Regulation of Allantoin and Allantoic Acid Degradation in the Yam and Sweet Potato

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In the regulation of allantoin and allantoic acid degradation in root tubers, allantoic acid is the substrate for allantoicase and allantoate amidohydrolase while allantoin is the substrate for allantoinase. The allantoate amidohydrolase is not influenced by urea and glyoxylate, whereas allantoicase is activated by both compounds in yam tubers but inhibited by glyoxylate in the sweet potato. Thus, allantoate amidohydrolase with a lower apparent K_m than allantoicase controls the anabolic degradation, whereas allantoicase controls the catabolic degradation of allantoic acid. Glyoxylate and urea inhibit the degradation of allantoin by allantoinase, which responds allosterically to varying concentrations of allantoin, urea, and glyoxylate. High concentrations of urea (ca. 0.4 mM) and glyoxylate (ca. 4 mM) induce the tuber allantoinase to catalyze the synthesis of allantoin using glyoxylate and urea as substrates. Allantoinase appears to be the enzyme that regulates the overall rate of purine catabolism and synthesis in root tubers.

Recent studies have shown that the yam tuber (*Dioscorea spp.*) loses up to 50% of its nonprotein nitrogen (NPN) on storage for only 6 months (Osuji and Ory, 1986). Since these tubers are the staple food of millions of people in West Africa, the loss of nitrogen by the tubers is critical for the dietary nitrogen of its consumers. The major component of the NPN of yam tuber is allantoin (Ozo et al., 1983). It has been shown that the purine degradative pathway of the tuber is the avenue through which NH_3 is lost from the tuber (Osuji and Ory, 1986). Preliminary investigations also suggested that the tuber has a feedback regulatory mechanism by which it attempts to conserve its purine nitrogen. The aim of this investigation was to understand the mechanism by which the yam tuber regulates the degradation of its major purines allantoin and allantoic acid. Regulation of the degradation of these ureides in the yam tuber was also compared with that in the sweet potato since these two root tubers are tropical staples and they are known to metabolize the ureides.

Allantoin and allantoic acid are the principal compounds for the storage and transportation of nitrogen in legumes, especially soybean and cowpea (Christensen and Jochimsen, 1983). These ureides are important in the nitrogen economy of plants, and research on their metabolism has attracted considerable attention (Fujihara et al., 1977; Fujihara and Yamaguchi, 1980; Herridge et al., 1978; Ory et al., 1969). Apart from a study on the ureide metabolism of banana leaves, all other studies of ureide metabolism in higher plants have been limited to the nitrogen-rich plant species (Freiberg et al., 1957; Thomas and Schrader, 1981).

In this paper we propose a mechanism whereby the end products of purine degradation, urea and glyoxylic acid, regulate the activities of allantoinase, allantoicase, and allantoate amidohydrolase and for the condensation of urea and glyoxylate to form allantoin by the root tubers.

MATERIALS AND METHODS

The yams (*Dioscorea spp.*) and sweet potato (*Ipomoea batatas* Jewel cultivar 8) were freshly harvested and procured as stated before (Osuji and Ory, 1986). The purine-degrading enzymes of the tubers were extracted as described earlier (Osuji and Ory, 1986) with 0.1 M K_2HPO_4 containing 0.1% mercaptoethanol and used for assays of the activities of allantoinase, allantoicase, and allantoate amidohydrolase. In some experiments, partial separation of allantoinase from allantoicase was accomplished by saturating the tuber extract up to 35% with solid $(NH_4)_2SO_4$. The precipitated protein was dissolved in a minimum volume of water and dialyzed against distilled water, while the supernatant was made 85% with solid $(NH_4)_2SO_4$ and the precipitated protein redissolved in minimum volume of water and similarly dialyzed as stated above. All tuber extracts were stored frozen. Enzyme assays were repeated three times on each tuber extract; results are averages of the triplicate assays. Standard errors were less than 3%.

