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Ureide Catabolism in Soybeans[1]

III. UREIDOGLYCOLATE AMIDOHYDROLASE AND ALLANTOATE AMIDOHYDROLASE ARE ACTIVITIES OF AN ALLANTOATE DEGRADING ENZYME COMPLEX

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ABSTRACT

We demonstrate that allantoate is catabolized in soybean seedcoat extracts by an enzyme complex that has allantoate amidohydrolase and ureidoglycolate amidohydrolase activities. Soybean seedcoat extracts released $^{14}CO_2$ from [ureido-^{14}C]ureidoglycolate under conditions in which urease is not detectable. CO_2 and glyoxylate are enzymically released in a one to one ratio indicating that ureidoglycolate amidohydrolase is the responsible activity. Ureidoglycolate amidohydrolase has a K_m of 85 micromolar for ureidoglycolate. Glyoxylate and CO_2 are enzymically released from allantoate at linear rates in a one to 2.3 ratio from 5 to 30 min. This ratio is consistent with the degradation of allantoate to two CO_2 and one glyoxylate with approximately 23% of the allantoate degraded reacting with 2-mercaptoethanol to yield 2-hydroxyethylthio, 2′-ureido, acetate (RG Winkler, JC Polacco, DG Blevins, DD Randall 1985 Plant Physiol 79: 787–793). That [^{14}C]urea production from [2,7-^{14}C]allantoate is not detectable indicates that allantoate-dependent glyoxylate production is enzymic and not a result of nonenzymic hydrolysis of a ureido intermediate (nonenzymic hydrolysis releases urea). These results and those from intact tissue studies (RG Winkler DG Blevins, JC Polacco, DD Randall 1987 Plant Physiol 83: 585–591) suggest that soybeans have a second amidohydrolase reaction (ureidoglycolate amidohydrolase) that follows allantoate amidohydrolase in allantoate catabolism. The rate of $^{14}CO_2$ release from [2,7-^{14}C]allantoate is not reduced when the volume of the reaction mixture is increased, suggesting that the release of $^{14}CO_2$ is not dependent on the accumulation of free intermediates. That [2,7-^{14}C]allantoate dependent $^{14}CO_2$ release is not proportionally diluted by unlabeled ureidoglycolate indicates that the reaction is carried out by an enzyme complex. This is the first report of ureidoglycolate amidohydrolase activity in any organism and the first *in vitro* demonstration in plants that the ureido-carbons of allantoate can be completely degraded to CO_2 without a urea intermediate.

The ureides[3], ALN[4] and ALL, are important nitrogen (N) transport and storage compounds in a number of plant species. Fixed N is transported as ureides in many legumes of tropical or subtropical origin, *e.g.* soybean, and account for the majority of the N transported in the xylem sap to the aerial portions of soybeans under N_2-fixing conditions (5). Thus, the majority of N in a soybean plant is made available by the catabolism of ALN and ALL.

The pathway of ALL catabolism has yet to be resolved fully. Previously it had generally been assumed that the subsequent release of N is entirely dependent on the urease-dependent hydrolysis of urea released from ALL (6, 7, 9). This would be consistent with allantoate amidinohydrolase (15) (EC 3.5.3.4.), which releases equimolar urea and ureidoglycolate and the subsequent metabolism of ureidoglycolate to urea and glyoxylate. Although there are several reports (1, 7, 9) of *in vivo* urea formation, the data presented are insufficient to establish this pathway. The reports of urea formation can be criticized because corrections for nonenzymic degradation of ALL (which is labile) were not made: (a) the urea found could have been a product of aphid metabolism; (b) urea could have been released nonenzymically from ALL by acid hydrolysis during the isolation procedure; (c) urea could have been released nonenzymically from ALL by the extraction procedure (boiling 80% ethanol); (d) the unlabeled urea reported could have resulted from sources other than ALN, such as arginine.

There are a number of papers (discussed in Ref. 12) that report an *in vitro* ALL degrading activity that is assumed to be allantoate amidinohydrolase; however, in all cases the products are insufficiently characterized to eliminate alternative interpretations. It is essential to quantitatively characterize the production of urea, relative to urease-independent NH_3 or CO_2 production to differentiate between the possible ALL degrading activities.

