Effects of α-Ketoglutarate on the Activities of the Glutamate Synthase, Glutamate Dehydrogenase, and Aspartate Transaminase of Sweet Potato, Yam Tuber, and Cream Pea

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The regulation of ammonium ion salvage by α-ketoglutarate (αKG) in crops was investigated by photometric assays of the activities of glutamate synthase (GOGAT), glutamate dehydrogenase (GDH), and aspartate transaminase (GOT), isolated from yam and sweet potato tubers (protein-deficient crops) and cream pea (protein-rich crop). Whereas the cream pea GDH and GOGAT were not inhibited by αKG, the yam tuber GDH was competitively inhibited (K_i of 1.3 mM) and the sweet potato GOGAT was noncompetitively inhibited (K_i of 1.0 mM) by αKG. The K_m values of GDH and GOGAT for αKG were higher in the yam and sweet potato than in the cream pea. The GOTs were not inhibited by αKG. By inhibiting the GDH of yam tuber and the GOGAT of sweet potato, αKG may be reducing the efficiencies of the crops in ammonium ion salvage and in protein storage.

INTRODUCTION

The storage organs of food crops constitute the major staples of the world because on the average they provide about 68% of the world's dietary protein. Furthermore, low-income countries derive up to 85% of their dietary protein from crops because of the increasing cost of animal food products (Meyer and Rask, 1984). But most food crops, especially tubers, are deficient in protein. Although success has been made in the enhancement of the essential amino acid composition of tubers by genetic engineering techniques (Dodds, 1989), success is yet to be achieved in the enhancement of the total proteins of crops. These data emphasize the need for the development of new technologies for increasing the protein storage capabilities of food crops. There have been attempts to understand the biochemical basis of the protein deficiency of tubers, especially yam and sweet potato (Osuji and Umezurike, 1985). Recently, it was found that yam and, to some extent, sweet potato lose ammonia during storage (Osuji and Ory, 1986, 1987; Osuji et al., 1986). Questions arising from this include whether the tubers have the ability to salvage the ammonium ions normally generated from other metabolic reactions and whether there is a relationship between their protein deficiency and their tendency to lose ammonia. Yam tuber proteins have a half-life of about 56 weeks (Osuji and Umezurike, 1985). This short half-life may be a consequence of the loss of ammonia by the tubers during storage. Yam tubers are usually stored at ambient temperatures for up to 9 months during consumption (Osuji et al., 1986). Onayemi and Idowu (1988) have also reported decreases in the protein contents of yam tubers during tuber storage.

The two pathways that salvage ammonia in plants utilize α-ketoglutarate as a common substrate (Lea and Miflin, 1974; Givan, 1979; Lea et al., 1990). The key enzymes that utilize α-ketoglutarate to salvage ammonia are glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH). The resulting glutamate is then utilized by transaminases, for example, aspartate transaminase (GOT), for the reversible synthesis of other amino acids (Ireland and Joy, 1990). The kinetics of the interaction of α-ketoglutarate with the enzymes of ammonium ion salvage would therefore indicate some aspects of the substrate-dependent regulation of the two pathways of ammonium ion salvage. The biological functions of the enzymes of the two pathways have been studied mainly from the point of view of their ammonium ion dependent activities (Blevins, 1989), but the comparative effects of α-ketoglutarate on ammonium ion salvage in different crop species have not been studied. In this paper we report the regulation by α-ketoglutarate of ammonium ion salvage in yam tuber and sweet potato (protein-deficient crops). The cream pea was used as a positive control since it does not lose ammonia during storage and it is a protein-rich crop.

MATERIALS AND METHODS

Materials. Yam tuber (*Dioscorea alata* L.), sweet potato tuber (*Ipomoea batatas* L. cv. Georgia red), and zipper cream pea (*Pisum sativum* L.) seeds were freshly harvested crops and were purchased from the green grocery. They were not allowed to dry before they were used for the experiments. The yam and sweet potato tubers were peeled and cut into roughly 1-cm^3 pieces and immediately stored in 10-g portions in plastic bags at −20 °C as described earlier (Osuji et al., 1986). The cream pea seeds were also stored frozen at −20 °C in plastic bags if they were not used soon after purchase. Biochemicals were purchased from Sigma Chemical Co., St. Louis, MO.

Extraction of Glutamate Dehydrogenase. Plant material (50 g), while still frozen, was homogenized with an equal volume of ice-cold extraction buffer (Loyola-Vargas and Jimenez, 1984) in a food blender at maximum speed for 3 min. All extractions were performed at below 3 °C. The homogenate was clarified by centrifugation at 5000*g* for 10 min. The supernatant liquid was decanted and frozen at −20 °C for 30 min. After thawing at 0 °C, it was recentrifuged at 10000*g* for 10 min. The supernatant liquid was treated with solid ammonium sulfate, and the protein which precipitated between 40 and 60% ammonium sulfate saturation (King and Wu, 1971) was collected by centrifugation (10000*g* for 10 min); after suspension in a minimum volume of extraction buffer, the protein was dialyzed against three changes (2 L each change) of 0.05 M Tris-HCl buffer (pH 8.2) at 2 °C to remove the ammonium ions. After dialysis, the extracts were 20, 36, and 25 mL for sweet potato, yam tuber, and cream pea, respectively. The dialyzed enzymes were used for glutamate dehydrogenase assays and for gel electrophoresis.