Allantoinase (EC 3.5.2.5) activity was determined as described earlier (Osuji and Ory, 1986) with allantoin as substrate. For determination of the effects of urea and glyoxylic acid on the K_m and V_{max} of the enzyme, allantoin concentrations from 0.3 to 3.0 μmol/mL were placed in separate test tubes. For the effect of urea, 2 mL of urea solution containing 10 μmol/mL of urea was added to each test tube of allantoin solution. For the effect of glyoxylic acid, 2 mL of glyoxylic acid solution containing 0.88 μmol/mL of glyoxylic acid was added to each test tube of allantoin solution. Solutions of allantoin, urea, and glyoxylic acid were prepared in 0.1 M Tris buffer, pH 7.4, and then 1 mL of the tuber extract was added to each reaction tube. A control containing no allantoin but with glyoxylic acid and enzyme was used for the glyoxylic acid experiments. The reactions were conducted at 40 °C for 60 min, then the tubes were placed in ice, and 2 mL of each reaction mixture was transferred to a centrifuge tube containing 1 mL of phenylhydrazine (10 mg/3 mL), followed by 1 mL of concentrated HCl. The mixture was heated in a boiling water bath for 5 min and then cooled rapidly in ice. Potassium ferricyanide solution (1 mL, 50 mg/3 mL) was added, and after being mixed thoroughly, the solution was centrifuged (1000g × 5 min) to remove the precipitated protein. Absorbance (525 nm) was measured immediately after centrifugation. A calibration curve was prepared with glyoxylic acid, to each tube was also added 1 mL of the tuber enzyme, and the resultant mixture was processed as before. Absorbance of the control experiment was subtracted from that of the glyoxylate experiments to derive the data for calculating the effect of glyoxylate on the activity of allantoinase.

Allantoicase (EC 3.5.3.4) and allantoate amidohydrolase (EC 3.5.3.9) were assayed by the differential glyoxylate method of Trijbels and Vogels (1966a) using allantoic acid as substrate. For the effects of urea and glyoxylate on their

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activities, 1-mL solutions of allantoic acid containing 1.5–7 μmol were placed in different test tubes. For the effect of urea, 2 mL of the urea solution (as was done for allantoinase) was added to each tube of allantoic acid. Similarly, for the effect of glyoxylate, 2 mL of the glyoxylate solution (as for allantoinase) was added to each tube of allantoic acid. Controls for the glyoxylate experiments contained 2 mL of glyoxylate solution but no allantoic acid. Then, 1 mL of the enzyme extract was added to each reaction mixture. A control containing no enzyme but only allantoic acid was treated as in the main experiments. The reactions were incubated at 40 °C for 1 h, after which the tubes were placed in ice. Half of each reaction mixture was transferred to a centrifuge tube containing 1 mL of the phenylhydrazine solution. A 1-mL portion of concentrated HCl was added, and after thorough mixing, 1 mL of the potassium ferricyanide was added and the mixture again thoroughly mixed. The mixture was centrifuged (10000g × 5 min) to remove the protein after which absorbance of the centrifugate was determined at 525 nm. Absorbance of the controls was subtracted from the appropriate experiments, and the amounts of glyoxylate produced under each set of experimental conditions were calculated from a calibration curve set up with glyoxylic acid. The data were plotted by the double-reciprocal plot of Lineweaver and Burk (1934). The results are contained in Tables II and III.

To examine utilization of urea as substrate for the synthesis of allantoin and/or allantoic acid, 1 mL of urea solution containing 5–20 μmol was placed in different test tubes followed by 1 mL each of glyoxylic acid solution (0.88 μmol/mL) and NH_4Cl solution (2.8 μmol/mL). One milliliter of the tuber enzyme was added and the reaction incubated at 40 °C for 60 min. From the reaction mixture were removed 1-mL aliquots for assays of allantoin and allantoic acid produced by the reaction and for free unreacted glyoxylic acid (Trijbels and Vogels, 1966a). Controls without the tuber enzyme were set up and treated as in the main experiments. Double-reciprocal plots of the allantoin or allantoic acid produced were made against the urea substrate.

