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De Novo Purine Synthesis in Nitrogen-Fixing Nodules of Cowpea (*Vigna unguiculata* [L.] Walp.) and Soybean (*Glycine max* [L.] Merr.)[1]

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ABSTRACT

Partially purified, cell-free extracts from nodules of cowpea (*Vigna unguiculata* L. Walp. cv. Caloona) and soybean (*Glycine max* L. Merr. cv. Bragg) showed high rates of *de novo* purine nucleotide and purine base synthesis. Activity increased with rates of nitrogen fixation and ureide export during development of cowpea plants; maximum rates (equivalent to 1.2 micromoles N_2 per hour per gram fresh nodule) being similar to those of maximum nitrogen fixation (1–2 micromoles N_2 per hour per gram fresh nodule). Extracts from actively fixing nodules of a symbiosis not producing ureides, *Lupinus albus* L. cv. Ultra, showed rates of *de novo* purine synthesis 0.1% to 0.5% those of cowpea and soybean. Most (70–90%) of the activity was associated with the particulate components of the nodule, but up to 50% was released from this fraction by osmotic shock. The accumulated end products with particulate fractions were inosine monophosphate and aminoimidazole carboxamide ribonucleotide. Further metabolism to purine bases and ureides was restricted to the soluble fraction of the nodule extract. High rates of inosine monophosphate synthesis were supported by glutamine as amide donor, lower rates (10–20%) by ammonia, and negligible rates with asparagine as substrate.

The ureides, allantoin and allantoic acid, are formed as major products of nitrogen fixation in a wide range of tropical legumes (8, 14). Studies with cell-free extracts of cowpea and soybean nodules have demonstrated that these compounds may be formed from oxidation of purine bases and nucleotides (2, 5, 22, 23) and that this activity is associated with the plant cell cytosol of the central, infected tissue of the nodule (5). A pathway for *de novo* synthesis of purine nucleotides has been suggested (3) as the source of these purines with currently fixed nitrogen being utilized from the amino groups of glycine and aspartate and the amide group of glutamine. While [^{14}C]glycine supplied to nodule slices was more effectively metabolized to ureides than was [^{14}C]glucose or acetate (5), consistent with an active purine pathway, the level of labeling was relatively low. Furthermore, of the eleven enzymes likely to be involved in purine biosynthesis (6), the activity of only two, ribose phosphate pyrophosphokinase (PRPP[2] synthetase, EC 2.7.6.1) and glutamine:PRPP amidotransferase (EC 2.4.2.14), have been detected in extracts of 'ureide-forming' legume nodules (15, 18).

This study reports the assay of a complete pathway of *de novo* purine nucleotide and base synthesis in cell-free extracts of nodules from cowpea and soybean.

MATERIALS AND METHODS

Chemicals. Radioactive substrates ([1-^{14}C]glycine, [3-^{14}C]serine, [U-^{14}C]serine, [8-^{14}C]IMP, and $NaH^{14}CO_3$) were purchased from Amersham (Aust.) Pty. Ltd. The labeled glycine and serine were purified prior to use (13). Nonradioactive amino acids, PEP, glucose 6-P, purine bases, nucleosides, nucleotides, creatine phosphate, creatine phosphokinase, and pyruvate kinase (type III) were obtained from Sigma; PRPP from P-L Biochemicals Inc. as the sodium salt (approx. 70% PRPP); and AICAR from Boehringer (Aust.). Ion-pair reagents (1-heptane sulfonic acid and tetrabutyl ammonium hydroxide) were supplied by Eastman Chemical Co. or Ajax Chemicals (Aust.). PEG 4000 was obtained from BDH (Aust.) and was not purified before use. 5,10-Methenyl THF was synthesized from *dl*-L-THF (Sigma grade III) (22) and stored in vacuo at −20°C.

Plant Material. Cowpea (*Vigna unguiculata* [L.] Walp. cv. Caloona) plants effectively nodulated with *Rhizobium* CB756, soybean (*Glycine max* [L.] Merr. cv. Bragg) with a commercial soybean peat inoculant (Nitrogerm, group H), and white lupin (*Lupinus albus* [L.] cv. Ultra) with *Rhizobium* WU425 were grown in nitrogen-free sand culture (9) in a naturally lighted glasshouse. Maximum day temperature for cowpea and soybean was 35°C, and for lupin, 30°C.

Preparation of Cell-Free Extracts. Freshly harvested nodules (4–9 g fresh weight) from plants of various ages were washed with deionized H_2O and crushed with 1 to 2 volumes of chilled 50 mM Hepes-NaOH buffer (pH 7.5) containing 0.25 M sucrose, 25 mM KCl, 5 mM $MgCl_2$, and 50 mM β-mercaptoethanol in a chilled mortar and pestle. The extract of the central, pigmented tissues of the nodule was separated from the largely intact cortical tissues (5) by filtration through 100 μm mesh followed by centrifugation (10,000*g*, 10 min, 3–4°C) to separate the soluble and particulate components. The supernatant was adjusted to 40% (w/v) PEG 4000 by dropwise addition of a chilled 50% (w/v) PEG solution with continuous stirring. The pellet, containing bacteroids and intact plant organelles (5), was resuspended in 5 to 10 ml breaking medium and twice passed through a chilled French pressure cell (1). Based on the solubilization of protein, disruption in this manner was more than 90% effective despite the high level of sucrose present. After centrifugation of the disrupted suspension (10,000*g*, 20 min, 3–4°C), the supernatant was adjusted to 40%

[1] Supported by funds from the Australian Research Grants Committee.

