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# Purine Degradative Pathway of the Yam and Sweet Potato

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The degradation of purines by nitrogen-deficient plants was investigated in the carbohydrate staples yam tubers (*Dioscorea spp.*) and sweet potatoes (*Ipomoea batatas*). The activities of adenosine deaminase, xanthine oxidase, uricase, allantoinase, allantoicase, and allantoate amidohydrolase were detected in extracts of the tubers, showing that purine degradation operates in these nitrogen-deficient plants as in the case of nitrogen-rich plants. The presence of allantoate amidohydrolase in the tubers suggested that much ammonia was lost from the pathway. Stored tubers also had increased activities of some enzymes of the pathway and experienced high losses of nitrogen. These results indicate that the nitrogen deficiency of root tubers was in part attributed to the ammonia lost via the purine degradative pathway. The implications of these reactions are discussed as they affect storage of the tubers for food uses.

## INTRODUCTION

The yam (*Dioscorea spp.*) and sweet potato (*Ipomoea spp.*) are staple food crops for millions of people (Coursey, 1967; Walter et al., 1984), especially in West Africa. They are a major source of carbohydrate and a minor source of nitrogen nutrition. Recent studies to understand the causes for the low nitrogen contents of yams showed that the proteins of the tubers were degraded to low molecular weight proteins during tuber storage (Osuji, 1981; Osuji and Umezurike, 1983) and the tuber accumulates large quantities of ureide nitrogen as allantoin (Ueda and Sasaki, 1956). The deposition of ureides in root tubers (known to be N deficient) is unusual because thus far the ureides are known to be important intermediates in the metabolism of nitrogen-rich plant species (Fosse, 1926; Mothes and Engelbrecht, 1952, 1954; Krupk and Towers, 1959; Reinbothe and Mothes, 1960). Thus, the deposition of ureides in root tubers could be a clue for a general understanding of the causes of low nitrogen contents of tubers.

It was considered necessary, therefore, to understand the purine degradative pathway in root tubers and to determine whether the nitrogen deficiency and allantoin accumulation are attributable to activities of the enzymes of the pathway, especially in stored yam tubers. Yam tubers are traditionally stored for several months to be used as food in many parts of the tropics. Further support for such a study of purine degradation in root tubers was based upon the fact that ureides are known to be metabolized mainly by plant roots (Brunel and Capelle, 1947; Kushizaki et al., 1964; Ishizuka et al., 1970) and nitrogen fixation and ureide synthesis are coupled via purine degradation (Fujihara and Yamaguchi, 1978a, 1978b, 1980).

The purpose of this investigation was to understand the purine degradative pathway of N-deficient plants. The purine pathway intermediates contain 50% N by weight, and their degradation would be critically important in nitrogen economy of the plant. The results of such a study should indicate the mechanism for regulation of the pathway and whether nitrogen deficiency in root tubers is attributed to activities of enzymes of the pathway, especially in stored yam tubers.

## MATERIALS AND METHODS

Sweet potato [*Ipomoea batatas* (L.) Lam.] (Jewel cv.) was purchased from a local grocery. Yam tubers (*Dioscorea spp.*) were provided by the National Root Crops Research Institute, Umudike, Nigeria; some were purchased from open markets in Benin City, Nigeria. The tubers were used about 1 month after harvest. For the effects of tuber storage on purine degradation, healthy yam tubers were placed in paper bags and stored in cupboards at room temperature. Tubers that sprouted during storage were not used for the enzyme assays.

**Homogenization of Tubers.** The cork layer of each tuber was carefully removed by scraping to minimize loss of the outer tissues since this layer contains much of the tuber protein (Walter et al., 1984; Eka, 1985). The tuber was cut into small pieces (approximately 1 in.$^3$) and frozen in 50-g portions with solid $CO_2$. The frozen pieces were transferred to a Waring blender containing 100 mL of ice-cold 0.1 M $K_2HPO_4$ and 0.1 mL of $\beta$-mercaptoethanol. Tuber pieces were homogenized at No. 1 speed for 3 min; the homogenate was squeezed through two layers of cheesecloth, and the filtrate was centrifuged at 20000*g* for 10 min. The pellet was discarded, and the supernatant liquid was dialyzed against three changes of deionized water for 24 h to remove low molecular weight sugars that

