3). Significant increases in bacteroid P and N concentrations occurred 2 and 7 d after increasing the external P concentration (Fig. 3). A significant decrease in bacteroid N concentration occurred 11 d after decreasing the external P concentration, but bacteroid P concentration was not significantly affected during the experimental period (Fig. 3).

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from P deficiency and decreased from 0.75 to 0.65 during onset of P deficiency (Fig. 6). Energy charge increased significantly 2 d after removal of external P limitation and decreased significantly 7 d after imposition of external P limitation (Fig. 6).

Concentrations of ATP and total adenylate in the bacteroid fraction were not significantly affected by P treatments (Fig. 7). Energy charge was altered to a slight but significant (P < 0.05) extent by the P treatments (Fig. 7). However, most of this effect was caused by bacteroids from plants recovering from P deficiency having a significantly lower energy charge than bacteroids from P-sufficient and P-deficient control plants at the 32 and 35 DAT samplings (Fig. 7).

Concentrations of ATP and total adenylate in the plant cell fraction of nodules from P-deficient control plants were only 9 and 38%, respectively, of those from P-sufficient control plants (Fig. 8). Significant increases in ATP and total adenylate occurred 4 and 7 d, respectively, after increasing the external P concentration (Fig. 8). Significant decreases in ATP and total adenylate concentrations in the plant cell fraction occurred 7 and 11 d, respectively, after imposition of external P limitation (Fig. 8).

Energy charge averaged 0.70 and 0.35 in the plant cell fraction of nodules from P-sufficient and P-deficient control plants, respectively (Fig. 8). A significant increase in the energy charge of the plant cell fraction occurred 2 d after increasing the external P concentration, whereas a significant

### Energy Status of Nodules

The concentration of ATP was relatively constant in whole nodules of P-sufficient and P-deficient control plants and was three- to fourfold greater in the P-sufficient plants (Fig. 6). The concentration of total adenylate was 2- to 2.7-fold greater in nodules of P-sufficient than of P-deficient plants (Fig. 6). Whole nodule ATP concentrations increased and decreased significantly 2 and 7 d after increasing and decreasing the external P concentration, respectively (Fig. 6). Energy charge of whole nodules increased from 0.55 to 0.71 during recovery

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**Figure 4.** Effects of alterations in external P concentration on the N and P concentrations in the plant cell fraction of nodules. Data points represent mean of three replicates. The bars indicate LSD0.05 values calculated on the basis of significant treatment by time interaction effects and can be used to compare any two treatment means. GDW, grams dry weight.

**Figure 5.** Effects of alterations in external P concentration on specific and total nitrogenase activity of soybean nodules. Data points represent means of four replicates. The bars indicate LSD0.05 values calculated on the basis of significant treatment by time interactions and can be used to compare any two treatment means. GFW, grams fresh weight.
ATP and bacteroid proliferation.

or concentration alteration processes.

Effects nodules. Data deficient AMP and weight dry grams relative in trations almost means. resulted by LSDo.05 not nodules also plant cell fraction although status indicates (Fig. 6).

This indicates that developing nodules of the P-deficient plants contained sufficient phosphate to support bacteroid proliferation. Concentrations of ATP in bacteroids were not significantly affected by onset and removal of P deficiency or by continuous P deficiency, and alterations in the external P supply had relatively small negative effects (10%) on energy charge of the bacteroids (Fig. 7).

These observations indicate that bacteroids in nodules of P-deficient plants contained sufficient Pi to support the energy transducing pathways that lead to ATP synthesis. The energy charge values of bacteroids from nodules of P-deficient plants (0.8–0.9) are comparable to those reported for several bacterial genera grown under nonlimiting substrate conditions (4).

The lower P status of nodules caused by external P deficiency had significant negative effects on bacteroid dry mass per unit nodule dry mass (Fig. 3) and on the bacteroid N concentration (Fig. 3). Lower bacteroid mass per unit nodule mass could indicate decreased cell size or density under P deficiency, because bacteroid number per unit nodule mass was not significantly affected by P treatment (data not shown). Decreased N concentrations in bacteroids from nodules of P-deficient plants indicate that protein synthesis in the bacteroids was inhibited by the P deficiency.