Extraction of Glutamate Synthase. Frozen plant material (50 g) was blended with an equal volume of ice-cold 0.1 M potassium phosphate buffer (pH 8) containing 0.1 M KCl, 0.1%

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Table I. Kinetic Constants of Reactions of αKG and Other Substrates with the GDH, GOGAT, and GOT of Yam Tuber, Sweet Potato, and Cream Pea

enzyme	varied fixed substrates[a]	kinetic constants	values (±SE) sweet potato	yam tuber	cream pea
GDH	NH_4^+, αKG	V_{max}, mmol min^{-1} g^{-1}	0.1 ± 0.01	0.2 ± 0.02	0.5 ± 0.03
GDH	αKG	K_i, mM		1.3 ± 0.1	
GDH	αKG	K_m, mM	8.0 ± 0.9		4.0 ± 0.4
GOGAT	Gln, αKG	V_{max}, mmol min^{-1} g^{-1}	0.1 ± 0.01	0.05 ± 0.004	0.12 ± 0.01
GOGAT	αKG	K_i, mM	1.0 ± 0.05		
GOGAT	αKG	K_m, mM		11.0 ± 1.5	0.5 ± 0.03
GOT	Asp, αKG	V_{max}, mmol min^{-1} g^{-1}	0.5 ± 0.07	0.13 ± 0.01	0.78 ± 0.06
GOT	αKG	K_m, mM	0.2 ± 0.01	0.07 ± 0.005	0.06 ± 0.008

[a] In the GDH assays, where αKG was the varied fixed substrate, NH_4^+ was the varied substrate; in GOGAT assays, where αKG was the varied fixed substrate, L-Gln was the varied substrate; in GOT assays, where αKG was the varied fixed substrate, L-Asp was the varied substrate.

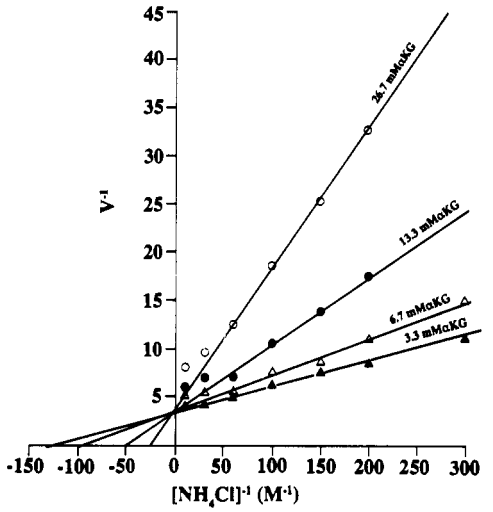

Figure 1. Double-reciprocal plots of velocity of GDH against varied NH_4Cl concentration in the presence of a constant concentration of NADH (0.1 mM) with αKG held at varied fixed levels. Assay was carried out with $(NH_4)_2SO_4$-precipitated GDH from yam tuber. Velocities are mmol min^{-1} (g of fw)$^{-1}$ of crop.

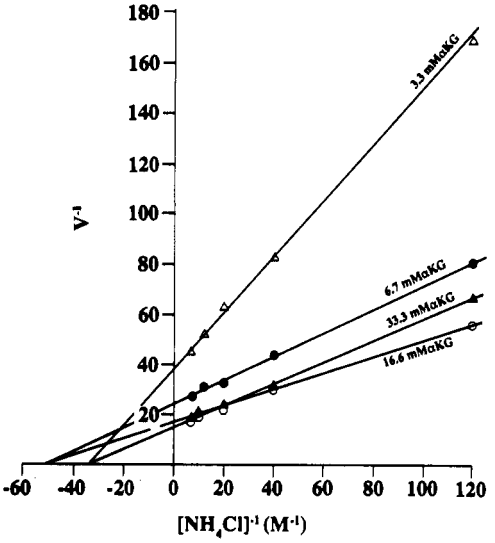

Figure 2. Double-reciprocal plots of velocity of GDH against varied NH_4Cl concentration in the presence of a constant concentration of NADH (0.1 mM) with αKG held at varied fixed levels. Assay was carried out with $(NH_4)_2SO_4$-precipitated GDH from sweet potato. Velocities are mmol min^{-1} (g of fw)$^{-1}$ of crop.