For the use of glyoxylic acid as substrate in the synthesis of allantoin and/or allantoic acid, 1-mL solutions of glyoxylic acid containing 1.5–10 μmol were placed in reaction tubes containing 1 mL each of urea solution (10 μmol/mL) and NH_4Cl (6 μmol/mL). Controls without the enzyme extract were set up and processed in parallel with the experiment. Incubation of the reaction and analysis of the reaction mixture for allantoin, allantoic acid, and unreacted glyoxylic acid were performed as described above. Double-reciprocal plots of the allantoin, allantoic acid, and unreacted glyoxylic acid were made vs. glyoxylic acid as substrate. Protein contents of the tuber extracts were determined by the method of Lowry et al. (1951), using bovine serum albumin as the standard.

RESULTS AND DISCUSSION

Activity of the Tuber Extracts. Specific activities of the tuber extracts and the $(NH_4)_2SO_4$-purified preparations were determined with respect to allantoinase, allantoicase, and allantoate amidohydrolase. Results in Table I show that the tuber extract contains activities of the three enzymes. The proteins precipitated with $(NH_4)_2SO_4$ differed in their enzyme contents from sweet potato to yams and from one yam cultivar to the other. $(NH_4)_2SO_4$ treatment did not effect a satisfactory partial separation of the three enzymes, probably because the ureide enzymes are located in glyoxysomes (St. Angelo and Ory, 1970) that are ruptured to form fragments of varying sizes during homogenization of the tubers. Precipitation of the three enzymes with $(NH_4)_2SO_4$ would therefore depend upon the extent to which the glyoxysomes were ruptured, whether the enzymes are bound to the glyoxysomal membrane and whether some of the enzymes are inside the glyoxysomes. Ory and St. Angelo (1971) demonstrated that, in castor seed glyoxysomes, allantoinase was bound to the membrane while isocitrate lyase was inside the glyoxysome. St. Angelo and Ory (1970) also observed that after castor seed glyoxysomes had been ruptured, activities of allantoinase and isocitrate lyase were distributed in several glyoxysomal fragments whose densities ranged from 1.01 to 1.21 in a Ficoll gradient. In the study reported here on yam tubers, no attempt was made to localize the three enzymes in glyoxysomes, so the tuber extracts were used most of the time without $(NH_4)_2SO_4$ purification. In some experiments (cf. Table III), the enzymes precipitated at 35% $(NH_4)_2SO_4$ saturation of the tuber extracts were used.

Table I. Distribution of Allantoinase, Allantoicase, and Allantoate Amidohydrolase Activities in Proteins Precipitated with $(NH_4)_2SO_4$ from Extracts of Some Root Tubers

tuber sp	$(NH_4)_2SO_4$ satn, %	protein rec, %	sp act., μmol/min per mg: allantoinase	allantoicase	allantoate amidohydrolase
D. cayenensis (water yam)	orig extr		0.86	2.81	0.78
	0–35	30	0.32	1.41	0.30
	35–55	50	0.95	1.32	0.65
	55–85	10	1.05	1.42	1.50
I. batatas (jewel)	orig extr		1.50	3.5	0.95
	0–35	25	1.20	2.0	0.80
	35–55	60	1.28	1.47	0.77
	55–85	5	0.80	1.20	1.40
D. cayenensis (Nkokpu)	orig extr		2.16	3.49	1.06
	0–55	70	2.06	3.10	1.30
	55–85	13	2.21	3.23	1.15

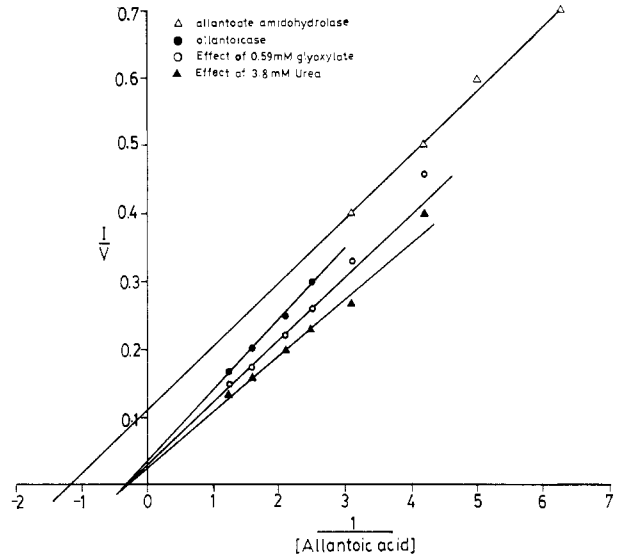

Figure 1. Lineweaver–Burk plot of allantoic acid degradation by *D. cayenensis* (Nkokpu) tuber extracts at high and low concentrations of allantoic acid.