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Table I. Specific Activities of the Purine-Degrading Enzymes of Yams and Sweet Potatoes

| species | cultivars (Nigerian names or sources) | adenosine deaminase, mmol/min per mg | xanthine oxidase, mmol/min per mg | uricase, mmol/min per mg | allantoinase, μmol/min per mg | allantoicase, μmol/min per mg |
|---|---|---|---|---|---|---|
| *Dioscorea alata* L. | umudike | 1.24 | 0.25 | 1.40 | 1.10 | 1.50 |
| *Dioscorea alata* L. | benin water yam | 1.45 | 0.20 | 0.98 | 0.81 | 2.50 |
| *Dioscorea cayenesis* Lam | *umudike nkokpu* | 0.11 | 0.3 | 1.72 | 0.93 | 4.1 |
| *Dioscorea dumetorum* (Kunth) | *umudike una* | 0.49 | 0.4 | 1.78 | 1.23 | 3.6 |
| *Dioscorea rotundata* Poir | *obiaturugo* | 0.54 | <0.01 | 1.32 | 1.03 | 1.7 |
| *Dioscorea rotundata* Poir | *nwokpoko* | 0.76 | 0.06 | 1.6 | 0.96 | 3.2 |
| *Dioscorea rotundata* Poir | *asukwu* | 0.76 | 0.12 | 6.3 | 3.6 | 11.8 |
| *Ipomoea batatas* | (jewel) | 0.25 | 0.01 | 1.4 | 1.21 | 3.9 |

would interfere with the phenylhydrazine assays for allantoinase and allantoicase of the tubers. The dialyzed extract was stored frozen at −20 °C.

**Enzyme Assays.** Adenosine deaminase (EC 3.5.4.4) was assayed by the method of Coddington (1965) at 25 °C, and the change in absorbance at 265 nm over the first 5 min of reaction was used for calculating enzyme activity.

Xanthine oxidase (EC 1.2.3.2) was assayed by the procedure of Bray (1963) at 25 °C using xanthine as substrate and $O_2$ as the electron acceptor. The change in absorbance at 293 nm from the 5th–10th min of reaction was used for calculating enzyme activity.

Uricase (EC 1.7.3.3) was assayed by the method of Mahler (1963) at 25 °C using $O_2$ as the electron acceptor. The change in absorbance at 293 nm over the first 5 min of reaction was used for calculating activity of the enzyme.

Allantoinase (EC 3.5.2.5) was assayed with allantoic acid substrate by a modification of the methods of Singh et al. (1970) and Trijbels and Vogels (1966). To 1 mL of allantoin solution (3 mM in 0.1 M Tris buffer, pH 7.4) was added 0.5 mL of the tuber extract and the resultant mixture incubated at 40 °C for 1 h. The reaction was stopped by adding 1 mL each of concentrated HCl and phenylhydrazine solution (100 mg/30 mL of deionized water) to react with glyoxylic acid (end product). The mixture was placed in boiling water for 5 min and then cooled rapidly by plunging into ice. After the reaction was brought to room temperature, 1 mL of potassium ferricyanide (500 mg in 30 mL of deionized water) was added. The reaction was centrifuged at 10000*g* for 5 min to remove the precipitated protein, and then the absorbance was measured at 525 nm. Control samples with allantoin but without the tuber extract were set up and processed as above, and the absorbance was subtracted from the test samples. A standard curve was prepared with glyoxylic acid.

Allantoicase (EC 3.5.3.4) was assayed with allantoic acid as substrate by a modification of the glyoxylate method described by Trijbels and Vogels (1966) and Ory et al. (1969). To 0.5 mL of allantoic acid solution (3.5 mM in 0.1 M Tris buffer, pH 7.4) was added 0.5 mL of the tuber extract and the resultant mixture incubated at 40 °C for 1 h. The reaction was stopped by placing the test tubes in ice; then, 1 mL of concentrated HCl and 1 mL of phenylhydrazine (100 mg dissolved in 30 mL of deionized water) were added, followed by 1 mL of the potassium ferricyanide solution (500 mg dissolved in 30 mL of deionized water) to react with the glyoxylic acid end product. The precipitated protein was centrifuged for removal, and absorbance of the supernatant liquid was determined at 525 nm. Controls with allantoic acid but without tuber extract were treated as above, and its absorbance was subtracted from that of test samples. A standard curve was prepared with glyoxylic acid.

The amounts of ureidoglycine and ureidoglycolate produced from allantoic acid by the tuber extracts were determined, based on the differential glyoxylate method of Trijbels and Vogels (1966), after the tuber extracts and allantoic acid solutions had been incubated as described under allantoicase assay.

Protein contents of the tuber extracts were determined by the method of Lowry et al. (1951) using BSA as the standard. Enzyme activities were expressed as moles of product released per minute per milligram of protein.