(v/v) Triton X-100, and 14 mM 2-mercaptoethanol (Lea et al., 1990) in a food blender at maximum speed for 2 min. The homogenate was centrifuged at 15000*g* for 10 min. For yam tuber and cream pea, the supernatants were treated with solid ammonium sulfate, and the protein which precipitated between

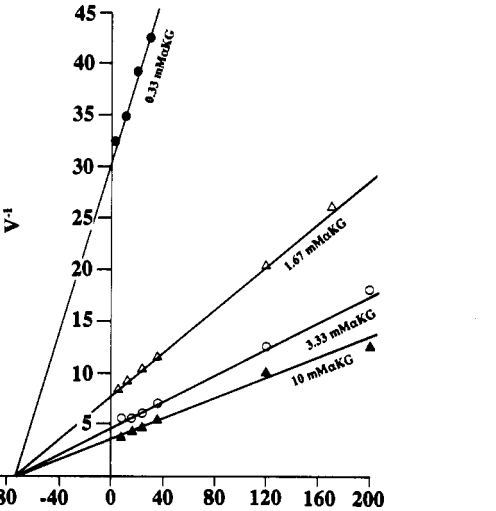

Figure 3. Double-reciprocal plots of velocity of GDH against varied NH_4Cl concentration in the presence of a constant concentration of NADH (0.1 mM) with αKG held at varied fixed levels. Assay was carried out with $(NH_4)_2SO_4$-precipitated GDH from cream pea. Velocities are mmol min^{-1} (g of fw)$^{-1}$ of crop.

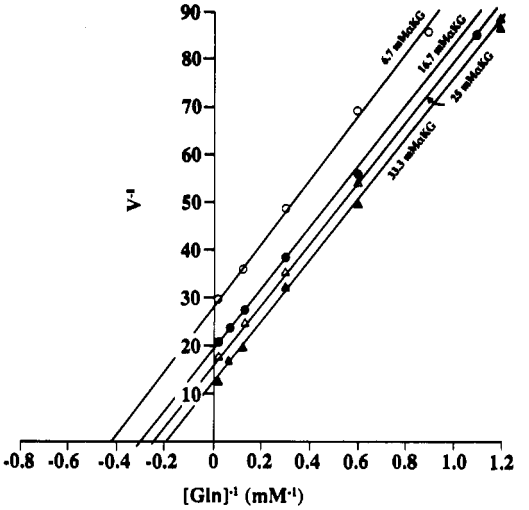

Figure 4. Double-reciprocal plots of velocity of GOGAT against varied L-glutamine concentrations in the presence of NADH (0.1 mM) with αKG held at varied fixed levels. GOGAT precipitated with $(NH_4)_2SO_4$ from yam tuber was used for the assay. Velocities are mmol min^{-1} (g of fw)$^{-1}$ of crop.

30 and 60% ammonium sulfate saturation contained the glutamate synthase. In the case of sweet potato, the protein which precipitated between 20 and 70% ammonium sulfate saturation contained the glutamate synthase. The protein precipitates containing the glutamate synthase activities were suspended in

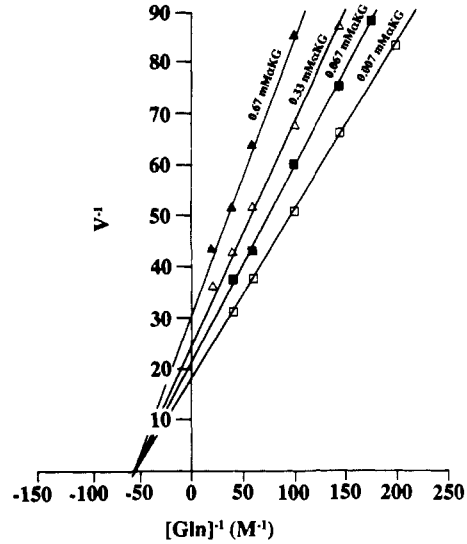

Figure 5. Double-reciprocal plots of velocity of GOGAT against varied L-glutamine concentrations in the presence of NADH (0.1 mM) with αKG held at varied fixed levels. GOGAT precipitated with $(NH_4)_2SO_4$ from sweet potato was used for the assay. Velocities are mmol min^{-1} (g of fw)$^{-1}$ of crop.