We have presented evidence that ALL is degraded by ALAH (14, 15) (EC 3.5.3.9) in soybean seedcoat extracts (18): urea was shown not to be the initial product of the reaction. Further 2-hydroxyethylthio, 2′-ureido, acetate was identified as an apparent nonenzymic reaction product between an enzymically generated ureido intermediate and βME. The subsequent metabolism of ALL was not defined in that report.

The catabolism of ALN was studied in intact leaf tissue to overcome the difficulties of labile and reactive intermediates. [2,7-^{14}C]Allantoin-dependent (labeled urea carbons) $^{14}CO_2$ release was not inhibited by the potent urease inhibitor, PPD (11), and no [^{14}C]urea could be recovered from the tissue indicating that urea was not a direct product of ALL or a product of the subsequent metabolism of its intermediates. [4,5-^{14}C]Allantoin (labeled acetate carbons) yielded [^{14}C]glyoxylate, $^{14}CO_2$, [^{14}C]glycine, and [^{14}C]serine. These results are consistent with ALAH and a second amidohydrolase, UGAH reaction that releases glyoxylate, NH_3 and CO_2 from a ureido intermediate, with the subsequent metabolism of glyoxylate through the photores-

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[3] Ureides refer only to allantoin and allantoate.

[4] Abbreviations: ALN, allantoin; ALL, allantoate; PPD, phenylphosphordiamidate; ALAH, allantoate amidohydrolase; UG, ureidoglycolate; UGAH, ureidoglycolate amidohydrolase, βME, 2-mercaptoethanol.

piratory pathway. The nature of the intermediates and their catabolism is not known. Ureidoglycine and UG would be the expected intermediates if each N was removed sequentially from ALL (Fig. 1).

Very little has been published on either UG or ureidoglycine catabolism, in part because UG is relatively unstable and there are no published reports on ureidoglycine synthesis. Wu *et al.* (19) reported that ureidoglycine accumulated in a mutant of *Pseudomonas acidovorans* that was unable to utilize ureidoglycine. Their conclusions were based on comparisons to synthesized ureidoglycine, but no details of the synthesis or identification of ureidoglycine were reported.

Two activities have been identified that degrade UG (15): ureidoglycolate urea-lyase (EC 4.3.2.3), which releases glyoxylate and urea from UG, and ureidoglycolate dehydrogenase which oxidizes ureidoglycolate to oxalurate (Fig. 2). Oxalurate is known to be catabolized to oxamate. Neither activity described previously would be consistent with our findings in intact soybean leaf tissue. However, a novel activity, UGAH would be consistent.

This paper focuses on the identification of ureidoglycolate amidohydrolase and its role in ALL catabolism.

MATERIALS AND METHODS

Plant Material. Nodulated plants of soybean (*Glycine max* L. Merr. cv Williams) were greenhouse grown with available light or field grown. Seed coats were harvested from developing beans at midpod fill and put directly into liquid N_2, and stored at −40°C.

Chemicals and Radiolabeled Compounds. Potassium allantoate was prepared by the method of Hermanowicz (2) and recrystalized twice from ethanol. [2,7-^{14}C]Allantoate was synthesized by the procedure of Winkler *et al.* (patent application in process).

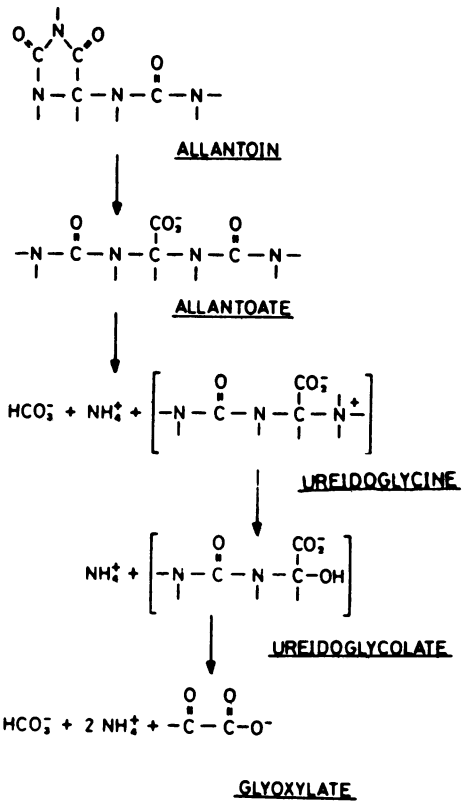

FIG. 1. Proposed pathway of allantoate catabolism. Pathway of allantoate catabolism in soybeans based on (17, 18) and this report: allantoinase, ALAH, ureidoglycine aminohydrolase, and UGAH.

