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Low Temperature Effects on Soybean (*Glycine max* [L.] Merr. cv. Wells) Free Amino Acid Pools during Germination[1]

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ABSTRACT

The free amino acid concentrations in cotyledons and axes of soybean (*Glycine max* [L.] Merr. cv. Wells) seedlings were determined by automated single column analysis after germination at 10 and 23 C. After 5 days germination at 10 C, glutamate and aspartate were in high concentration in both cotyledons and axes (38 and 24% of total free amino acids recovered, respectively), whereas the concentrations of their amide derivatives, asparagine and glutamine, were low in cotyledons (4.4%) and high in axes (21%). In contrast, after 5 days germination at 23 C, asparagine and glutamine accounted for 22 and 45% of total free amino acids in cotyledons and axes respectively, and aspartate and glutamate concentrations were low. The activities of glutamine synthetase and asparagine synthetase were considerably lower in tissues from the 10 C treatment than those from the 23 C treatment.

Aspartate and glutamate concentrations were nearly equal in all but one sample. Both glutamate oxaloacetate transaminase and glutamate dehydrogenase activities were much higher in axis tissues at 23 C as compared to 10 C. Arrhenius plots of axis glutamate oxaloacetate transaminase and glutamate dehydrogenase activities were biphasic and triphasic, respectively, with energies of activation for both increasing with low temperature. Energies of activation were identical for glutamate oxaloacetate transaminase from 10 and 23 C treatments but much higher for glutamate dehydrogenase from 23 C-treated axes. This indicates a difference in enzyme complement for glutamate dehydrogenase with the two treatments.

Hydrolysis of free amino acid sample (basic fraction) aliquots showed large quantities of peptides in 23 C-treated axes at 2 days, while few or no peptides were found in the 10 C treatment. Amino acid residues most prevalent in peptides were aspartate, threonine, serine, glutamate, and glycine.

In legume germination the proportions of amino acids in storage proteins are not reflected by the proportions of free amino acids which occur during storage protein mobilization of such species as *Glycine max* (5, 15). This is largely due to interconversions of amino acids released from storage proteins to produce both new nonprotein and protein amino acids (2).

Slower soybean germination (as measured by radicle emergence from the testa) at low temperature appears to be due in part to high E_a[3] values at suboptimal germination temperatures for the enzymes involved in energy transduction (10). Inasmuch as amino acid interconversions are in many cases energy-requiring processes, we decided to determine the effects of low temperature on free amino acid pools in soybean seedlings. Also, because we found high levels of aspartate, glutamate, asparagine, and glutamine, we surveyed several enzymes which are considered primary in the metabolism of these amino acids (GDH, GS, AsnS, and GOT). The activities of these enzymes from soybean seedlings grown at low and optimal temperatures appear to be responsible for many of the observed differences in free amino acid pools.

MATERIALS AND METHODS

Plant Material. Soybean (*G. max* [L.] Merr. cv. Wells) seeds were imbibed and germinated at 10 and 23 C as previously described (10). Seeds or seedlings were collected and separated into cotyledons and axes at 2 and 5 days after the beginning of imbibition.

Amino Acid Determinations. Cotyledon and axis samples (2–5 g/sample) were ground in a mortar with 20 ml of warm (45 C) ethanol (80%, w/v). Homogenates were allowed to stand for 10 min and filtered through Whatman No. 1 filter paper. Insolubles were rinsed with 2 to 5 ml of warm (45 C) ethanol (80%, w/v). Flash-evaporated samples (to about 10 ml) were loaded on glass columns (7 × 200 mm) containing 7 to 8 cm of Dowex 50 resin (pH 7.0). Amino acids were eluted with 40 ml of 2 N NH_4OH. Samples were flash-evaporated (45 C) to dryness and dissolved into a volume of 50% (w/v) ethanol so that the free amino acids from 0.1 g of tissue were in 0.2 ml of ethanol. Aliquots (0.2 ml) of samples were flash-evaporated to dryness and dissolved in 0.2 ml of citric acid buffer (67 mM, pH 2.0) containing 1% thiodiglycol. Due to the loss of glutamine in citrate buffer (about 10% loss/day) aliquots were immediately divided into 0.01- to 0.16-ml fractions and loaded on cartridges for analysis on a Technicon TSM amino acid analyzer and analyzed as previously described (24). Due to poor resolution of GABA with some samples on the Technicon analyzer, aliquots of some samples were also analyzed on a Durrum D-500 amino acid analyzer. Determinations of amino acid concentrations with the two amino acid analyzers yielded