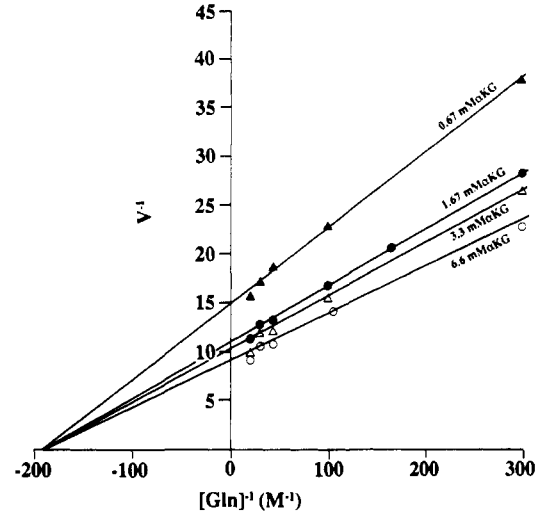

Figure 6. Double-reciprocal plots of velocity of GOGAT against varied L-glutamine concentrations in the presence of NADH (0.1 mM) with αKG held at varied fixed levels. GOGAT precipitated with $(NH_4)_2SO_4$ from cream pea was used for the assay. Velocities are mmol min^{-1} (g of fw)$^{-1}$ of crop.

minimum volumes of 0.05 M Tris-HCl buffer (pH 8) and dialyzed against three changes (2 L each change) of the 0.05 M Tris-HCl buffer containing 0.01 M KCl and 0.1% (v/v) 2-mercaptoethanol at 2 °C. The dialyzed enzyme volumes were 64, 27, and 42 mL for sweet potato, yam tuber, and cream pea, respectively. The dialyzed enzymes were used for glutamate synthase assays.

Extraction of Aspartate Transaminase. Frozen plant material (50 g) was homogenized with 150 mL of ice-cold extraction buffer [0.5 M borate buffer (pH 8.5) containing 0.1% (v/v) 2-mercaptoethanol and 1% poly(vinylpyrrolidone)], at maximum speed for 2 min in a food blender. The homogenate was centrifuged at 10000*g* for 15 min, and the pellet was discarded. To the supernatant was added cold acetone (−30 °C) to 25% (v/v) saturation (Kermasha et al., 1990). Protein was allowed to precipitate at −80 °C for 15 min and then centrifuged out at 10000*g* for 10 min. The pellet was discarded. To the supernatant was added cold acetone (−30 °C) to 60% (v/v) saturation. Transaminase was allowed to precipitate at −80 °C for 15 min and was collected by centrifugation at 15000*g* for 10 min. The pellet was suspended in a minimum volume of 0.1 M borate buffer (pH 8.5) and dialyzed against the same buffer at 2 °C. There were three changes of buffer during dialysis, each change being 2 L. Enzyme volumes after dialysis were 50, 20, and 42 mL for

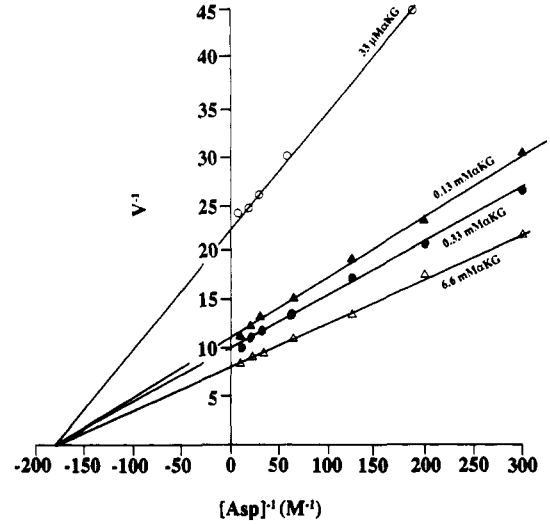

Figure 7. Double-reciprocal plots of the velocity of GOT against varied L-aspartate concentrations with αKG held at varied fixed levels. GOT precipitated with acetone from yam tuber was used for the assay. Velocities are mmol min^{-1} (g of fw)$^{-1}$ of crop.

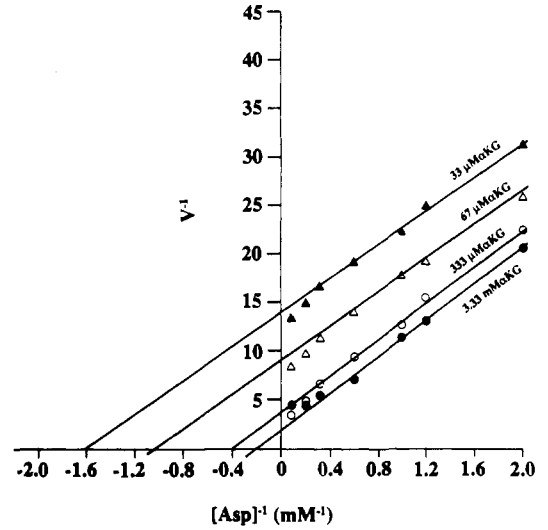

Figure 8. Double-reciprocal plots of the velocity of GOT against varied L-aspartate concentrations with αKG held at varied fixed levels. GOT precipitated with acetone from sweet potato was used for the assay. Velocities are mmol min^{-1} (g of fw)$^{-1}$ of crop.

cream pea, sweet potato, and yam tuber, respectively. The dialyzed extracts were used for aspartate transaminase assays.

All the partially purified extracts were stored frozen in 2-mL aliquots at −20 °C, and there were no significant (±2%) changes in activity for about 2 weeks during which the assays were conducted.