Purity was ascertained by HPLC analysis (17). The effluent was monitored by UV detection at 210 nm and analysis of collected fractions for urea and glyoxylate. Purity was also ascertained on TLC-silica plates with *n*-butanol, acetic acid, and H_2O (12:3:5).

Potassium ureidoglycolate was synthesized by a procedure modified from Valentine and Wolfe (13). Glyoxylic acid, 10.0 g, was added to 5 ml water at 4°C and 6.0 g solid KOH was added. The pH was adjusted to 7.0. Urea, 14 g, was added with stirring and the reaction was incubated at room temperature. The reaction was monitored by ion exclusion HPLC (*i.e.* glyoxylate loss and UG synthesis). After the reaction reached equilibrium (2–3 h), 9 volumes of 95% ethanol were added with stirring. The precipitate was collected on filter paper, and redissolved in 100 ml H_2O and recrystallized with 9 volumes ethanol. The resulting precipitate was collected, washed with ethanol, and dried under vacuum. The dried salt was aliquoted and stored at −20°C in a desiccator. [Ureido-^{14}C]UG was synthesized similarly except that 1 mCi [^{14}C]urea was included per 1.5 g unlabeled urea. HPLC organic acid and organic base columns and ^{13}C-NMR were utilized to determine the purity of UG.

PPD was purchased from ICN (Plainview, NY). All other chemicals were purchased from Sigma, unless otherwise stated.

Preparation of Enzyme Extract. Seedcoats were homogenized with a Polytron in 10 volumes of cold 50 to 100 mM Tris-H_2SO_4 (pH 8.8) containing 2 mM $MnSO_4$, and 14 mM βME. The crude homogenate was centrifuged at 14,000*g* for 30 min and subsequently filtered through Miracloth (VWR Scientific, St. Louis, MO).

Solid $(NH_4)_2SO_4$ was added to the crude extract to 45% of saturation and stirred for 45 min at 4°C. The extract was then centrifuged at 14,000*g* for 30 min and the pellet was redissolved in 1 volume of the extraction buffer and centrifuged at 14,000*g* for 30 min. The extract was desalted on Sephadex G-50 (the extraction buffer plus 250 mM Na_2SO_4 was used as mobile phase) and stored as 1 to 4 ml aliquots at −20°C. All analyses were performed within 2 weeks.

Assay of Enzymic Activity. Enzyme extract (50–500 μl) was added to one to four volumes of 50 to 100 mM Tris-H_2SO_4, 2 mM $MnSO_4$, 14 mM βME with ALL or UG as substrate. The buffer was degassed prior to βME addition. Extracts were preincubated at 4°C for 15 min with PPD (0.1–1 mM final concentration).

Chemical Analysis. $^{14}CO_2$ was quantitated similarly to Kerr *et al.* (4) by capturing CO_2 on a glass fiber filter impregnated with 150 μl of 20% saturated NaOH prepared fresh each day from a saturated solution. Reactions were terminated by the addition of 1 volume 1 N H_2SO_4. $^{14}CO_2$ was captured for 3 h. Samples were counted after 18 to 20 h and data were corrected for CO_2 capturing and counting efficiency.

Glyoxylate was analyzed by the methods of Vogels and Van der Drift (16), except that all volumes were halved. Sodium glyoxylate monohydrate was dried under vacuum and stored in a desiccator at −20°C. Standard curves were prepared from sodium glyoxylate monohydrate in the reaction buffer with enzyme extract. Urea was analyzed by quantitation of its diacetylmonoxime derivative (3).