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[3] Abbreviations: E_a: energy of activation; GDH: glutamate dehydrogenase (EC 1.4.1.3); GS: glutamine synthetase (EC 6.3.1.2); AsnS: asparagine synthetase (EC 6.3.1.1); GOT: glutamate oxaloacetate transaminase (EC 2.6.1.1); GABA: γ-amino butyric acid; OAA: oxaloacetic acid; MDH: malate dehydrogenase (EC 1.1.1.37); α-KG: α-ketoglutarate; ϕ-Ala: phenylalanine; Asx: aspartate including hydrolyzed asparagine; Glx: glutamate including hydrolyzed glutamine.

data which were the same. Also, free amino acid concentrations were determined for each sample by TLC (11). TLC values were near those for the two amino acid analyzers in the majority of cases although amino acids found in low concentrations could not be detected in every case by TLC. Some samples were subjected to hydrolysis (24) to determine if peptides were present. All amino acid data are expressed as μmol g^{-1} fresh weight. Abbreviations of amino acids in the table and figures are as suggested by the IUPAC-IUB Commission (14) with the exceptions of those listed under footnotes and where noted in the text

Amino Acids From [^{14}C]Aspartate. Small holes were made in the center of cotyledons (one hole/seedling) of 5-day-old seedlings grown at 23 C by the removal of a plug of tissue with a Pasteur pipette. Seedlings were then placed, hole up, in Petri dishes containing moist filter paper, and 10 μl containing 0.5 μCi of L-[^{14}C-U]-aspartate (2.63 μmol titrated to pH 7.0 with NaOH) were added to each hole. Seedlings were harvested at 0, 15, 60, 120, and 180 min after the introduction of [^{14}C]aspartate, washed thoroughly with distilled H_2O, and divided into axes and cotyledons. Samples contained two or three axes or cotyledons. Amino acids were extracted as described for amino acid determinations with the exception that after the final flash evaporation they were dissolved with 0.5 ml of 80% (w/v) ethanol. Amino acids were separated by TLC (12) using 20-μl aliquots. Labeled amino acids were located on TLC plates by autoradiography as described before (13) and were quantified by lightly spraying plates with 1% (w/v) ninhydrin in acetone, removing spots, eluting spots in 0.5 ml of 80% (w/v) ethanol, and counting each amino acid in a Beckman liquid scintillation spectrometer in a cocktail containing 750 ml of xylene, 250 ml of Triton X-114, and 6 g of 2,5-diphenyloxazole. All ^{14}C-amino-acid data are expressed as dpm g^{-1} fresh weight.

Enzyme Extraction and Assay. Seedlings were separated into cotyledons and axes at 2 and 5 days from the beginning of imbibition with samples (5–36 seedlings) taken from the 10 and 23 C treatments. Axes (0.2–2.1 g) were ground in a mortar with 5.0 ml of extraction medium (25 mM Tricine, 25 mM HEPES [pH 8.0], 2% [w/v] casein [30 mesh, Sargent Welch Chem. Co.], 0.4 M sucrose). Cotyledons (1.7–2.0 g) were ground for 1 min in a VirTis 60K homogenizer at 80% top speed with 20 ml of extraction medium. Homogenates were filtered through four layers of cheesecloth and then centrifuged at 500g for 3 min to remove cellular debris. Supernatants (500g) were centrifuged at 15,000g for 30 min to pellet mitochondria. These pellets were resuspended in 2 to 5 ml of extraction medium and freeze-thawed three to five times to solubilize enzymes (8). All operations before freeze-thawing were at about 0 C. GDH[3] and GS assays were as previously described (6) except that routine assays were at 23 C. Hydroxyamate AsnS assays were the same as for GS except that 0.2 ml of 0.4 M aspartate was substituted for glutamate. GOT assays were spectrophotometric (340 nm) by the coupling of GOT production of OAA to MDH production of malate. Endogenous MDH activity was 5 to 50 times higher than that for GOT activity in all preparations. MDH activity was measured as before (9). GOT reaction mixtures contained 2.5 ml of 0.1 M Tris-HCl (pH 7.5), 0.2 ml of 1.0 mM NADH, 0.1 ml of 0.33 M α-ketoglutarate (α-KG) at pH 6.5, 0.1 ml of 0.33 M L-aspartate (pH 6.5), and 0.1 ml of enzyme preparation. Order of addition of substrate had little or no effect on GOT activity. Endogenous NADH oxidation was very low in all preparations (0 to 5% of GOT or GDH activity). Enzyme assays for Arrhenius plots were conducted with 0.1 M HEPES (pH 7.5) as the reaction buffer to avoid the larger changes in pH with temperature change that occur when using Tris. E_as were calculated as before (10) except where noted. All enzyme activities are expressed as either nmol or μmol of NADH oxidized or product formed min^{-1} g^{-1} fresh weight or $axis^{-1}$ with the exceptions of Arrhenius plots where $V_{max(apparent)}\Delta OD$ min^{-1} for a given volume of enzyme extract is used.