Glutamate Dehydrogenase (EC 1.4.1.3) Assay. The method of Garland and Dennis (1977) was used. All substrates were prepared in 0.1 M Tris-HCl buffer (pH 8.2). Concentration ranges of 0.3–33 mM α-ketoglutarate and 3–530 mM NH_4Cl were used. The concentrations of NADH and $CaCl_2$ were fixed at 0.1 and 1.3 mM, respectively. Reaction was started by addition of 0.2 mL of dialyzed enzyme. Final volume of the reaction mixture was 3.0 mL. Rates of reaction were determined by the decrease of absorbance at 340 nm in a Beckman DU-64 spectrophotometer.

Glutamate Synthase (EC 1.4.1.13) Assay. The method of Beevers and Storey (1976) was used. All substrates were prepared in 0.1 M potassium phosphate buffer (pH 7.5). Concentration ranges used were 0.3–33.3 mM α-ketoglutarate and 0.3–67 mM L-glutamine. The concentration of NADH was fixed at 0.1 mM. Reaction was started by addition of 0.2 mL of dialyzed enzyme. Final volume of the reaction mixture was 3.0 mL. Rates of reaction were determined by measuring the decrease of absorbance at 340 nm.

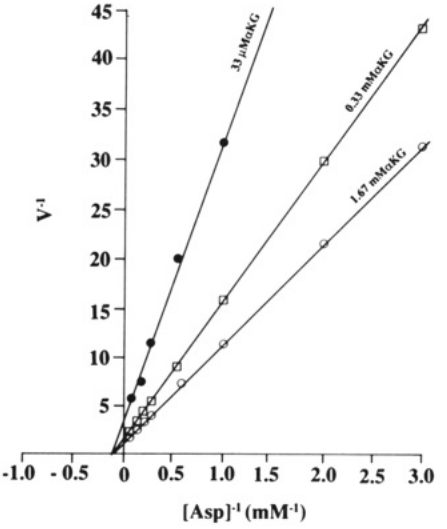

Figure 9. Double-reciprocal plots of the velocity of GOT against varied L-aspartate concentrations with αKG held at varied fixed levels. GOT precipitated with acetone from cream pea was used for the assay. Velocities are mmol min^{-1} (g of fw)$^{-1}$ of crop.

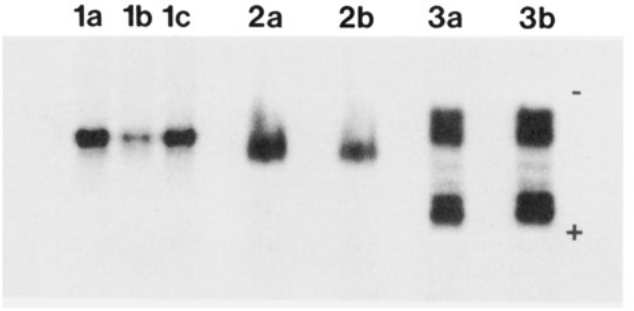

Figure 10. Nondenaturing PAGE patterns of the GDHs precipitated with $(NH_4)_2SO_4$ from sweet potato (1), yam tuber (2), and cream pea (3). Different concentrations of sweet potato GDH (1a–c), yam tuber GDH (2a and 2b), and cream pea GDH (3a and 3b) were electrophoresed to define clearly the isoenzyme bands. After electrophoresis, the gel was activity stained for GDH with tetrazolium bromide reagent.

Aspartate Transaminase (EC 2.6.1.1) Assay. The method of Murray (1980) was used. Concentration ranges of 30 μM–16.7 mM α-ketoglutarate and 0.3–67 mM L-aspartate were used. Fixed concentrations of NADH (0.1 mM), pyridoxal phosphate (1.0 μM), and malate dehydrogenase (10 units) were used. All the substrates were prepared in 0.2 M Tris-HCl buffer (pH 7.5). Reaction was started by addition of 0.2 mL of the dialyzed enzyme. Final volume of the reaction mixture was 3.0 mL. Rates of reaction were followed by measuring the decrease in absorbance at 340 nm.

Enzyme activities were expressed as millimoles of NADH oxidized per minute per gram of fresh weight (fw) of crop material. Each assay was carried out in triplicate on the same dialyzed enzyme, and reproducibility was generally within 5%.