For nonprotein nitrogen (NPN), 100 g of tuber was homogenized in a Waring Blender at speed No. 1 for 3 min in 90% ethanol. The homogenate was filtered through two layers of cheese cloth and the filtrate centrifuged at 15000*g* for 5 min. The volume of the supernatant liquid was measured and analyzed by Kjeldahl analysis.

## RESULTS AND DISCUSSION

Table I shows the activities of five enzymes of the purine degradative pathway in four species of yams compared with those of the sweet potato. The presence of the enzymes indicates that the root tubers have the capacity of metabolize ureides. Legumes like peanuts (Singh, 1968; Singh et al., 1970), castor beans (St. Angelo and Ory, 1970; Ory et al., 1969), cowpea (Herridge et al., 1978), and soybean (Matsumoto et al., 1977; Mothes and Engelbrecht, 1952, 1954) have already been demonstrated to possess these enzymes. The purine degradative pathway is therefore potentially present in low-nitrogen plant species as it is in N-rich species (Thomas and Schrader, 1981).

Table I also shows that, upon going from uricase to allantoinase, there is an apparent 1000-fold drop in enzyme activity along the purine degradative pathway. Such a phenomenon has not been observed in any other plants (Christensen and Jochimsen, 1983). Activities of the allantoinase of peanut cotyledons (Singh et al., 1970) and of castor bean (Ory et al, 1969) were found to be 4.9 mmol/min per mg and 0.1–1.28 mmol/min per mg, respectively. Thus, the allantoinase of oilseeds appears to be more active than that of root tubers. Such low activity of allantoinase in the tubers should lead to accumulation of its substrate, allantoin, in the tuber. Thus, low activity of allantoinase may account for the accumulation of allantoin in tubers. Since the tubers are quite low in nitrogen, a reduction in activity of the tuber's allantoinase could be a metabolic device for conserving the nonprotein nitrogen.

Allantoicase is only marginally higher in activity than allantoinase in the tubers, so that the two enzymes may be cooperating in the controlled release of nitrogen from the ureides. Furthermore, the five enzymes in Table I appear to alternate between high and low specific activities going from adenosine deaminase to allantoicase, such that adenosine deaminase and uricase have relatively high activities than xanthine oxidase and allantoicase more than allantoinase. Since the five enzymes of the purine degradative pathway were assayed in the same buffer extracts of tubers, the specific activities are directly comparable, based upon the same nitrogen contents of the extracts.

**Table II. Rates of Production of Glyoxylate, Ureidoglycine, and Ureidoglycolate from Allantoic Acid by Extracts of Sweet Potato and Yam Tubers**

| species | cultivars (Nigerian name or sources) | rate of prodn,[a] μmol/min: glyoxylate | glyoxylate, ureidoglycine, and ureidoglycolate | ureidoglycine and ureidoglycolate | ratio of glyoxylate to ureidoglycine and ureidoglycolate |
|---|---|---|---|---|---|
| *Dioscorea cayenensis* | *nkokpu* | 16.3 | 21 | 4.7 | 3.47 |
| *Ipomoea batatas* | jewel | 17.8 | 21.7 | 3.9 | 4.56 |
| *Dioscorea dumetorum* | *una* | 14.5 | 18 | 3.5 | 4.14 |
| *Dioscorea alata* | water yam | 12.7 | 19.7 | 7.0 | 1.81 |
| *Dioscorea alata* | *umudike* | 8 | 21.3 | 13.3 | 0.6 |
| *Dioscorea rotundata* | *asukwu* | 14.7 | 22 | 7.3 | 2.01 |

[a] Enzyme activity expressed as μmoles produced/minute per milligram protein in tuber extract.

Thus, the alternating high and low specific activities should reflect the in vivo relationship between the five enzymes. Such relationships suggest that there may be a general metabolic regulation of these enzymes. It would be of interest to study the mechanism of this regulation.

The three enzymes in Table I with higher activities hydrolyze amide bonds, which leads to the release of ammonia from the ureide molecules. It therefore appears that the purine degradative pathway of the tubers may have an overall net loss of nitrogen. Accumulation of $NH_3$ in the tissues is reported to cause the accumulation of ureides in plants (Thomas et al., 1979). Thus, besides the low activity of the allantoinase of root tubers, the accumulation of allantoin could also be caused by the $NH_3$ released from purine degradation. These observations indicate that the purine degradative pathway in root tubers could be under the control of a feedback inhibitory mechanism.