Analysis of Neutral ^{14}C Products. Reaction mixture (75 μl) was applied to a 0.5 ml Dowex AG1-X8 resin (200–400 dry mesh, acetate form) in a 1 ml tuberculin syringe. Neutral products were eluted by washing the column with 500 μl 20 mM urea and then three times with 750 μl 20 mM urea. ^{14}C-Neutral products were quantitated by liquid scintillation spectrometry. All samples were quench corrected.

K_m Determination. Desalted seed coat extract (100 μl) was incubated with [ureido-^{14}C]ureidoglycolate at 30°C with a final reaction volume of 1.0 ml. Final concentrations of 0.0625, 0.125, 0.5, 1.0, 2.5, and 5.0 mM [ureido-^{14}C]ureidoglycolate were analyzed for $^{14}CO_2$ at 15, 30, and 45 min.

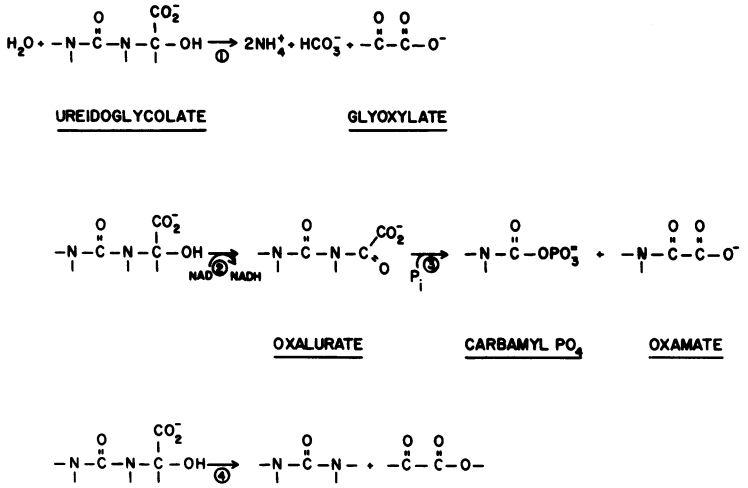

FIG. 2. Possible pathways of UG catabolism. 1) UGAH (this work and [17]); 2) UG dehydrogenase (15); 3) Carbamoylphosphate:oxamate carbamoyltransferase (15); 4) UG urea-lyase (15).

NMR Spectroscopy. ^{13}C-NMR spectroscopy was performed by Dr. W. Guo on a Nicolet NT300 spectrometer at 75.45 MHz. Dioxane (67.4 ppm) was used as an external standard. Samples were dissolved in D_2O and analyzed in a 5 mm probe.

RESULTS AND DISCUSSION

Purity of Synthesized Compounds. Preliminary experiments established that potassium ureidoglycolate was more readily crystallized by ethanol than sodium ureidoglycolate, which generally yielded a syrup upon addition of ethanol. This modified synthesis gave a 70% yield of UG after two recrystallizations with ethanol.

UG was determined to be highly pure by both HPLC and ^{13}C-NMR analysis. UG (30 μmol) was injected onto an HPLC organic base column, fractions were collected and analyzed for urea. Less than 0.12 μmol urea (0.4% of the injected UG) was detected in the urea fraction by this method. Other than UG, no other urea containing peaks were observed by this procedure. The acidic conditions of the urea assay should degrade any noncyclic ureides to urea. Less than 2% glyoxylate contamination was observed when UG was analyzed by the method of Vogels and Van der Drift (16). Less than 0.7% [^{14}C]urea contamination was observed in [ureido-^{14}C]UG by organic base HPLC separation with detection by scintillation spectroscopy. Unlabeled urea (45 μmol) was included to prevent possible adsorption of low levels of [^{14}C]urea. [^{14}C]Ureidoglycolate was recovered as 80% of the label injected, while 10% of the label was recovered as a nearly level background from 11 to 50 min. Fractions were not collected after 50 min, but the background label was still high at this time. Other than 0.7% [^{14}C]urea, no other labeled impurities were observed. The background represents the nonenzymic degradation of [ureido-^{14}C]UG to [^{14}C]urea and adsorption of urea to the column.