Gel Electrophoresis. The dialyzed glutamate dehydrogenases of the crops were subjected to polyacrylamide gel electrophoresis (PAGE) in nondenaturing (without SDS) 7.5% polyacrylamide slab gel (Laemmli, 1970) in Tris–glycine buffer (pH 8.9). The enzyme solutions were mixed with equal volumes of sample buffer without SDS (Davis et al., 1986) and loaded into gel wells: from the 250 μmol min^{-1} mL^{-1} solution of sweet potato glutamate dehydrogenase, activities of 3.1, 0.6, and 2.5 μmol min^{-1} were prepared and loaded into gel wells 1a, 1b, and 1c, respectively; from the 278 μmol min^{-1} mL^{-1} solution of yam tuber glutamate dehydrogenase, activities of 3.5 and 1.4 μmol min^{-1} were loaded into wells 2a and 2b, respectively; from the cream pea glutamate dehydrogenase solution of activity of 1000 μmol min^{-1} mL^{-1}, 4 and 7 μmol min^{-1} activities were prepared and loaded into gel wells 3a and 3b, respectively. The gel electrophoresis was for 4 h at 100 V (Davies et al., 1986). After activity staining of the gel for 1 h with the tetrazolium bromide reagent (Garland and Dennis, 1977), the gel was destained by three rapid changes of 0.015 M Tris-HCl buffer.

Protein Assay. The protein contents of the enzyme preparations were determined with the Biuret reagent (Gornal et al., 1949) using bovine serum albumin as standard.

RESULTS

The purification by fractional precipitation of the enzymes gave about 10-fold purification of the glutamate synthases and about 20-fold purification of the glutamate dehydrogenases and aspartate transminases. The exception was the glutamate synthase of sweet potato, whose purification was only 5-fold because the activities of the enzyme were spread over a wide percentage (20–70%) of ammonium sulfate saturation. The partial purification of the enzymes minimized such interfering enzyme activities as the NADH oxidases (Beevers and Storey, 1976).

The initial velocities of each enzyme from each crop were used to construct Lineweaver–Burk double-reciprocal plots. The plots are arranged as shown in Figures 1–9. Replots (not shown) were then constructed as described by Michal (1973) and Segel (1975).

Effect of α-Ketoglutarate on Glutamate Dehydrogenase Activity. Figure 1 shows that α-ketoglutarate competitively inhibited the glutamate dehydrogenase of yam tuber. Stone et al. (1980) had demonstrated that glutarate, an analogue of α-ketoglutarate, inhibited the glutamate dehydrogenase of lupin nodules competitively. Replot of slopes against α-ketoglutarate concentrations gave a straight line from which the inhibition constant of 1.3 mM was deduced (Table I). Figure 2 shows an ordered mechanism in which high concentrations of α-ketoglutarate (>33 mM) (Segel, 1975) inhibited the sweet potato glutamate dehydrogenase but low concentrations were normal. Replot of $1/v$ axis intercepts against the reciprocals of α-ketoglutarate concentrations gave a straight line and a K_m of 8 mM (Table I). The double-reciprocal plots for cream pea glutamate dehydrogenase (Figure 3) intersected like those of safflower GDH (Errel et al., 1973). Replot of $1/v$ axis intercepts vs the reciprocals of α-ketoglutarate concentrations gave the K_m of 4 mM.

Therefore, α-ketoglutarate did not inhibit the glutamate dehydrogenase of cream pea (protein-rich crop) but did inhibit that of yam tuber and caused that of sweet potato to possess a high K_m.

Effect of α-Ketoglutarate on Glutamate Synthase Activity. The double-reciprocal plots for yam tuber glutamate synthase (Figure 4) were parallel, and the replot of the $1/v$ axis intercepts vs the reciprocals of α-ketoglutarate concentrations gave the K_m of 11 mM (Table I). The double-reciprocal plots for sweet potato glutamate synthase (Figure 5) intersected on the horizontal axis, but α-ketoglutarate, even at low concentrations, noncompetitively inhibited the enzyme. A replot of the $1/v$ axis intercepts against α-ketoglutarate concentrations gave the inhibition constant of 1 mM. The double-reciprocal plot for cream pea glutamate synthase (Figure 6) shows that α-ketoglutarate did not inhibit the enzyme. A replot of the $1/v$ axis intercepts against the reciprocals of α-ketoglutarate concentrations gave a K_m of 0.5 mM.

Therefore, as with the glutamate dehydrogenase of the crops, α-ketoglutarate did not inhibit the glutamate synthase of the cream pea but inhibited that of the sweet potato (protein-deficient crop) and caused that of the yam tuber (protein-deficient crop) to possess a high K_m.

Effect of α-Ketoglutarate on Aspartate Transaminase Activity. The double-reciprocal plots of the yam tuber aspartate transaminase (Figure 7) and cream pea