It has been clearly demonstrated in 31P-NMR experiments that Pi and other P metabolites are not uniformly distributed among the different cell types of soybean nodules (25). Therefore, in our experiment, the adenylate concentrations represent the averages of all cell types.

The P and energy status of the plant cell fraction of nodules was seriously impaired by continuous P deficiency, as P and ATP concentrations and energy charge were decreased by 50,

**DISCUSSION**

Although whole plant adjustments under P deficiency resulted in maintenance of relatively high P concentrations in nodules relative to other plant organs, P deficiency impaired the energy status of the nodules (Figs. 6 and 8). Decreased ATP concentrations (Fig. 6) in response to P deficiency were due almost entirely to decreased ATP concentrations in the plant cell fraction of the nodule (Fig. 8), as the ATP concentrations in bacteroids were not significantly affected (Fig. 7). Total adenylate concentrations in the plant cell fraction of nodules also decreased significantly in response to P deficiency, although not to the same extent as the ATP concentration (Fig. 8). Therefore, Pi was apparently released from AMP and ADP for utilization in other essential cellular processes.

Phosphorus deficiency decreased the bacteroid P concentration by approximately 25% to 9 mg per gram bacteroid dry weight (Fig. 3). Phosphorus treatments did not significantly alter the bacteroid number per unit nodule mass (data not shown). This indicates that developing nodules of the P-deficient plants contained sufficient phosphate to support bacteroid proliferation. Concentrations of ATP in bacteroids were not significantly affected by onset and removal of P deficiency or by continuous P deficiency, and alterations in

**Figure 6.** Effects of alterations in external P concentration on the ATP and total adenylate concentrations and energy charge of whole nodules. Data points represent means of three replicates. The bars indicate LSD0.05 values calculated on the basis of significant treatment by time interactions and can be used to compare any two treatment means. GFW, grams fresh weight.

decrease occurred 7 d after imposition of external P limitation (Fig. 8).

**Figure 7.** Effects of alterations in external P concentration on the ATP and total adenylate concentrations and energy charge of bacteroids isolated from soybean nodules. Data points represent means of three replicates. The treatment effect was not significant for ATP and total adenylate concentrations. The bar indicates LSD0.05 value calculated on the basis of significant treatment by time interaction for energy charge and can be used to compare any two treatment means. GDW, grams dry weight.
deficient plants (Figs. 3 and 9). Because nitrogenase is localized in the bacteroids, lower bacteroid mass per unit nodule mass and N concentrations could account for decreased specific nitrogenase activity under P deficiency. However, the observation that significant increases in bacteroid mass per unit nodule mass and bacteroid N concentration upon alleviation of external P limitation were delayed 5 d relative to significant increases in specific nitrogenase activity (Figs. 3 and 5) is not consistent with this idea. On the other hand, ATP concentrations and energy charge in the plant cell fraction of nodules increased significantly 2 d after alleviation of external P deficiency (Fig. 8), as did the specific nitrogenase activity (Fig. 5), and significant decreases in these parameters preceded significant decreases in specific nitrogenase activity during onset of P deficiency. These observations are consistent with the inference that P deficiency decreases the specific nitrogenase activity by inhibiting an ATP-dependent reaction(s) in the plant cell fraction of nodules.

Leaf P concentration and specific nitrogenase activity increased to the level of P-sufficient controls within 2 d of increasing the external P concentration available to P-deficient plants (Figs. 2 and 5). This could be interpreted as an indication that increased specific nitrogenase activity was linked with increased input of photosynthate into nodules from the leaves. However, in another experiment, we have observed that P deficiency significantly decreased hexose and sucrose concentrations in soybean nodules, but total nonstructural carbohydrate concentration was not affected, because starch concentrations in the nodules increased in response to P deficiency (our unpublished results). This indicates that P deficiency may have decreased the efficiency of carbohydrate utilization within the nodules as well as carbohydrate input from the leaves. Decreased oxidative phosphorylation in the plant cell component of nodules, as inferred from adenylate measurements (Fig. 8), may have been associated with this apparent decrease in carbohydrate utilization efficiency. Collectively, these results indicate that P deficiency has a direct impact on the energy metabolism of nodules.