Ureidoglycolate is unstable under the acidic conditions of the organic acid column, but this analysis is useful to establish possible contamination by ALL. [ureido-^{14}C]UG (25 μmol) was injected on an organic acid column and fractions were collected for 50 min. No [2,7^{14}C]-ALL or ALN was detected by this analysis.

Although not sensitive enough to detect impurities at very low concentrations (unless long analyses are performed), ^{13}C-NMR is probably the most unbiased way to establish the minimum purity of UG. Carbons were detected at 74.509 ppm (C-2), 161.161 ppm (C-4), and 176.427 ppm (C-1), consistent with the structure of UG by comparison to ALL and chemical shift calculations. No carbons from impurities could be detected. The signal to noise ratio suggested that UG was at least 95% pure.

[2,7-^{14}C]Allantoate was greater than 99.5% pure by the HPLC and TLC analyses described. Allantoate synthesized by the method of Winkler *et al.* (unpublished data) co-migrated with ALL synthesized by the method of Hermanowicz (2) on the organic base and organic acid columns as well as TLC-silica plates.

Ureidoglycolate Degradation. In the presence of PPD, one of the most potent urease inhibitors known (4, 11), [ureido-^{14}C]UG was hydrolyzed to $^{14}CO_2$ by crude seed coat extracts at a rate of 1.7 nmol·min^{-1}·mg^{-1} protein (Fig. 3). Urease activity was less than 0.02 nmol·min^{-1}·mg^{-1} protein. Boiled enzyme controls had a rate of $^{14}CO_2$ release less than 0.1% that of the enzymic rate. Additionally, if 100 mM urea was included in the incubation mixture (no PPD included) the rate of $^{14}CO_2$ release was 1.9 nmol·min^{-1}·mg^{-1} protein. These results eliminate the possibility that $^{14}CO_2$ release is dependent on a urea intermediate and are consistent with catabolism by UGAH. An alternative explanation would be carbamoylphosphate:oxamate carbamoyltransferase (EC 2.1.3.-) activity (Fig. 2) (13, 15). This is unlikely as NAD would be required for this reaction and oxamate or oxalate would be the expected product instead of glyoxylate.

Analysis of Concentrated Fractions. Ureidoglycolate-dependent glyoxylate production was studied to distinguish between the two possible urease-independent CO_2 releasing reactions: UGAH should release glyoxylate; while the oxidative degradation of UG would release oxalurate, oxamate, or oxalate (Fig. 2). Because the rate of nonenzymic degradation to urea and glyoxylate is high (Fig. 4; [13]), it is necessary to use concentrated extracts to quantitate glyoxylate production. The enzymic activity was concentrated by $(NH_4)_2SO_4$ precipitation so that a minimal reaction mixture volume could be used. A rate of enzymic UG-dependent glyoxylate production of 6.8 nmol·min^{-1}·mg^{-1} protein was found and minus enzyme samples gave a rate of 2.9 nmol·min^{-1} on an equivalent basis (Fig. 4). Thus, the enzymic rate was 3.9 nmol·min^{-1}·mg^{-1} protein. In a parallel experiment $^{14}CO_2$ production was 3.9 nmol·min^{-1}·mg^{-1} protein, yielding the expected one to one ratio of glyoxylate to $^{14}CO_2$. These results are entirely consistent with UG amidohydrolase and are not consistent with other UG degrading activities. This is the first report of UGAH activity in any organism.

Desalted Seed Coat Extracts. Desalted 45% $(NH_4)_2SO_4$ pellets show urease-independent $^{14}CO_2$ release consistent with the results in crude extracts. Rates from 5.0 to 16 nmol $^{14}CO_2$·min^{-1}·mg^{-1} protein (V_{max}) were obtained from different seed coat stocks. As with crude extracts, addition of 100 mM urea (final concentration) did not inhibit $^{14}CO_2$ release.