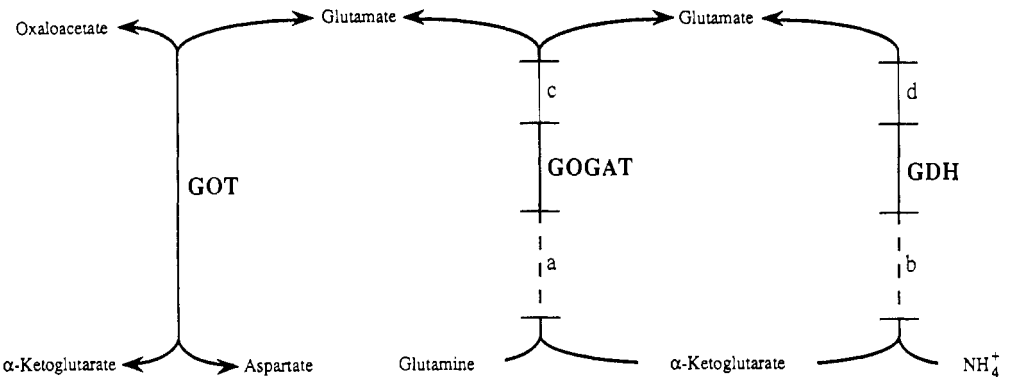

Figure 11. Regulation of ammonium ion salvage by αKG in the sweet potato, yam tuber, and cream pea: (a) inhibition of sweet potato GOGAT and (b) inhibition of yam tuber GDH by αKG; (c) high K_m of yam tuber GOGAT and (d) high K_m of sweet potato GDH for αKG. The cream pea GDH and GOGAT are not inhibited by αKG, and they have lower K_m values for αKG as compared to those of yam and sweet potato.

aspartate transaminase (Figure 9) intersected, whereas those of the sweet potato (Figure 8) were parallel. There was no inhibition of the enzyme by α-ketoglutarate. Replots gave the K_m for α-ketoglutarate of 0.07, 0.2, and 0.06 mM for the aspartate transaminase of yam, sweet potato, and cream pea, respectively. The kinetic constants of the aspartate transaminases of the crops therefore deviated from the patterns of the glutamate dehydrogenases and glutamate synthases in that the K_m of the aspartate transaminase of yam was the same as that of the cream pea even though cream pea has more protein than yam tuber.

Electrophoretic Fractionation Patterns of the Glutamate Dehydrogenases. The partially purified glutamate dehydrogenases of the crops were electrophoresed, and their isoenzymic patterns are shown in Figure 10. Different concentrations of the enzyme were applied on the gel to define clearly the number of isoenzyme bands. Sweet potato glutamate dehydrogenase gave only one band, yam tuber gave one band in addition to a slow moving high molecular weight material that persistently appeared as a smear and cream pea gave six isoenzyme bands. The glutamate dehydrogenases of higher plants are known to possess multiple electrophoretic bands (Errel et al., 1973; Cammaerts and Jacobs, 1985; Lauriere and Daussant, 1983). The advantages of the multiple isoenzymes of glutamate dehydrogenase are not yet known, but the results in Figure 10 together with the kinetic properties of the enzyme in Table I appear to indicate that multiple isoenzymes ensure high enzyme activities.

DISCUSSION

The kinetic constants shown in Table I for the glutamate dehydrogenases are generally in the range obtained for higher plants (Errel et al., 1973; Garland and Dennis, 1977; Yamaya et al., 1984). Cream pea glutamate synthase has a K_m which is within the range obtained for other plants (Beevers and Storey, 1976; Lea et al., 1990), but the K_m for yam tuber glutamate synthase is higher than the range reported for other plants. The α-ketoglutarate inhibition of sweet potato glutamate synthase is the first time the substrate has been observed to inhibit the enzyme.

Table I also shows that α-ketoglutarate inhibits either the glutamate dehydrogenase or the glutamate synthase of the protein-deficient crops. Yam and sweet potato tubers lose ammonia during storage (Osuji and Ory, 1986, 1987). The inhibition by α-ketoglutarate of the glutamate dehydrogenase of yam tuber and the glutamate synthase of sweet potato could be responsible for the inefficiencies of the tubers in ammonium ion salvage.

Table II. Protein Contents of the Partially Purified GDHs, GOGATs, and GOTs of Yam Tuber, Sweet Potato, and Cream Pea Used in the Enzyme Assays

	protein contents of enzyme preparations,[a] mg/mL		
enzyme	yam tuber	sweet potato	cream pea
GDH	5.5 (3.96)	3.5 (1.26)	14.0 (7.0)
GOGAT	6.2 (3.35)	8.6 (11.0)	8.6 (7.2)
GOT	3.0 (2.52)	4.0 (1.6)	11.5 (11.5)

[a] In parentheses are the protein contents of the enzyme preparations expressed in mg (g of fw)$^{-1}$ of crops.

Cream pea glutamate dehydrogenase and glutamate synthase are not inhibited by α-ketoglutarate, and consequently the crop does not lose ammonia during storage. These results suggest that the storage organs of crops are dependent on the activities of glutamate synthase and glutamate dehydrogenase for ammonium ion salvage. If either of the two enzymes were dispensable in ammonium ion salvage, yam tuber or sweet potato tuber would have been efficient in ammonium ion salvage.