Table II shows the rates by which tuber extracts convert allantoic acid to glyoxylic acid, ureidoglycine, and ureidoglycolate. When allantoicase hydrolyzes allantoic acid, the initial products are urea and (−)-ureidoglycolate. Allantoicase then hydrolyzes (+)-ureidoglycolate to glyoxylic acid and urea. The enzymic production ratio between glyoxylic acid and (±)-ureidoglycolate is 1:2 (Van der Drift and Vogels, 1970; S-Gravenmade, 1970). Table II shows that the ratio of glyoxylate to ureidoglycolate produced by tuber extracts is above 0.5, suggesting that more glyoxylate than ureidoglycolate is being produced from allantoic acid. This finding suggests that there may be another enzyme in the tuber that converts allantoic acid to (+)-ureidoglycolate, and this is hydrolyzed readily to urea and glyoxylic acid. The apparent enzyme is believed to be allantoate amidohydrolase, which has been found in some bacteria (Vogels, 1966; Thomas and Schrader, 1981) but not reported in higher plants. It degrades allantoic acid in two steps. In the first step, ureidoglycine, $CO_2$, and $NH_3$ are produced; in the second step the ureidoglycine is hydrolyzed to $NH_3$ and (+)-ureidoglycolate. The alternate degradation of allantoic acid by allantoate amidohydrolase would produce more $NH_3$. It has already been noted above that accumulation of allantoin in the tuber could be caused by $NH_3$ liberated during purine degradation. Activity of allantoate amidohydrolase in the tuber could lead to further production of $NH_3$ that could be sufficiently toxic to cause the accumulation of allantoin in the tuber's tissues.

The differential glyoxylate method used for determination of the rates of ureidoglycolate and ureidoglycine production and hydrolysis does not allow calculation of the separate activities of allantoate amidohydrolase and ureidoglycolase, but the high production ratios of glyoxylate to ureidoglycine and ureidoglycolate in *Dioscorea dumetorum*, *I. batatas*, and *Dioscorea cayenensis* (Table II) suggest that allantoate amidohydrolase may be degrading allantoate to (+)-ureidoglycolate, which is then hydrolyzed by allantoicase to yield the high levels of glyoxylic acid relative to the ureidoglycolate and ureidoglycine that were measured.

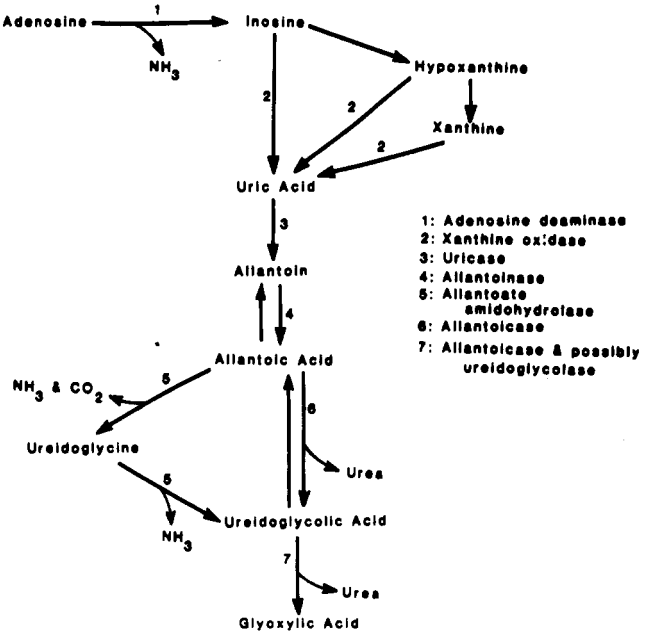


**Figure 1.** Proposed scheme for the purine catabolic pathway of the sweet potato and yam tuber.

From the results discussed thus far, the scheme in Figure 1 is proposed as the purine degradative pathway of root tubers. The presence of allantoate amidohydrolase in the pathway is biologically important because it has not been reported in N-rich plant species. The hydrolysis of allantoic acid by allantoate amidohydrolase liberates 2 molecules of $NH_3$. Since the ammonia absorbed by roots is not reassimilated directly into the purine molecule (Fujihara and Yamaguchi, 1978a), it follows that allantoate amidohydrolase could cause the loss of nonprotein nitrogen (NPN) from root tubers. Ammonia toxicity appears to be the primary factor causing the deposition of allantoin in root tubers (Ueda and Sasaki, 1956).