When 100 μl desalted extract was incubated with 100 μl 1.0 mM [ureido-^{14}C]UG for 2 h, 26% of the added label was re-

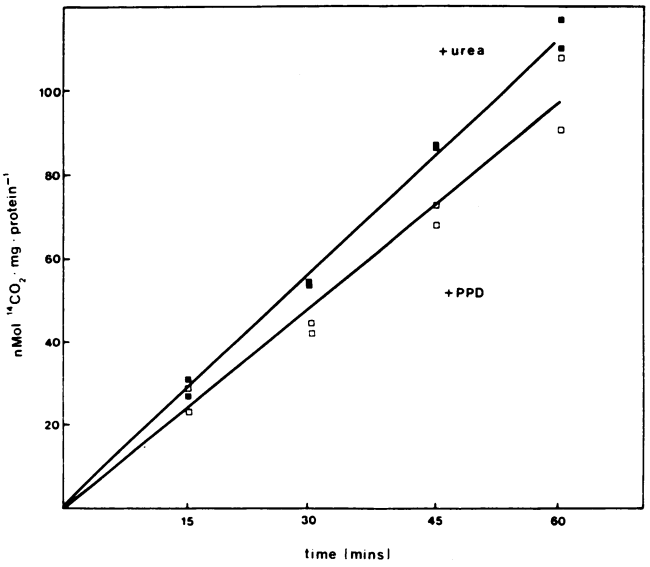

FIG. 3. [Ureido-^{14}C]UG dependent $^{14}CO_2$ release from crude extracts of seed coats. Crude extract, 0.5 ml, was incubated with 0.5 ml 20 mM [ureido-^{14}C]UG in 50 mM Tris (pH 8.8) buffer containing 2 mM $MnSO_4$ and 8 mM βME. Reactions were terminated and $^{14}CO_2$ captured at 15, 30, 45, and 60 min. In one set of samples the enzyme was preincubated with 0.5 mg·ml^{-1} PPD for 15 min and filtered through Miracloth prior to addition to the assay mixture, while urea was added to 100 mM (final concentration) in the other set.

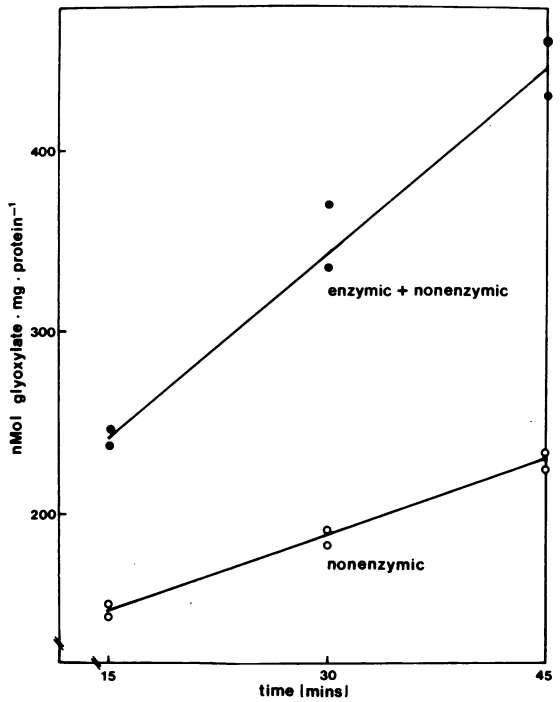

FIG. 4. Comparison of enzymic and nonenzymic rates of UG dependent glyoxylate production. UG-dependent glyoxylate production was measured at 15, 30, and 45 min for both plus and minus enzyme samples. A redissolved 0 to 45% $(NH_4)_2SO_4$ pellet from seed coats, 50 μl was incubated with 50 μl 10 mM UG in 100 mM (pH 8.8) Tris buffer containing 2 mM $MnSO_4$ and 14 mM βME. Analyses were in duplicate. The correlation coefficient was 0.99 for both lines. An analysis in parallel gave a rate of $^{14}CO_2$ release from [ureido-^{14}C]UG of 3.9 nmol·min^{-1}·mg^{-1} protein, with a correlation coefficient of 0.98.

covered as $^{14}CO_2$. Assuming that only one optical isomer of UG is hydrolyzed by soybean UGAL, then 52% of the added substrate (stereoisomer) was recovered as $^{14}CO_2$. These data eliminate the possibility that a minor contaminant could be the $^{14}CO_2$ releasing substrate.