Evidence for the Presence of Allantoate Amidohydrolase. Double-reciprocal plots of allantoic acid degradation by the tuber extracts to yield free glyoxylate gave two curves in each case (cf. Figure 1): one curve corresponding to the degradation of low concentrations the other to high concentrations of allantoic acid. This suggests that allantoic acid in root tubers binds to two enzyme sites during degradation. One site is in allantoate ami-

Table II. Allantoicase Activities

	apparent K_m, mM			V_{max}, μM/min		
	allantoate minus effectors	in pres of glyoxylate (589 μM)	in pres of urea (3.8 mM)	allantoate minus effectors	in pres of glyoxylate (589 μM)	in pres of urea (3.8 mM)
D. cayenensis (water yam cv.)	12.1			18.2		
D. cayenensis (Nkokpu cv.)	2.7	2.67	2.67	22.86	32	26.67
I. batatas (jewel cv.)	2.8	2.8		63.5	190.5	

Table III. Allantoinase Activities

	apparent K_m, mM			V_{max}, μM/min		
	allantoin minus effectors	in pres of glyoxylic acid (589 μM)	in pres of urea (3.8 mM)	allantoin minus effectors	in pres of glyoxylic acid (589 μM)	in pres of urea (3.8 mM)
D. cayenensis (water yam)	0.05	0.25	0.05	4.17	2.56	3.51
D. cayenensis (Nkokpu)	0.15	0.30		8.18	0.25	
I. batatas (jewel)	0.74	0.44		18.20	10.60	

dohydrolase and the other in allantoicase. Previously (Osuji and Ory, 1986), the high ratio of glyoxylate to ureidoglycolate produced from allantoic acid was used to identify the presence of allantoate amidohydrolase in the extracts of tubers. The kinetic data in Figure 1 are additional evidence for the presence of allantoate amidohydrolase in root tubers. Since allantoicase is known to be the catabolic enzyme for allantoic acid, the enzyme in Figure 1 with the higher K_m and V_{max} is allantoicase, whereas the enzyme with the lower K_m and V_{max} is allantoate amidohydrolase. Activity of allantoate amidohydrolase could not be assayed by monitoring the release of NH_3 from allantoic acid because the tuber extract also contains urease, which liberates NH_3 from urea, a product of allantoic acid degradation. Urease was not removed in order to monitor the release of NH_3 by allantoate amidohydrolase because, in so doing, the relative activities of allantoicase and allantoate amidohydrolase in the extract would have been altered. The relative activities of allantoicase and allantoate amidohydrolase in the same tuber extract were first noted in our previous paper (Osuji and Ory, 1986). The apparent K_m and V_{max}, respectively, of the allantoate amidohydrolase of *Dioscorea cayenensis* (water yam cv.) are 4 mM and 4.5 μM/min; of *D. cayenensis* (Nkokpu cv.), 0.7 mM and 7.6 μM/min; and of *I. batatas* (Jewel cv.), 1.8 mM and 41.7 μM/min. Table II shows that the apparent K_m and V_{max} values for allantoicase of tubers are higher than those of allantoate amidohydrolase. Since allantoate amidohydrolase has a lower apparent K_m than allantoicase, this suggests that it performs the anabolic degradation of allantoic acid while allantoicase performs catabolic degradation. A similar regulatory mechanism exists in the metabolism of aromatic amino acids (Welch and Gaertner, 1980). This appears to be an important regulatory device for the conservation of ureide nitrogen by root tubers. Other higher plants are not known to possess this capability for differentiating anabolic from catabolic degradation of allantoic acid (Thomas and Schrader, 1981).