[2] Abbreviations: PRPP, phosphoribosyl pyrophosphate; IMP, inosine 5′-monophosphate; PEP, phosphoenolpyruvate, AICAR, aminoimidazole carboxamide ribonucleotide; PEG 4000, polyethylene glycol (average mol wt, 4000); THF, tetrahydrofolic acid; CAIR, aminoimidazole carboxylate ribonucleotide; FGAR, formylglycinamide ribonucleotide, XMP, xanthosine 5′-monophosphate; AIR, aminoimidazole ribonucleotide.

(w/v) PEG as above. The protein precipitated by PEG from both the soluble and particulate fractions was collected by centrifugation (30,000*g*, 20 min, 3–4°C) and the pellets dissolved in 1 to 4 ml breaking medium containing 40 mM KCl. For cowpea nodules from 4- to 6-week plants, this fractionation and partial purification yielded around 5 mg protein/ml in the soluble extract and 3 to 6 mg protein/ml from disruption of the particulate components. In some experiments, the nodule homogenate obtained after filtration was not separated into soluble and particulate fractions but was passed through a French pressure cell, and after centrifugation the total protein was precipitated with PEG as above.

Assay of *De Novo* Purine Synthesis. Incorporation of [1-^{14}C] glycine into purine bases, IMP, and intermediates of the pathway was measured using 0.1 to 0.2 ml partially purified extracts in a reaction mixture containing 0.08 mM [1-^{14}C]glycine (1 μCi ^{14}C), 2 mM PRPP, 1.5 mM 5,10-methenyl THF, 1 mM L-aspartic acid, 2.2 mM sodium formate, 5 mM ATP, 10 mM $KHCO_3$, 10 mM $MgCl_2$, 10 mM KCl, 10 mM PEP, 25 units pyruvate kinase, 50 mM Hepes-NaOH (pH 7.5), 20 mM L-mercaptoethanol, and either 10 mM L-glutamine, 10 mM L-asparagine, or 40 mM NH_4Cl in a final volume of 0.7 ml. All components except for enzyme extract were mixed and held on ice. The metabolism was initiated by addition of enzyme extract and immediate transfer of the mixture to a shaking bath at 30°C. After varying periods (15–120 min), duplicate reactions were terminated by the addition of 0.05 ml ice cold 10 M $HClO_4$ and the precipitated protein removed by centrifugation. Excess perchlorate was neutralized with KOH and the precipitate removed by centrifugation. A portion of the neutralized supernatant was mixed with $HClO_4$ acid to a final concentration of 2 M and heated to 95°C for 1 h to cleave all purine derivatives to their component bases and all purine pathway intermediates, up to and including CAIR, to yield glycine (15). Excess perchlorate was removed as above.

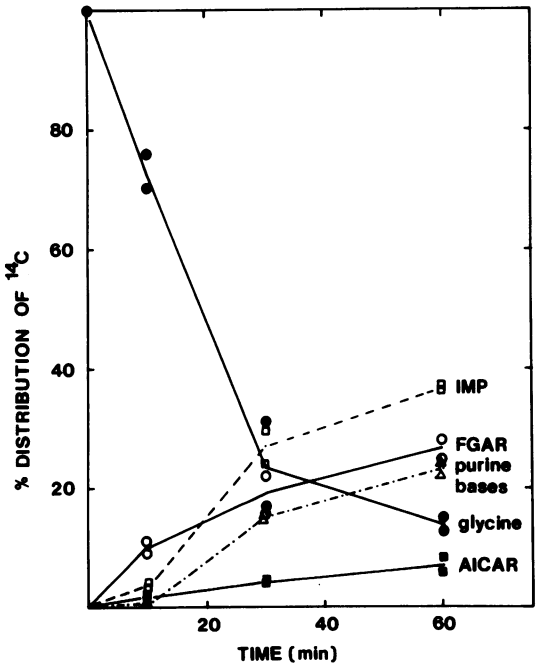

FIG. 1. Distribution of ^{14}C with time among products and intermediates of *de novo* purine synthesis from [1-^{14}C]glycine metabolized by extracts of cowpea nodules. Nodule homogenate was passed through a French press to disrupt particulate components. These assays contained both the soluble and particulate components of the nodule, which in other experiments, were separated and assayed separately. The individual labeling of AICAR and IMP was determined following acid hydrolysis of a mixture of the parent compounds to their corresponding bases. Purine bases were principally hypoxanthine with small amounts of xanthine. The duplicate data points are values from two separate experiments.