RESULTS

Free Amino Acids. The concentration of total free amino acids extracted from cotyledons of soybeans germinated for 2 days at 10 C was only 25% of that from 2-day-old 23 C-treated cotyledons; whereas, total concentrations of free amino acids from axes of the two treatments were nearly the same (Fig. 1). After 5 days, the cotyledon concentration of total free amino acids had increased 113% at 10 C, but decreased 62% at 23 C as compared to 2 days. Axis free amino acid concentrations showed little change between 2 and 5 days in the 10 C treatment, contrasting with a 202% increase at 23 C.

Cotyledons after 2 days at 10 C had few small peptides in that only small changes in the concentrations of total free amino acids occurred with hydrolysis (Table I). However, with the 23 C

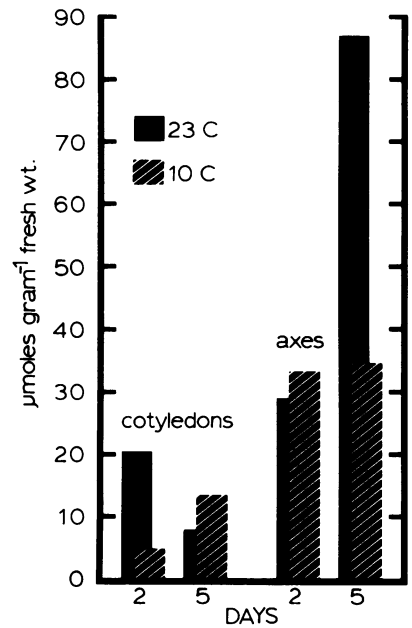

FIG. 1. Total free amino acids from germinating *G. max* seeds at 10 and 23 C. Data were computed by the addition of concentrations of individual free amino acids (Figs. 2 and 3).

Table I. Hydrolyzed aliquots of basic fraction samples from 2-day-old soybean seedlings germinated at 10 and 23 C. Concentrations are in μmoles g^{-1} fresh weight (% change from unhydrolyzed control, Fig. 2). Totals with per cent change from control were calculated only from the amino acids shown. Tryptophan degraded with hydrolysis.

	10 C		23 C	
	cotyledons	axes	cotyledons	axes
Asx	1.07(0)	4.84(0)	4.93(0)	18.61(131)
Thr	0.15(200)	1.27(30)	0.80(43)	1.31(285)
Ser	0.17(-6)	1.73(-64)	1.02(-19)	2.17(178)
Glx	1.22(36)	5.82(0)	6.31(6)	5.84(36)
Pro	0.07(250)	0.18(350)	0.27(35)	0.31(72)
Cys	<0.01(0)	0.15(-53)	0.04(-20)	0.15(-6)
Gly	0.07(600)	0.45(150)	0.16(78)	0.50(257)
Ala	0.18(38)	2.83(29)	1.06(0)	2.75(10)
cystine	0.12(0)	0.27(0)	0.22(69)	0.24(71)
Val	0.31(0)	0.58(33)	0.94(-24)	1.23(6)
Met	0.06(0)	0.36(0)	0.25(25)	0.22(175)
unknown	<0.01(0)	0.01(0)	0(0)	0(0)
Ile	0.08(33)	0.27(0)	0.56(4)	0.57(27)
Leu	0.04(33)	0.23(-34)	0.54(4)	0.56(33)
Tyr	0.24(300)	1.05(69)	0.25(0)	0.22(-260)
β-Ala	<0.01(0)	0.03(0)	0.28(4)	0.05(0)
φ-Ala	0.28(300)	0.93(675)	0.73(31)	0.75(27)
ethanolamine	0.46(2)	1.98(-26)	0.98(-11)	1.92(-10)
ammonia	4.10(163)	6.48(70)	7.21(59)	18.32(1320)
Orn	0.04(∞)	0.27(93)	0.15(∞)	0.40(298)
unknown	0(0)	0.02(0)	0(0)	<0.01(0)
Lys	0.11(83)	0.39(11)	0.30(15)	0.32(60)
His	0.07(75)	0.55(-4)	0.41(0)	1.77(9)
unknown	0(0)	0(0)	0(0)	0.01(-83)
Trp	-	-	-	-
Arg	0.16(14)	4.59(-33)	0.26(13)	2.48(6)
TOTAL	4.93(16)	28.80(-2)	20.68(5)	42.39(61)