Effects of Glyoxylate and Urea on the Degradation of Allantoic Acid. Table II and Figure 1 show the apparent K_m and V_{max} values for allantoicase of some species of root tubers and the influence of glyoxylate and urea on the values. The apparent K_m values in Table II, which range from 2.7 to 12.1 mM for tuber allantoicase in the absence of effectors are slightly lower than the K_m of allantoicase of bacteria (9.5–28.6 mM) but higher than allantoicase of peanut cotyledons (0.85 mM) (Trijbels and Vogels, 1966a,b; Singh, 1968). The K_m of allantoicase of *Candida utilis* is 5.5 mM (Cho et al., 1968), in the range of values for the tubers.

Glyoxylate and urea do not seem to affect the apparent K_m of allantoicase in *D. cayenensis* but appear to increase the V_{max} values (Table II; Figure 1). Glyoxylate and urea, therefore, noncompetitively activate the allantoicase (Segel, 1976). Glyoxylate and urea have been reported to inhibit the allantoicase of *Pseudomonas aeruginosa* (Thomas and Schrader, 1981), but in *C. utilis*, the allantoicase is inhibited by urea but not by glyoxylate. Activators of the allantoicase of *C. utilis* include uric acid, allantoin, and allantoate (Cho et al., 1968). In higher plants there are no reports of activation of allantoicase.

In *I. batatas*, glyoxylate lowers the V_{max} value of allantoicase. Glyoxylate, therefore, noncompetitively activates the allantoicase of sweet potato (Segel, 1976), similar to its effect on allantoicase of *D. cayenensis* (Nkokpu CV) in Figure 1. The effect of urea on allantoicase of sweet potato was not investigated here, but the results in Table II suggest that regulation of allantoicase activity in sweet potato is similar to that in the yam. Results in Figure 1 also show that the noncompetitive activation of allantoicase by glyoxylate and urea does not affect the allantoate amidohydrolase. A similar result was obtained for allantoate amidohydrolase of the sweet potato. The data in Table II show that the K_m values for allantoicase of both *D. cayenensis* and *I. batatas* are approximately equal and the range of V_{max} values for the enzyme in *D. cayenensis* is very close to the inhibited V_{max} value of the *I. batatas* enzyme. Thus, in vivo, both yam tuber and sweet potato allantoicase may have approximately identical activities. This was confirmed by earlier results (Osuji and Ory, 1986) where the specific activities of the enzyme were 4.1 and 3.9 μmol/min per mg for *D. cayenensis* and *I. batatas*, respectively.

Effects of Glyoxylate and Urea on Degradation of Allantoin. In Table III, the apparent K_m values for the tuber allantoinase range from 0.05 to 0.74 mM in the absence of effectors. These values contrast sharply with the high values for legumes, which range from 3.8 mM in peanuts (Singh, 1968), through 13.8 mM in castor beans (Ory et al., 1969) and 14 mM in *Glycine hispida*, to 46 mM in *Phaseolus* (Vogels et al., 1966). On the other hand, V_{max} values and specific activities of the uninhibited allantoinase of tubers are strikingly higher than those of legumes (Osuji and Ory, 1986). For castor bean allantoinase the specific activity was 1.28 nM/min per mg (Ory et al., 1967), pea root was 22.5 nM/min per mg, and soybean root was 87.2 nM/min per mg (Christensen and Jochimsen, 1983). The specific activity of tuber allantoinase ranges from 0.81 to 3.6 nM/min per mg (Osuji and Ory, 1986).