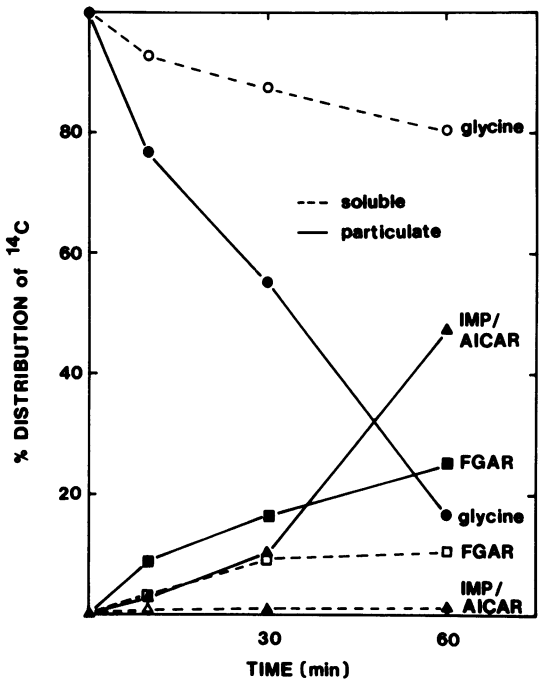

FIG. 2. Distribution of ^{14}C with time among products and intermediates of *de novo* purine synthesis from [1-^{14}C]glycine metabolized by the soluble (open symbols) or particulate (closed symbols) components of extracts of soybean nodules. Particulate components were disrupted by passage through a French press. IMP/AICAR was a single chromatographic peak containing both compounds. The results are taken from a single, typical experiment.

Table I. *Metabolism of [8-^{14}C]IMP by Extracts of Cowpea Nodules*

Nodules were fractionated into soluble and particulate components and the latter disrupted by passage through a French press. The same volumes of each fraction as assayed singly were mixed and assayed together. In all cases, the reactions were incubated for 30 min. The results are from a single, typical experiment.

Fraction	^{14}C Recovered as			
	IMP	Inosine	Hypoxanthine	Xanthine
			%	
Soluble	31	3	57	9
Particulate	81	16	3	ND[a]
Soluble + Particulate	32	7	47	13

[a] Not detectable in the assay.

Labeling of purine pathway intermediates was also assessed in the same reaction mixture as above but containing unlabeled glycine and $KH^{14}CO_3$ (1 mM, 1 μCi ^{14}C) or with formate and methenyl-THF replaced with [3-^{14}C]serine (0.1 mM, 1 μCi ^{14}C), THF (1.5 mM), and NADP (1.5 mM).

Other Assays. Metabolism of [8-^{14}C]IMP (1 mM, 1 μCi) to purine bases was measured in the same reaction mixtures used for *de novo* purine assays. Alanine dehydrogenase (EC 1.4.1.1) activity was measured according to Stripf and Werner (18), hydroxybutyrate dehydrogenase (EC 1.1.1.30) according to Hanks *et al.* (7), protein, after precipitation with TCA, by the method of Lowry *et al.* (11), and ureides in xylem sap as the phenylhydrazone of glyoxylate (8). Measurement of total nitrogen by Kjeldahl analysis was used to estimate nitrogen fixation.

Chromatographic Analysis of ^{14}C-Labeled Products. Nucleo-

tides and pathway intermediates were separated by paired-ion reverse-phase HPLC using a C_{18} Micropak column (MCH-10, 300 × 4 mm; Varian) equilibrated with an aqueous phase containing 5 mM tetrabutyl ammonium hydroxide (adjusted to pH 6.5 with phosphoric acid) and eluted with a linear increase in methanol to 55% over 45 min at 1.0 ml/min. The following retention times (min) were reproducibly obtained: glycine (3.0), hypoxanthine (8.5), FGAR (12.0), IMP (14.8), AICAR (15.0), AMP (20.0), XMP (20.2), ADP (25.0), and ATP (28.0). IMP and AICAR were not sufficiently resolved, and their separate analysis required acid hydrolysis and separation of the resultant bases as described below.

Bases and nucleosides were separated using ion-suppression reverse-phase HPLC with a C_{18} Micropak column (MCH-10, 300 × 4 mm; Varian) equilibrated with 20 mM (pH 7.5) ammonium phosphate buffer and eluted with a linear increase in methanol to 35% after 23 min at a flow rate of 1.0 ml/min. The following reproducible retention times (min) were observed: glycine (1.6), IMP and XMP (2.0), AMP (3.2), xanthosine (4.0), xanthine (8.2), hypoxanthine (10.9), guanine (11.5), inosine (12.5), guanosine (13.5), adenosine (19.7), and adenine (21.0). The base derived from acid hydrolysis of AICAR eluted at 8.5 min.

The effluent from the HPLC columns was collected at 1-min intervals, and ^{14}C in the fractions was determined by liquid scintillation spectrometry with quench correction by the channel's ratio method.

Labeled amino acids were separated from purines and purine pathway intermediates with an amino acid analyzer operated in the 'physiological fluids' mode with lithium buffers and assayed in the column effluent with a flow-through scintillation spectrometer (Coruflow, Tracerlab). The efficiency of the flow cell was determined using ^{14}C-labeled standards of glycine and serine.