The effect of yam tuber storage on enzymes of the purine degradative pathway is shown in Figure 2. The water yam cultivar of *Dioscorea alata* and the *obiaturugu* and *asukwu* cultivars of *Dioscorea rotundata* were assayed for these enzymes after the tubers had been stored for 6 months, while the *umudike* cultivar of *D. alata* was assayed after the tubers had been stored for 12 months. Figure 2 shows that all five enzymes of purine degradation in the water yam after storage increased in activity. This would cause a corresponding increased degradation of the purines and increased the loss of $NH_3$. This suggests that the root tubers could lose nitrogen during storage. Figure 2 also shows that, for the other three yam cultivars, while some of the enzymes increased in activity, others decreased

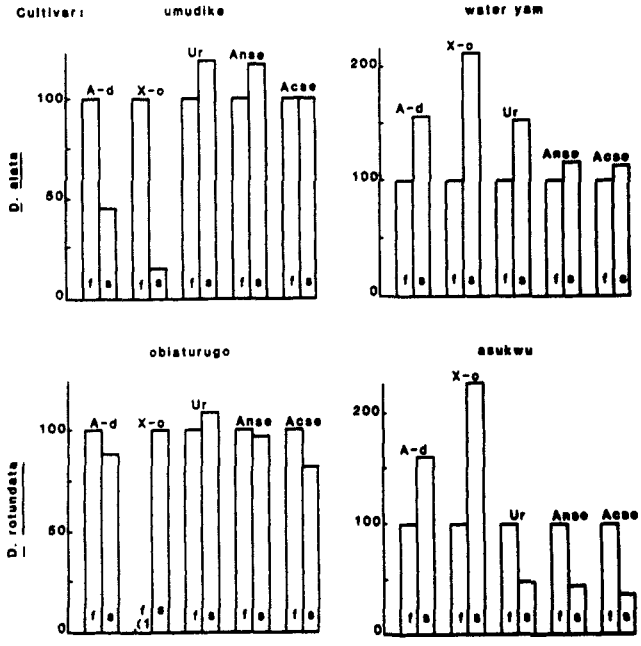


**Figure 2.** Patterns of changes in activities of five enzymes of the purine degradative pathway in freshly harvested and stored yam tubers. Freshly harvested tubers (less than 1 month from harvest) were assayed for the enzymes, and these activities were arbitrarily assigned 100% value. The tubers after 6-months storage were again assayed for activities of the enzymes. These activities were expressed as the percentage of activities of the enzymes from freshly harvested tubers. For each cultivar, activities of the five enzymes were assayed from the same extract made from the same tubers. Activities of an enzyme from different tubers of the same cultivar were not averaged, but the patterns of changes in enzyme activities in each cultivar between freshly harvested and stored tubers were always reproducible. Abbreviations: f = freshly harvested tuber; s = stored tuber; A-d = adenosine deaminase; x-o = xanthine oxidase; UR = uricase; Anse = allantoinase; Acse = allantoicase. Other conditions are as described in text.

in activity, possibly an attempt by the cultivar to minimize the loss of nitrogen. This regulation of purine degradation may be one index for selection in breeding of tubers for high nitrogen content; tubers with decreasing activities of the purine degradative enzymes would be preferred to those that increase in activity during storage.

These losses of nitrogen from the purine degradative pathway prompted us to determine the nitrogen contents of yam tubers before and after storage. The tubers are traditionally stored in barns in the tropics for several months and are eaten as a major carbohydrate staple, but as a minor source of nitrogen (Ikediobi, 1985). On the basis of Kjeldahl analyses of protein and nonprotein nitrogen before and after storage, the *asukwu* cultivar lost 15% of its total nitrogen and 50% of the NPN during 6-months storage, while the *umudike* cultivar lost 31% of its total nitrogen and 65% the NPN during 12-months storage. The loss of ammonia via purine degradation results in a net loss of nitrogen from the tubers. Such losses of nitrogen are significant for the tubers because they are already low in nitrogen (<8% nitrogen by dry weight) and they do not assimilate nitrogen during storage.

These tubers are staples for many people in the tropics (Coursey, 1967). Since they lose a high percentage of their already low nitrogen content during storage through purine degradation, it becomes necessary either to consume all tubers soon after harvest or to process them to products that can retain the nitrogen upon storage. The traditional habit of yam storage practiced in the yam zone of West Africa must now be seen as a wasteful preservation, since the tubers lose significant amounts of nitrogen during the period. Although the tubers are eaten as food because of their carbohydrate contents, the little protein present can also be conserved for improved nutritional benefits, if the tubers are processed to stable products immediately after harvest.

**Registry No.** 1, 9026-93-1; 2, 9002-17-9; 3, 9002-12-4; 4, 9025-20-1; 5, 37289-13-7; 6, 9025-21-2; $N_2$, 7727-37-9; purine, 120-73-0.

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