Glyoxylate and urea reduce the V_{max} of allantoinase for all of the tubers examined; thus, they appear to inhibit

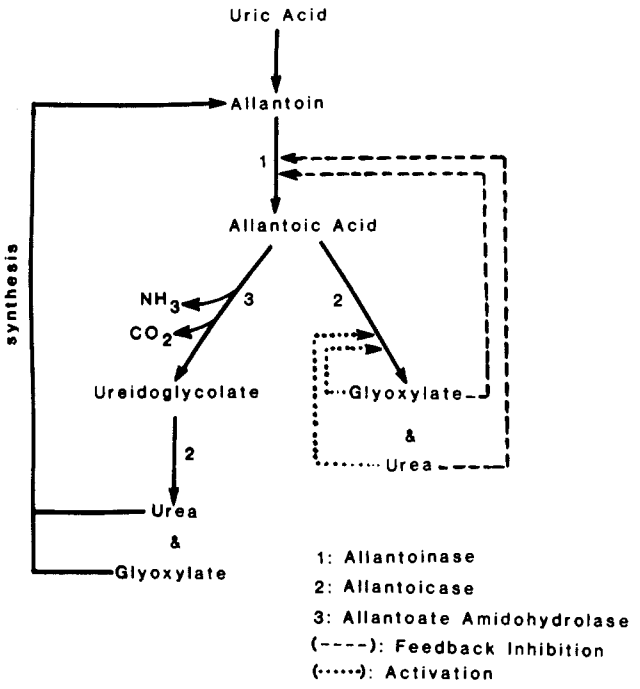

Figure 2. Proposed mechanism for differential-feedback control of allantoinase and allantoicase activities in yams by glyoxylate and urea.

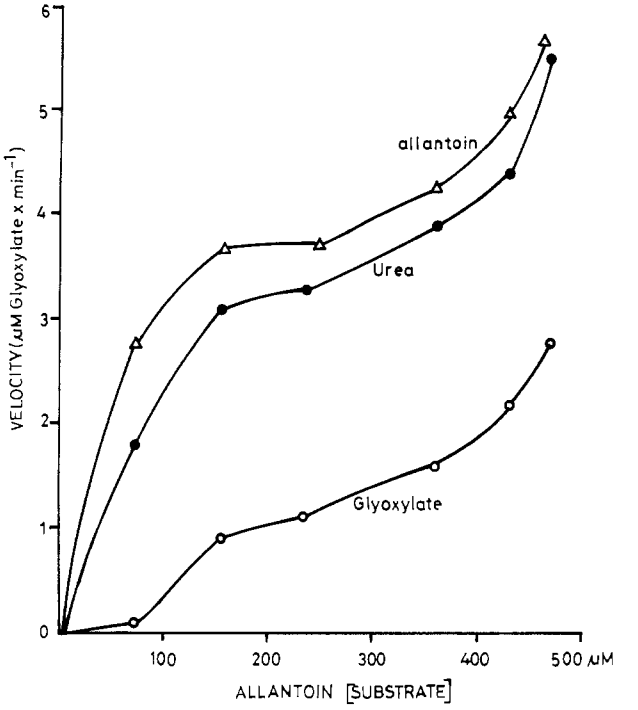

Figure 3. Degradation of allantoin by yam allantoinase at different concentrations of allantoin, urea, and glyoxylate.

allantoin degradation. Vogels et al. (1966) reported the inhibition of allantoinase in some legumes by high concentrations of urea. Glyoxylate increased the apparent K_m for allantoinase of yams but decreased the value in sweet potato. Thus, glyoxylate appears to be an inhibitor of allantoinase in sweet potato. Urea is a noncompetitive inhibitor of allantoinase of *D. cayenensis*. This suggests that glyoxylate, urea, and allantoin may bind at different sites on the allantoinase molecule.

Allantoin has been reported to accumulate in yam tubers (Ozo et al., 1983; Ueda and Sasaki, 1956). Feedback inhibition of allantoinase of the tubers now provides an adequate explanation for this deposition of allantoin. Also, the reported (Osuji and Ory, 1986) low activity of allantoinase compared with the high activity of allantoicase of tubers now becomes understandable in view of the fact that both enzymes are at the same time under differential control of the end products of the pathway. While urea and glyoxylate activate the allantoicase, they inhibit allantoinase of the tubers. The overall effect of this differential influence of glyoxylate and urea on the two enzymes is retardation of the rate of catabolism of purines in the tubers, which subsequently leads to conservation of nitrogen by the tuber. The mechanism by which the root tuber attempts to regulate the degradation of its purines and thereby conserve nitrogen is through the differential-feedback influence of glyoxylate and urea on the activities of allantoinase and allantoicase. This differential feedback control is shown in Figure 2. It is noteworthy that glyoxylate and urea have no influence on allantoate amidohydrolase (Figure 1). In this way the enzyme is allowed to continue to produce low levels of NH_3, ureidoglycine, and ureidoglycolate for synthetic uses. It is not clear, however, whether this NH_3 is salvaged or allowed to diffuse out and is lost from the tuber.