RESULTS AND DISCUSSION

Nodule extracts, passed through a French press, and thus including proteins of both the soluble and particulate components of the tissue, readily metabolized [1-^{14}C]glycine to form the intermediates of purine synthesis, FGAR and AICAR, the initial purine nucleotide of the pathway, IMP, and purine bases, principally hypoxanthine (Fig. 1). The duplicate data points shown in Figure 1 are values from two separate experiments. The kinetic labeling pattern indicated a possible order of ^{14}C transfer of glycine to FGAR, AICAR, IMP, and purine bases (Fig. 1) consistent with the position of these intermediates in the pathway of *de novo* purine synthesis found in animal tissues (6, 17). The disappearance of labeled glycine with time was accounted for by the ^{14}C content of the recovered compounds (Fig. 1) indicating negligible metabolism of the carboxyl carbon of glycine by reactions outside the purine pathway. Accumulation of FGAR and AICAR indicated rate-limiting steps at the levels of FGAR amidotransferase (EC 6.3.5.3.) and AICAR transformylase (EC 2.1.2.2). Further metabolism of hypoxanthine to ureides would have required the addition of NAD (2).

Identification of FGAR was based on the chromatographic mobility of authentic [^{14}C]FGAR with similar ion-pair reverse-phase separations used in a previous study (17) and the quantitative recovery of ^{14}C as glycine following acid hydrolysis of the FGAR peak from the HPLC. IMP and AICAR were likewise identified by their co-chromatography with authentic compounds and with their basic products following acid hydrolysis. The product of IMP hydrolysis, hypoxanthine, was identified using both ion pair and ion suppression HPLC.

Fractionating nodule extracts into soluble and particulate components showed that most of the purine synthesis activity was associated with the particulate fraction. Figure 2 shows the products of [1-^{14}C]glycine metabolism for the two fractions from soybean nodules, but similar results were obtained for cowpea (Fig. 3). IMP was formed as one of the end products in this case (Fig. 2), with further metabolism of the nucleotide to form purine bases, a property of the soluble fraction (Table I). While some breakdown of IMP to inosine was found using the particulate preparation, negligible hypoxanthine was formed in the absence of the soluble extract (Table I). A previous study, using desalted prepa-

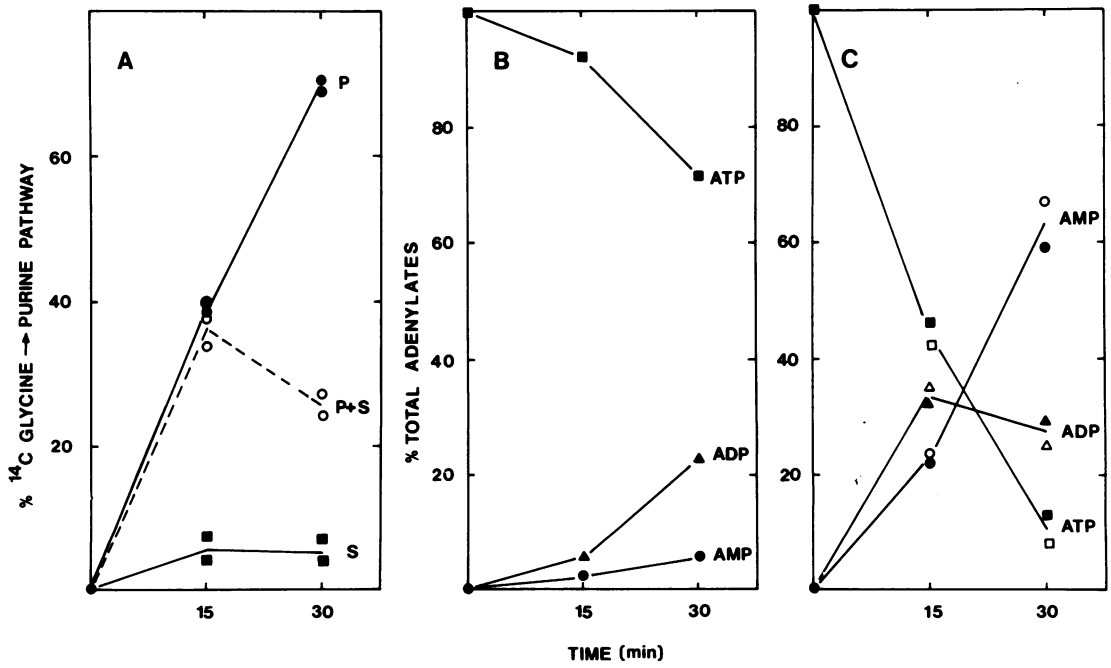

FIG. 3. A, Metabolism of [1-^{14}C]glycine to purine pathway products and intermediates with time using soluble (S), particulate (P), and a mixture of the two (P + S) components from cowpea nodule extracts. The particulate fraction was disrupted by passage through a French press. The duplicate data points are values from two separate experiments. B, Distribution of adenylates during incubation of particulate fractions as in A. C, Distribution of adenylates during incubation of soluble fractions (closed symbols) or a mixture of particulate and soluble fractions (open symbols) as in A.