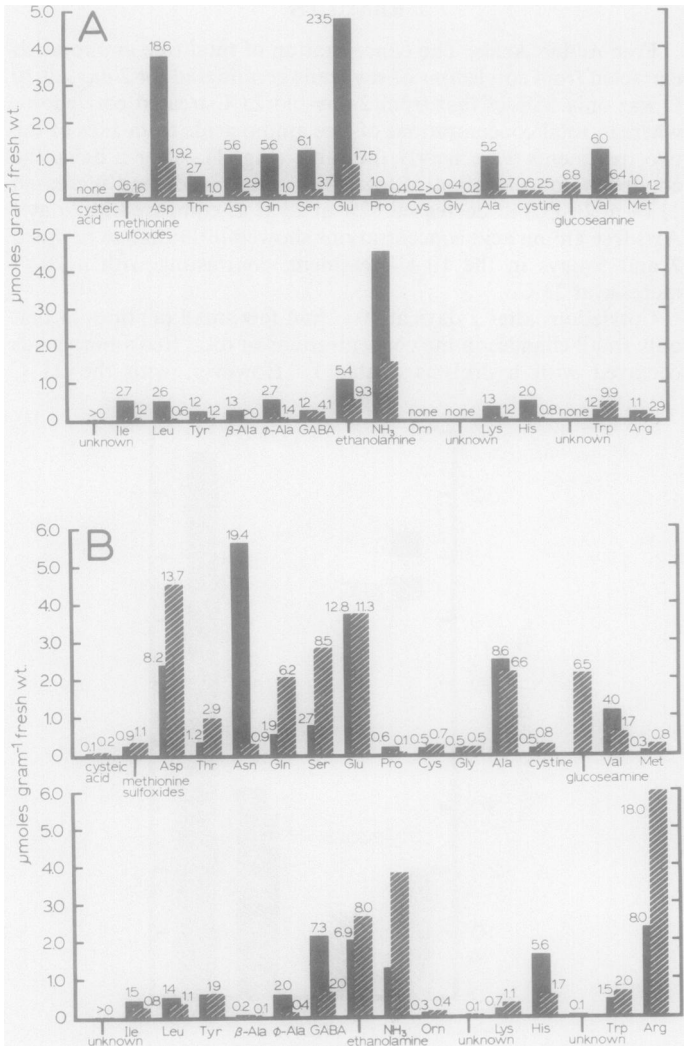

FIG. 2. Distribution and concentrations of free amino acids in *G. max* cotyledons (A) and axes (B) after 2 days germination at 10 C (▨) and 23 C (■). Order of elution on the Technicon TSM amino acid analyzer is left to right beginning with cysteic acid. Percentage of total free amino acids for each amino acid is indicated above each bar.

treatment, concentrations increased by 0.95 and 16.14 μmol g^{-1} fresh wt in acid hydrolysates of axes and cotyledons, respectively, indicating the presence of large quantities of peptides.

At 2 days, cotyledons from the 23 C treatment had large concentrations of free aspartate and glutamate, with the two amino acids accounting for 42.1% of the total (Fig. 2A), whereas concentrations in the 10 C treatment were much lower, yet still accounted for a large proportion of free amino acids (36.7%). At 2 days asparagine and glutamine accounted for 11.2% of the 23 C-treated cotyledon free amino acids as compared to 3.9% for the 10 C treatment. Only glucosamine and tryptophan were higher in concentration in the 10 C-treated cotyledons. Upon hydrolysis large increases in threonine, proline, glycine, tyrosine, phenylalanine, and lysine were seen in the 10 C cotyledon free amino acids; however, all of these were in low concentration before hydrolysis as well as after (Table I and Fig. 2A). Hence, although percentages of change were high, the actual amounts of these amino acids found in the form of peptides were low.