Allosteric Property of Allantoinase of Root Tubers. The observed feedback inhibition of allantoinase of root tubers by glyoxylate and urea prompted us to investigate further the properties of the enzyme. Plots of allantoin, glyoxylate, and urea concentrations vs. the velocities of allantoin degradation were made, to derive velocity curves for the degradation of allantoin in the presence and absence of the two effectors. Figure 3 shows that allantoinase exhibits a sigmoidal response to the presence of urea and glyoxylate. Even in the absence of the two effectors, allantoin alone confers some sigmoidicity to the velocity curve. Glyoxylate is the most potent effector compared to urea and allantoin. Thus, these three compounds serve as substrates for the tuber allantoinase.

The allosteric property of the tuber's allantoinase provides a further mechanism for regulation of the rate of catabolism of purines. The sigmoidal response (Segel, 1976) enables the enzyme to operate as an off–on switch to various concentrations of allantoin and, in the presence of its effectors, to act as a sensitive control of the rate for the entire purine degradative pathway. These properties of tuber allantoinase became sufficiently interesting, and we attempted to purify the enzyme to study its molecular structure. Filtration of the tuber extracts containing allantoinase activity through Sephadex G-200 (Trijbels and Vogels, 1966a) did not yield sufficiently pure enzyme preparations suitable for a study of its subunit structure. Allosteric enzymes are known to be multisubunit in structure (Segel, 1976). Allantoinase is not known to be a multisubunit enzyme. Thus, additional details of the structure of the allantoinase of root tubers will be needed in order to completely understand its allosteric properties.

Synthesis of Allantoin from Urea and Glyoxylic Acid. Since allantoin, urea, and glyoxylic acid induce allosteric responses in the tuber allantoinase, we investigated the capability of the enzyme to catalyze the condensation of urea and glyoxylate and form allantoin. Synthesis of allantoin was studied by keeping the concentration of urea constant while varying the concentrations of glyoxylic acid and vice versa. Double-reciprocal plots of the data gave the apparent K_m and V_{max} shown in Table IV. Allantoic acid was not detected in the reaction mixtures, showing that enzymic condensation of urea and glyoxylate produces allantoin directly. Extracts of some fungi (Brunel and Brunel-Capelle, 1951) and banana leaves (Freiberg et al., 1957) condense urea and glyoxylate to form allantoic acid. No other organism has

Table IV. K_m and V_{max} for the Synthesis of Allantoin from Urea and Glyoxylic Acid as Substrates (Enzyme Extract of Water Yam Used)

	app K_m, mM	V_{max}, μM/min
glyoxylate	4.0	1.07
urea	0.36	1.35

been reported to synthesize allantoin from urea and glyoxylate.

Tables III and IV show that whereas the apparent K_m values for allantoin degradation in the presence of glyoxylate and urea are many-fold lower than the apparent K_m for the synthesis of allantoin from glyoxylate and urea, the corresponding V_{max} values are only about 2.5 times higher than those for allantoin synthesis. This indicates that the rate of allantoin degradation is only 2.5 times faster than allantoin synthesis, so that the synthetic route makes a very significant contribution to the conservation of nitrogen by root tubers. This synthesis of allantoin from the end products of purine catabolism is a further explanation for the accumulation of allantoin in the tubers. It was this ability of the tuber extract to use glyoxylate and, thereby, reverse the hydrolysis of allantoin that made it necessary during allantoinase assay to add tuber extract also in the glyoxylate calibration curve tubes and to treat the calibration solutions like experimental assay solutions: i.e., incubation at 40 °C for 1 h, addition of phenylhydrazine solution and concentrated HCl, boiling at 100 °C for 5 min, addition of potassium ferricyanide solution, and centrifugation of the reaction solution to remove precipitated protein. When these precautions were taken, the derived activities of allantoinase increased almost 20-fold, with a corresponding decrease in the apparent K_m of the enzyme. It now appears necessary to redetermine the activities and apparent K_m of the allantoinase in legumes, which had been previously reported because reversal of the hydrolysis of allantoin was not taken into account in the assay methods employed for those studies.