Table II. *Release of Protein, Alanine Dehydrogenase Activity, Hydroxybutyrate Dehydrogenase Activity, and Enzymes of De Novo Purine Synthesis from the Particulate Fraction of Cowpea Nodule Extracts by Osmotic Shock*

The particulate components were isolated in a buffer containing 0.25 M sucrose, collected by centrifugation, and resuspended in buffer without sucrose. This buffer wash was collected by centrifugation, and the residual particulate material was resuspended in buffer with 0.25 M sucrose and disrupted by passage through a French press. All assays are values from an experiment using the same nodule extract.

Assay	Fraction	
	Released from particulate	Residual particulate
	mg/ml	
Protein	0.16 (7.1)[a]	2.10
	μmol/h·g fresh wt	
Alanine dehydrogenase	0.2 (3.2)	6.0
Hydroxybutyrate dehydrogenase	1.3 (4.6)	26.7
	nmol/h·g fresh wt	
Purine synthesis	54.3 (50.8)	52.5

[a] Values in parentheses are percentage of total.

rations of cowpea nodules concluded that ureide synthesis from purine nucleotides was located in the plant cytosol fraction of the central, infected tissue of the nodule (2) and that xanthine dehydrogenase (EC 1.2.3.2), uricase (EC 1.7.3.3), and allantoinase (EC 3.5.2.5) activities were present in this fraction (5).

Despite the inclusion of an ATP-regenerating reaction (PEP/pyruvate kinase) in all assays, ATP was readily hydrolyzed to ADP and AMP with time. Addition of adenylate kinase, other readily hydrolyzed phosphate esters (10 mM glucose 6-P), or the use of creatine phosphate/creatine phosphokinase were all ineffective in reducing ATP loss. Hydrolysis was most marked with assays containing the soluble fraction (Fig. 3C) in which little or no ATP remained at 30 min with 65% (3.25 mM) converted to AMP. By contrast, less than 20% ATP hydrolysis occurred after 30 min assays with the particulate fraction with only 6% (0.3 mM) as AMP. Because of the considerable ATP requirement for purine synthesis (3, 6) as well as the possible inhibition by AMP of steps early in the pathway (17), hydrolysis of ATP may have resulted in reduced glycine incorporation, and so assays of the soluble fraction markedly underestimated the enzymic activity. A mixture of the same volumes of the particulate and soluble fractions as assayed separately showed, however, that despite ATP hydrolysis within the first 15 min, a considerably higher rate of metabolism was possible in these assays if in fact the enzymes had been present in the soluble fraction (Fig. 3A). After a long period of time, glycine metabolism was inhibited, presumably because of the unfavorable supply of ATP or possibly PRPP to the component reactions. Thus, the relatively low rates of purine synthesis, consistently found in using soluble fractions from both cowpea (Fig. 3A) and soybean (Fig. 2) nodules, during the initial 15 min of reaction at least, probably reflected the level of pathway activity present in these fractions.

The soluble activity, which was between 14 and 28% of that found associated with the particulate components, could have been derived from the plant cytosol or from some breakage of the particulate components at the initial tissue disruption. Subjecting the particulate fraction to osmotic shock by resuspension in a medium without sucrose prior to passage through the French press released considerable purine synthetic activity (Table II), indicating the fragility of some membrane-bound component of the