K_m for Ureidoglycolate. A Lineweaver-Burk plot of $^{14}CO_2$ production catalyzed by a desalted extract of seed coats with respect to [ureido-^{14}C]UG concentration gave an apparent K_m of 170 μM for UG. We would expect that only one of the two stereoisomers of UG would be degraded, so the K_m is 85 μM for the optical isomer of UG that is metabolized in this pathway.

Analysis of Allantoate Degradation. Glyoxylate was released from ALL at linear rates (5–30 min) of 2.3 nmol·min^{-1}·mg^{-1} protein in desalted $(NH_4)_2SO_4$ precipitated seed coat extracts. Parallel incubations released $^{14}CO_2$ from [2,7-^{14}C]ALL at linear rates of 5.4 nmol·min^{-1}·mg^{-1} protein. PPD was included in both assays. Seedcoat extracts preincubated with PPD hydrolyzed urea at a rate less than 0.02 nmol·min^{-1}·mg^{-1} protein. These results are consistent with ALAH and UGAH action. The fact that $^{14}CO_2$ and glyoxylate are produced in a 2.3:1 ratio, instead of the expected 2:1 ratio is consistent with the accumulation of an intermediate. This is explained by the observation that some of the expected glyoxylate product is lost in the reaction of βME with an intermediate to produce 2-hydroxyethylthio, 2′-ureido, acetate (18). $^{14}CO_2$ and glyoxylate are produced in a constant ratio which is not consistent with the production of either being dependent on the accumulation of a free intermediate. In a previous study (18) we reported that CO_2 and pH 7.0 labile glyoxylate derivatives (ureidoglycine + UG; [16]) were released in approximately a 1:1 ratio. However, the stock glyoxylate solutions used in preparing standard curves were stored in pH 8.8 Tris buffer with 1.0 mM $MnSO_4$ prior to analysis. This caused a loss of glyoxylate (R Winkler, unpublished observations) in the standard and thus an overestimation of glyoxylate derivatives in the reported results. After correction for peak D formation (18) we find no significant difference in the rate of glyoxylate production and the rate of pH 7.0 labile glyoxylate derivative production.

Is There [2,7-^{14}C]Allantoate-dependent [^{14}C]Urea Production? [2,7-^{14}C]ALL-dependent [^{14}C]urea production was not detected in the presence of PPD (Table I). These results establish that glyoxylate is released directly from a ureido-intermediate by an amidohydrolase reaction. If glyoxylate were a product of nonenzymic degradation of ureidoglycine or UG then one equivalent of urea should be present for each equivalent of intermediate nonenzymically degraded. Further, if a labeled intermediate accumulated (*e.g.* UG), we would expect to observe an accumulation of urea from nonenzymic degradation of the intermediates.

Accumulation of Intermediates. That there is no detectable accumulation of [^{14}C] neutral products (Table I) strongly suggests that the hypothesized neutral intermediate, ureidoglycine, does not accumulate in the reaction mixture. Results from previous studies suggest that UG does not accumulate to detectable levels either in extracts or intact tissue (17, 18). The lack of intermediate accumulation may indicate that allantoate is degraded by an enzyme complex (reviewed by Srivastava and Bernhard, 10).

Is Allantoate Degraded by an Enzyme Complex? The rate of [2,7-^{14}C]ALL dependent $^{14}CO_2$ release was compared to the dilution of the enzyme (Table II). If $^{14}CO_2$ release were dependent on a free intermediate then the rate of $^{14}CO_2$ production should decrease by as much as 40 to 50% as the reaction (and therefore the intermediate) is diluted. $^{14}CO_2$ production (Table II) is slightly greater in the more dilute reaction and may indicate a slight activation of the enzyme. That there is no decrease in $^{14}CO_2$ release upon dilution as well as no apparent lag indicates that

Table I. *Analysis of ^{14}C-Neutral Products from [2,7-^{14}C]Allantoate*

Desalted $(NH_4)_2SO_4$ seed coat extract (preincubated with 0.1 mM PPD) was incubated in a buffered solution with (final concentration): 25 mM Tris-H_2SO_4, 2 mM $MnSO_4$, 14 mM βME, 5 mM urea, and 5 mM [2,7-^{14}C]All (specific activity of 1 mCi $mmol^{-1}$). The unlabeled urea did not affect $^{14}CO_2$ release and was included to prevent possible [^{14}C]urea loss. At 1 min seven aliquots of 75 μl of reaction mixture were applied to Dowex 1-X8 (acetate) columns and washed as in the "Materials and Methods" section. After 60 min, four aliquots were analyzed for $^{14}CO_2$ production and seven aliquots were again analyzed for ^{14}C-neutral components. Parallel samples incubated with [^{14}C]urea yielded 99.3% of the added [^{14}C]urea in the neutral fraction. All samples were quench corrected.