Table I shows that the kinetic constants of glutamate synthase and glutamate dehydrogenase increased from the protein-rich crop to the protein-deficient crops. The V_{max} in particular of the glutamate dehydrogenases increased from sweet potato and yam tuber to the cream pea. The expression of the velocities of the enzyme reactions in terms of the fresh weight of the crops (Table I) rather than of the concentrations of the protein contents of the enzyme extracts (Table II) permitted the demonstration of the relationship between the capabilities of crops to salvage ammonia and the protein contents of the crops. The protein contents of the dialyzed enzyme extracts (Table II) do not have a direct relationship to the protein contents of the crops. These results, taken together with the inhibition of the glutamate dehydrogenase of yam tuber and of the glutamate synthase of sweet potato tuber, suggest that the activities of the two enzymes are a direct function of the protein contents of the crops. Yam tuber, sweet potato tuber, and the leguminous seeds could therefore be useful experimental materials for probing the biological roles of the two enzymes in ammonium ion salvage and storage protein contents of food crops. Rhodes et al. (1989) have also suggested that glutamate dehydrogenase could be involved in the photorespiratory assimilation of ammonia.

The kinetic constants of the aspartate transaminases (Table I) of the crops do not fit the trends of the glutamate dehydrogenases and glutamate synthases since the yam tuber aspartate transaminase has the same K_m for α-ketoglutarate as the cream pea aspartate transaminase. Aspartate transaminase is not involved in the primary

step of ammonium ion salvage. This may explain the deviation of its kinetic constants from the patterns of the glutamate dehydrogenases and glutamate synthases of the crops.

Glutamate synthase and glutamate dehydrogenase catalyze the formation of an α-iminoglutarate intermediate Schiff base (central complex) (Smith et al., 1975; Miflin et al., 1981). Schiff bases are regulators in enzyme-catalyzed reaction mechanisms because they function as electron sinks (Fersht, 1985). The α-ketoglutarate-dependent Michaelis constants in Table I can therefore be examined as the dissociation constants (K_s) of the Schiff base complexes (Michal, 1983; Dalziel, 1975), because hydrolysis of the complex regenerates α-ketoglutarate, whereas reduction by NADH yields glutamate (Smith et al., 1975). Since the glutamate synthases and glutamate dehydrogenases of protein-deficient crops generally have higher dissociation constants as compared to those of the protein-rich crops, it follows that the protein-deficient crops have greater tendency than the protein-rich crops to hydrolyze their intermediate Schiff base complexes back to α-ketoglutarate. This is the case with sweet potato glutamate dehydrogenase, which has a higher K_m than cream pea glutamate dehydrogenase for α-ketoglutarate; also, yam tuber glutamate synthase has a higher K_m than cream pea glutamate synthase (Table I). When protein deficiency of a crop becomes extreme, the glutamate synthase and glutamate dehydrogenase may become inhibited by α-ketoglutarate. This is the case with yam tuber glutamate dehydrogenase (Figure 1) and sweet potato glutamate synthase (Figure 5), which are inhibited competitively and noncompetitively, respectively, by α-ketoglutarate. The glutamate synthase and glutamate dehydrogenase of cream pea were not inhibited by α-ketoglutarate (Table I). Garland and Dennis (1977) also did not observe any inhibition of the glutamate dehydrogenase of pea seeds by α-ketoglutarate. By inhibiting some of the enzymes of ammonium ion salvage of the protein-deficient crops, α-ketoglutarate makes less of the enzymes available to catalyze ammonium ion salvage. Therefore, both the higher K_m values and the inhibition of the enzymes of ammonium ion salvage of the protein-deficient crop species achieve the same objective, which is to reduce the efficiencies of such crops in the salvage of ammonium ion. Storage protein deficiency of a crop is therefore a function of the ability of α-ketoglutarate to inhibit the enzymes of ammonium ion salvage of the crop. The function of α-ketoglutarate in ammonium ion salvage seems to be the regulation of the activities of glutamate dehydrogenase and glutamate synthase such that protein-deficient crop species are inefficient while protein-rich crop species are efficient in ammonium ion salvage. Therefore, even though the two enzymes catalyze their respective specific reactions in the different crop species, they function under the differential regulatory regimes of α-ketoglutarate. The in vivo concentration of α-ketoglutarate in plants is in the millimolar range (Bowman et al., 1976; Yamaya et al., 1984). The K_m values for α-ketoglutarate of glutamate dehydrogenase and glutamate synthase are also in the millimolar range (Table I). Under normal physiological conditions, therefore, there is sufficient α-ketoglutarate concentration to regulate the activities of the two enzymes as postulated above. Figure 11 summarizes the pathways by which α-ketoglutarate regulates ammonium ion salvage in the storage organs of protein-deficient and protein-rich crop species. Any physiological situation that relieves the inhibitory effects of α-ketoglutarate on the enzymes of ammonium ion salvage would lead to the enhancement of both the ammonium ion salvage and the protein storage capabilities of the protein-deficient crops (unpublished results).

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