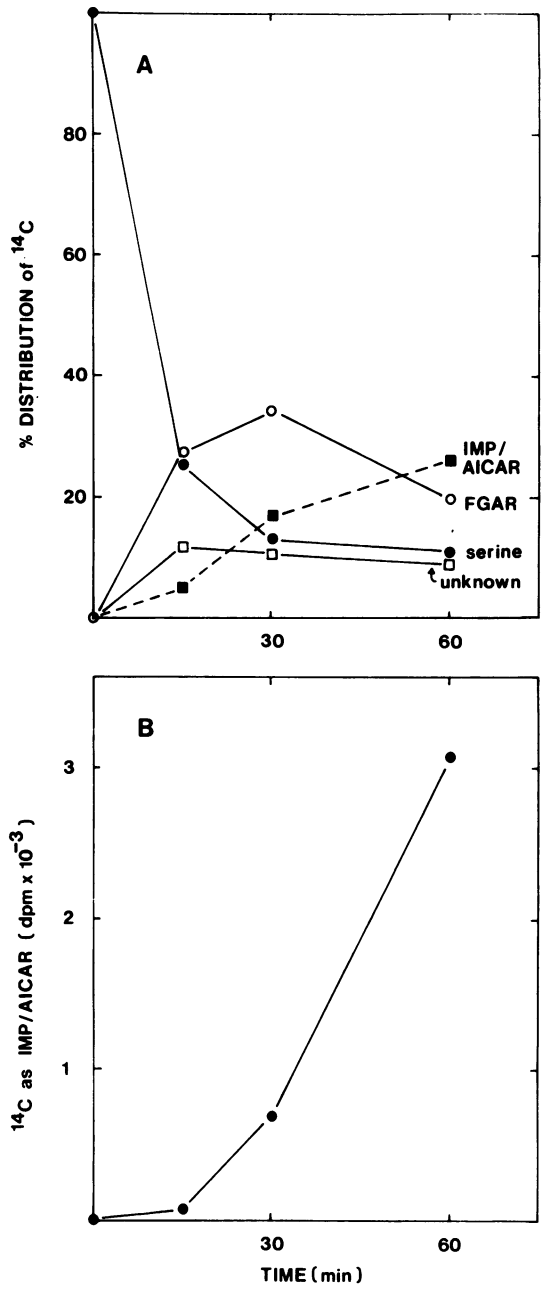

FIG. 4. A, Distribution of ^{14}C with time among products and intermediates of *de novo* purine synthesis from [3-^{14}C]serine metabolized by the particulate components of extracts of cowpea nodules. Particulate components were disrupted by passage through a French press. The data shown are from a single, typical experiment. B, Labeling of IMP/AICAR with time from $H^{14}CO_3$ in assays of *de novo* purine synthesis by the particulate components of extracts of cowpea nodules disrupted by passage through a French press. IMP/AICAR was a single chromatographic peak containing both compounds. The data shown are mean values from two separate experiments.

fraction which enclosed the purine enzymes. This suggests that the soluble activity was at least in part due to contamination with enzymes released from the particulate components of the nodule. Particulate fractions prepared in this manner contained marker enzymes for proplastids, mitochondria, and microbodies as well as for bacteroids (5), and preliminary electron microscopic examination has shown a large amount of vesiculated membrane material also present. Interestingly, more than half of the purine

Table III. *Substrates for the Amidotransferase Steps of De Novo Purine Synthesis in Extracts of Cowpea and Nodules*

Nodules were fractionated into soluble and particulate components and the latter disrupted by passage through a French press. *De novo* purine synthesis was assayed by [1-^{14}C]glycine metabolism for 30 min. The data are from a single, typical experiment.

Amide Donor Substrate	Fraction	^{14}C Recovered as		
		Glycine	FGAR	IMP + AICAR
Glutamine, 10 mm	Soluble	86.4	10.4	2.1
	Particulate	5.8	18.0	67.7
NH_4Cl, 40 mm	Soluble	89.4	7.4	1.7
	Particulate	9.9	81.8	8.6
Asparagine, 10 mm	Soluble	89.2	5.3	3.8
	Particulate	94.8	5.2	ND[a]

[a] Not detectable in the assay.

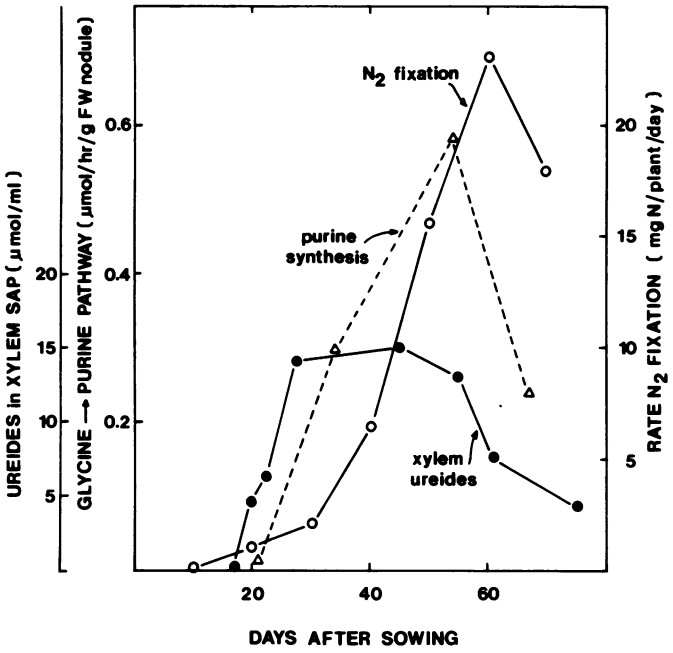

FIG. 5. Changes in rate of nitrogen fixation, concentration of ureides in root bleeding xylem sap, and in the specific activity of *de novo* purine synthesis of nodule extracts of cowpea plants during growth. Measurements of nitrogen fixation and xylem ureide content are mean values of duplicate assays from a bulked sample of twenty plants. Assays of purine synthesis are mean values of duplicate assays from single nodule extracts at each sampling time and are the conversion of glycine to purine nucleotide plus purine pathway intermediates.

synthesis but only 7.1% of the total protein and 3.2% and 4.6% of the activity of the two 'bacteroid-marker' enzymes (19), alanine dehydrogenase and hydroxybutyrate dehydrogenase, were released. Although this may indicate that these dehydrogenases and the purine pathway enzymes were not released from the same compartment, it does not preclude the existence of the pathway in bacteroids. Subpopulations of bacteroids have been recognized (7, 21), and these could contain different complements of enzymic activities and show differential sensitivity of osmotic shock (20).