The mechanism by which inhibition of energy-dependent reactions in the plant cell fraction of nodules restricts the specific activity of nitrogenase has not been elucidated. Glutamine synthetase, which catalyzes incorporation of ammonium into glutamine, and four enzymes in the purine biosynthetic pathway, which catalyze synthesis of ureides for transport to the shoot, are localized in the plant cell fraction of nodules and require ATP. Therefore, decreased ATP concentrations and energy charge may inhibit one or more of those enzymatic reactions and cause accumulation of N metabolites, perhaps ammonium and/or glutamine. The ammonium concentration in the plant cell cytoplasm of nodules from soybean plants grown under adequate P nutrition has been shown to be nil (29). Ammonia concentrations in the plant cell cytoplasm of nodules from P-deficient plants have not been measured. The biosynthetic activity of glutamine synthetase purified from soybean nodules has been reported to increase as the energy charge of the reaction medium increased (21). Therefore, if this energy charge sensitivity of glutamine synthetase is expressed in vivo, ammonium assimilation into glutamine could be decreased, and higher levels of ammonium could accumulate in nodules of P-deficient

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**Figure 8.** Effects of alterations in external P concentration on ATP and total adenylate concentrations and energy charge of the plant cell fraction of soybean nodules. Data points represent means of three replicates. The bars indicate LSD values calculated on the basis of significant treatment by time interactions and can be used to compare any two treatment means. GDW, grams dry weight.

60, and 50%, respectively (Figs. 4 and 8). These results are consistent with the inference that oxidative phosphorylation in plant cell fraction of nodules was inhibited by P deficiency. The capacity for oxidative phosphorylation in a tissue is dependent upon the total mitochondrial number, inner membrane area, and the composition, density, and optimal arrangement of energy conserving units (13). Phosphorus deprivation has been shown to decrease oxidative phosphorylation in coleoptile and root tissues of germinating corn seedlings (30). This decrease in oxidative phosphorylation was associated with a decrease in formation of cristae in the mitochondria and an increased swelling of the mitochondria (30). Significant increases in ATP concentrations and energy charge within 2 d of alleviating external P deficiency (Fig. 8) are consistent with the inference that Pi availability for transport into the mitochondria, rather than altered mitochondrial biogenesis and structure, was the major factor limiting oxidative phosphorylation in the plant cell fraction of nodules. Nitrate application and water stress have been shown to decrease the permeability of legume nodules to oxygen (22, 31). Thus, the possibility that oxidative phosphorylation was limited by oxygen diffusion into the inner cortex of P-stressed nodules must also be considered.

Several physiological and metabolic properties were associated with lower specific nitrogenase activity in nodules of P-deficient plants. Bacteroid mass per unit nodule mass, bacteroid N concentrations, plant cell ATP concentrations, and energy charge were significantly lower in nodules of P-
plants. Accumulation of these metabolites in plant cells could lead to increased transport into the bacteroids or slow the export of ammonium from the bacteroids resulting in repression of nitrogenase genes and, thereby, synthesis of less nitrogenase protein. Ammonium has been shown to repress transcription of nitrogenase genes in free-living bacteria such as *Klebsiella pneumoniae* (17). The observation that a significant increase in bacteroid N concentration (Fig. 3) upon alleviation of P deficiency was delayed 5 days relative to significant increases in specific nitrogenase activity (Fig. 5) is not consistent with this proposal. However, it is conceivable that synthesis of specific proteins such as nitrogenase and nitrogenase reductase could occur without a measurable increase in total protein synthesis.

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LITERATURE CITED