The allosteric property of allantoinase offers an alternate pathway for ureide synthesis, as proposed in Figure 2. Previously, only the catabolic pathway from purines was known for the synthesis of ureides (Thomas and Schrader, 1981). In the regulation of allantoin and allantoic acid degradation, urea and glyoxylate retard the rate of purine degradation and are also substrates for the synthesis of allantoin. The conservation of nitrogen by root tubers is regulated by allantoinase, so that efforts to increase nitrogen contents of these tubers should be directed toward genetic alteration of the structure of this enzyme, to enhance its capability to synthesize allantoin.

Registry No. EC 3.5.2.5, 9025-20-1; EC 3.5.3.4, 9025-21-2; EC 3.5.3.9, 37289-13-7; NH_2CONH_2, 57-13-6; allantoin, 97-59-6; allantoic acid, 99-16-1; glyoxylic acid, 298-12-4.

LITERATURE CITED

Brunel, A.; Brunel-Capelle, G. *C. R. Hebd. Seances Acad. Sci.* **1951**, *232*, 1130.
Cho, K. S.; Lee, K. W.; Yu Hi Co, S. C.; Roush, A. H. *Arch. Biochem. Biophys.* **1968**, *126*, 261.
Christensen, T.; Jochimsen, B. *Plant Physiol.* **1983**, *72*, 56.
Freiberg, S. R.; Bollard, E. G.; Hagarty, M. P. *Plant Physiol.* **1957**, *32*, Suppl. 1ii.
Fujihara, S.; Yamaguchi, M. *Agric. Biol. Chem.* **1980**, *44*, 2569.
Fujihara, S.; Yamamoto, K.; Yamaguchi, M. *Plant Soil* **1977**, *48*, 233.
Herridge, D. F.; Atkins, C. A.; Pate, J. S.; Rainbird, R. *Plant Physiol.* **1978**, *62*, 495.
Lineweaver, H.; Burk, D. *J. Am. Chem. Soc.* **1934**, *56*, 658.
Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
Ory, R. L.; St. Angelo, A. J. USDA-ARS, New Orleans, LA, unpublished data, 1971.
Ory, R. L.; Gordon, C. V.; Singh, R. *Phytochemistry* **1969**, *8*, 401.
Osuji, G.; Ory, R. L. *J. Agric. Food Chem.* **1986**, *34*(4), 599.
Ozo, O. N.; Robinson, J.; Reeves, S. Tropical Development and Research Institute, London, England, unpublished data, 1983.
St. Angelo, A. J.; Ory, R. L. *Biochem. Biophys. Res. Commun.* **1970**, *40*, 290.
Segel, I. H. *Biochemical Calculations*, 2nd ed.; Wiley: New York, 1976; p 305.
Singh, R. *Phytochemistry* **1968**, *20*, 1503.
Thomas, R.; Schrader, L. *Phytochemistry* **1981**, *20*, 361.
Trijbels, R.; Vogels, G. D. *Biochim. Biophys. Acta* **1966a**, *113*, 292.
Trijbels, F.; Vogels, G. D. *Biochim. Biophys. Acta* **1966b**, *118*, 387.
Ueda, H.; Sasaki, T. *J. Pharm. Soc. Jpn.* **1956**, *76*, 745.
Vogels, G. D.; Trijbels, F.; Uffink, A. *Biochim. Biophys. Acta* **1966**, *122*, 482.
Welch, R. G.; Gaertner, F. H. *Curr. Top. Cell. Regul.* **1980**, *10*, 113.

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