The end products of purine synthesis by the particulate fraction (IMP + AICAR) were also readily labeled using [3-^{14}C]serine (Fig. 4A). Methenyl-THF was replaced by THF, and NADP was added so that metabolism of the 3-carbon atom of serine would yield ^{14}C-labeled 'activated C_1' units via serine hydroxymethyl transferase (EC 2.1.2.1) and methylene tetrahydrofolate dehydrogenase (EC 1.5.1.5). ^{14}C-Glycine was isolated from similar assays employing [U-^{14}C]serine, supporting the involvement of serine hydroxymethyl transferase and providing a suitable mechanism for the supply of both C_1 units and glycine in purine synthesis. The pH of the reaction mixture (7.5) would have caused rapid non-enzymic conversion of added methenyl-THF to 10-formyl-THF (16). Thus, if the methenyl derivative was used by either or both of the transformylation reactions of purine synthesis (6), the extracts would have required an active cyclohydrolase (5,10-methenyl-THF 5-hydrolase; EC 3.5.4.9) to ensure interconversion of the 10-formyl and 5,10-methenyl derivatives (16). A requirement for folates was demonstrated by assaying purine synthesis from [1-^{14}C]glycine without added methenyl-THF. Although inhibition of glycine incorporation into purines was not complete, possibly due to the presence of significant reduced folate bound to the partially purified proteins (17), IMP formation was reduced to less than 20% of controls.

IMP and AICAR were also readily labeled using $H^{14}CO_3$ and the particulate fraction (Fig. 4B). Labeled FGAR was however, not detected, consistent with a carboxylation reaction distal to steps incorporating carbon from glycine and 'activated C_1' in the purine ring. The probable site of HCO_3^- incorporation was the carboxylation of AIR to form CAIR, as in avian liver (6, 17).

Glutamine and ammonia were equally effective in supporting glycine incorporation by the purine pathway in particulate fractions from nodules (Table III). While glutamine provided a high rate of [^{14}C]IMP accumulation with ammonia, a relatively low rate of IMP synthesis resulted and most of the labeled glycine was recovered as FGAR (Table III). Presumably, under the assay conditions used, the first amidotransferase of the purine pathway (PRPP:amidotransferase) but not the second (FGAR: amidotransferase), was able to utilize glutamine and ammonia equally. The very low level of metabolism with asparagine as amide donor (Table III) indicated that this amide was not readily utilized in purine synthesis in nodules, although in some plant tissues asparagine has proved a superior substrate to glutamine for PRPP:amidotransferase (10).

The specific activity of the *de novo* purine pathway in the particulate fractions of extracts from the primary root nodules of cowpea increased with nodule development roughly parallel to increasing rates of nitrogen fixation (Fig. 5). While the peak levels of xylem-borne ureides occurred at a time when rates of purine synthesis were relatively low (Fig. 5), the total amount of ureide nitrogen formed and exported at this time was also low, and presumably did not increase markedly until 30 d or more when rates of nitrogen fixation increased markedly. By this time, nodules exhibited high rates of purine synthesis. The maximum rate of purine synthesis found in the particulate fraction of cowpea nodules was approximately 0.6 μmol/h·g fresh nodule tissue (Fig. 5) or equivalent to 1.2 μmol N_2/h·g. These rates were similar to those of ureide production by comparable nodules during the period of maximum nitrogen fixation (equivalent to 1–2 μmol N_2/h·g fresh nodule tissue) in cowpea plants (2). Nodules of *Lupinus albus*, which export asparagine as the major product of nitrogen fixation (4), do not form ureides and show negligible activity for purine oxidation (R. M. Rainbird and C. A. Atkins, unpublished results). Extracts of nodules, harvested during the period of maximum nitrogen fixation and prepared in the same way as those from cowpea or soybean, showed markedly lower rates of *de novo* purine synthesis. The activity of the soluble fraction from lupin was 1.1 nmol/h·g fresh nodule tissue and 5.8 nmol/h·g for the particulate fraction; that is, at rates 0.1% to 0.5% those found in the 'ureide-forming' nodules of cowpea and soybean.

Recovery of a complete pathway of purine nucleotide synthesis which utilized nitrogenous substrates likely to be readily formed

in nodules as products of nitrogenase activity (ammonia, glutamine, glycine, serine, and aspartate), coupled with the capacity of the plant cell cytosol fraction to metabolize IMP and XMP to form allantoin (2, 23, 24), strongly indicates that purine synthesis followed by oxidation is the mechanism for high rates of ureide formation in cowpea and soybean nodules. A clear understanding of the relationship between purine synthesis and nitrogen fixation will require a closer definition of the cellular location of the metabolism described in this report and of the nature of the nitrogenous substrates utilized, especially for the amidotransferase steps. Although $^{15}N_2$ reduction by intact nodules to form labeled ureide (8, 12) has demonstrated that currently assimilated nitrogen is used in the synthesis of these molecules, implication of the purine pathway in their formation, though compelling, remains circumstantial. More direct proof will require ^{15}N or ^{13}N labeling of purine pathway intermediates using molecular nitrogen.