Product	[^{14}C]Product
	nmol · h^{-1} · aliquot^{-1}
$^{14}CO_2$	6.8 ± 0.2[a]
Neutral fraction	−0.1 ± 0.1

[a] ± SE.

Table II. *$^{14}CO_2$ Release verus Concentration of Extract*

Desalted $(NH_4)_2SO_4$-precipitated extract of soybean seed coats was incubated in a buffered solution containing 5 mM [2,7-^{14}C]ALL (final concentration). Two hundred μl of extract was used in all samples but the final reaction volume was varied from 400 μl to 1.0 ml. Samples were assayed for $^{14}CO_2$ in triplciate at 10, 20, and 30 min.

Reaction Volume	$^{14}CO_2$
μl	*nmol · min^{-1} mg^{-1} protein*
400	5.0 ± 0.2[a]
1000	6.2 ± 0.3

[a] ± SE.

$^{14}CO_2$ production is not dependent upon the accumulation of intermediates.

More direct evidence for a complex is provided by the observation that [2,7-^{14}C]ALL dependent $^{14}CO_2$ production is not proportionally diluted by unlabeled UG. A reaction mixture containing 5 mM [2,67-^{14}C]ALL was incubated with desalted seed coat extract with or without 1 mM UG and analyzed for $^{14}CO_2$ release. [2.7-^{14}]ALL-dependent $^{14}CO_2$ release was inhibited 25% by UG. These levels of UG would be expected to inhibit [2.7-^{14}C]allantoate-dependent $^{14}CO_2$ release by 35 to 50% (depending on the level of 2-hydroxyethylthio, 2′-ureido, acetate) if UG were a free intermediate. Definitive evidence for an enzyme complex would require an analytical system, such as ^{13}C-NMR, that could quantitate all products and intermediates derived from the 4 and 5 carbons of allantoate. Unfortunately, the levels of enzymatic activity observed are not sufficient for ^{13}C-NMR analysis.

SUMMARY AND CONCLUSIONS

We demonstrate that ALL is degraded *in vitro* by an enzyme complex that has ALAH and UGAH activity. UGAH releases CO_2 and glyoxylate from UG in a 1:1 ratio and has an apparent K_m of 85 μM for UG. Allantoate is catabolized to two CO_2 and one glyoxylate without detectable urea accumulation.

A critical observation suggesting that ALAH and UGAH act to degrade ALL is that glyoxylate is produced enzymically from ALL without detectable urea formation. Our results are not consistent with the accumulation of either proposed intermediate, UG (17, 18), or ureidoglycine.

Our data are consistent with previous reports that establish: (a) ALN can be degraded by soybean cell cultures without urease action (8), (b) leaf discs degrade ALN without a urea intermediate (17), (c) ALAH is the first step of ALL degradation (17, 18). Further, R Stahlhut (personal communication) has shown that ALN can be completely degraded by soybean cell cultures without urease action, thus confirming and extending the work of Polacco *et al.* (8).

Although these and previous results (8, 17, 18) clearly establish that, in soybean, ureides can be degraded by ALAH and UGAH without a urea intermediate, our results do not completely eliminate the possibility that urea may be a product of ureide catabolism in other plant species or that soybean plants may have a second activity (not evident under our experimental conditions) that degrades ALL via a urea intermediate. However, the assumption that all N released from ureide catabolism is derived from a urea intermediate (6, 9) is not yet supported by clear experimental evidence. The recent isolation of urease-negative soybeans (J Polacco, personal communication) will help clearly establish the *in vivo* role, if any, of urease and urea in soybean ureide catabolism.

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