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LITERATURE CITED

1. ATKINS CA 1974 Occurrence and some properties of carbonic anhydrase from legume root nodules. Phytochemistry 13: 93–98
2. ATKINS CA 1981 Metabolism of purine nucleotides to form ureides in nitrogen-fixing nodules of cowpea (*Vigna unguiculata* L. Walp). FEBS Lett 125: 89–93
3. ATKINS CA, DF HERRIDGE, JS PATE 1978 The economy of carbon and nitrogen in nitrogen-fixing annual legumes. *In* CN Welsh, ed, Isotopes in Biological Dinitrogen Fixation. International Atomic Energy Agency, Vienna, pp 211–242
4. ATKINS CA, JS PATE, PJ SHARKEY 1975 Asparagine metabolism—key to the nitrogen nutrition of developing legume seeds. Plant Physiol 56: 807–812
5. ATKINS CA, RM RAINBIRD, JS PATE 1980 Evidence for a purine pathway of ureide synthesis in N_2-fixing nodules of cowpea (*Vigna unguiculata* L. Walp). Z Pflanzenphysiol 97: 249–260
6. FLAKS JG, LN LUKENS 1963 The enzymes of purine nucleotide synthesis *de novo*. Methods Enzymol 6: 52–95
7. HANKS JF, NE TOLBERT, KR SCHUBERT 1981 Localization of enzymes of ureide biosynthesis in peroxisomes and microsomes of nodules. Plant Physiol 68: 65–69
8. HERRIDGE DF, CA ATKINS, JS PATE, RM RAINBIRD 1978 Allantoin and allantoic acid in the nitrogen economy of the cowpea (*Vigna unguiculata* L. Walp). Plant Physiol 62: 495–498
9. HOCKING PJ, JS PATE 1978 Accumulation and distribution of mineral elements in the annual lupins, *Lupinus albus* L. and *Lupinus angustifolius* L. Aust J Agric Res 29: 267–280
10. KAPOOR M, ER WAYGOOD 1962 Initial steps of purine biosynthesis in wheat germ. Biochem Biophys Res Commun 9: 7–10
11. LOWRY OH, NJ ROSEBROUGH, AL FARR, RJ RANDALL 1951 Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275
12. MATSUMOTO T, M YATAZAWA, Y YAMAMOTO 1977 Incorporation of ^{15}N into allantoin in nodulated soybean plants supplied with $^{15}N_2$. Plant Cell Physiol 18: 459–462
13. MOLLOY GR, TO SITZ, RR SCHMIDT 1973 Evidence for continuous availability of the phosphoribosyl glycinamide synthetase structural gene for transcription during the cell cycle of the eucaryote *Chlorella*. J Biol Chem 248: 1970–1975
14. PATE JS, CA ATKINS ST WHITE, RM RAINBIRD, KC WOO 1980 Nitrogen nutrition and xylem transport of nitrogen in ureide-producing grain legumes. Plant Physiol 65: 961–965
15. RAO KP, DG BLEVINS, DD RANDALL 1980 Glutamine-phosphoribosyl pyrophosphate amidotransferase from soybean nodules. Plant Physiol 65: S–110
16. ROWE PB, GP LEWIS 1973 Mammalian folate metabolism. Regulation of folate interconversion enzymes. Biochemistry 12: 1962–1979
17. ROWE PB, E MCCAIRNS, G MADSEN, D SAUER, H ELLIOTT 1978 *De novo* purine synthesis in avian liver. Co-purification of the enzymes and properties of the pathway. J Biol Chem 253: 7711–7721
18. SCHUBERT KR 1981 Relationship between *de novo* synthesis of purines and the export of ureides in soybeans. Plant Physiol 57: S–70
19. STRIPF R, D WERNER 1978 Differentiation of *Rhizobium japonicum*. II. Enzymatic activities in bacteroids and plant cytoplasm during the development of nodules of *Glycine max*. Z Naturforsch 33: 373–381
20. SUTTON WD, NM JEPSEN, BD SHAW 1977 Changes in the number, viability, and amino-acid-incorporating activity of *Rhizobium* bacteroids during lupin nodule development. Plant Physiol 59: 741–744
21. SUTTON WD, P MAHONEY 1977 Preparation and fractionation of *Rhizobium* bacteroids by zone sedimentation through sucrose gradients. Plant Physiol 60: 800–802
22. TATUM CM, PA BENKOVIC, SJ BENKOVIC, R POTTS, E SCHLEICHER, E FLOSS 1977 Stereochemistry of methylene transfer involving 5,10-methylenetetrahydrofolate. Biochemistry 16: 1093–1102
23. TRIPLETT EW, DG BLEVINS, DD RANDAL L 1980 Allantoic acid synthesis in soybean root nodule cytosol via xanthine dehydrogenase. Plant Physiol 65: 1203–1206
24. WOO, KC, CA ATKINS, JS PATE 1980 Biosynthesis of ureides from purines in a cell-free system from nodule extracts of cowpea (*Vigna unguiculata* L. Walp.). Plant Physiol 66: 735–739