Axes from the 10 C treatment at 2 days had high concentrations of aspartate and glutamate with the two accounting for 25% of the total free amino acids (Fig. 2B). The concentration of glutamate in 23 C axes was near that found in the 10 C treatment, whereas aspartate was considerably lower. Concentrations of asparagine were high and glutamine low in comparison (about 10% of asparagine) in 2-day 23 C axes. The opposite was true, but the difference was not so great for the 10 C axes. Also, threonine, serine, cysteine, cystine, glucosamine, methionine, ethanolamine, lysine, tryptophan, and arginine were higher in concentration in the 2-day-old 10 C treated axes. At 2 days, arginine was in much higher concentration in axes than in cotyledons with both treatments, as was the case to a lesser extent for most other amino acids. Hydrolysis of peptides in the 2-day-old 10 C axes (Table I) revealed data similar to those for 10 C cotyledons, whereas hydrolysis of 23 C axes produced large increases in aspartate including asparagine hydrolyzed to aspartate (Asx) and glutamate including glutamine hydrolyzed to glutamate (Glx) of 10.56 and 1.55 μmol g^{-1} fresh wt, respectively. Hence, aspartate, glutamate, and/or their amide derivatives are abundant in peptides and account for most of the large increase in free amino acid concentrations in 23 C axes following hydrolysis.

Between 2 and 5 days, concentrations of aspartate and glutamate in cotyledons from the 10 C treatment more than doubled (Figs. 2A and 3A). The only free amino acids that increased between 2 and 5 days in 23 C cotyledons were asparagine, proline, alanine,

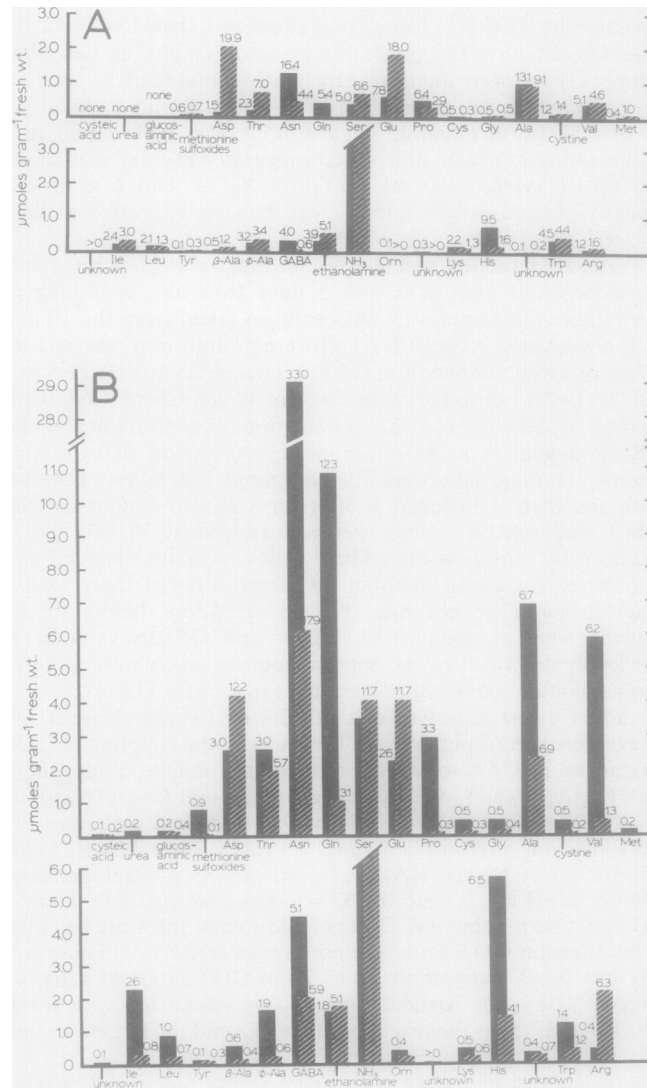

FIG. 3. Distribution and concentrations of free amino acids in *G. max* cotyledons (A) and axes (B) after 5 days germination at 10 C (▨) and 23 C (■). Order of elution on the Technicon TSM amino acid analyzer is left to right beginning with cysteic acid. Percentage of total free amino acids for each amino acid is indicated above each bar.

cystine, GABA, and tryptophan, whereas in the 10 C treatment nearly all free amino acids increased in concentration.

Axis concentrations of asparagine and glutamine increased 415 and 1,876%, respectively, in the 23 C treatment from 2 to 5 days (Figs. 2B and 3B). These two amides accounted for 45.3% of the total free amino acids and were in a concentration of 39.96 μmol g^{-1} fresh wt in the 5-day-old 23 C axes. Axes from the 10 C treatment showed a large increase in the concentrations of asparagine (1950%) and a decrease in glutamine (−49%) from 2 to 5 days. Concentrations of all of the sulfur amino acids and their derivatives (cysteic acid, methionine sulfoxides, cysteine, methionine, and cystine), lysine, tryptophan, and arginine decreased in the 5-day 10 C axes as compared to those of 2 days.

Average weights of axes at 23 C for 2 days and at 10 C for 5 days were 27 and 24 mg, respectively. If differences in free amino acid concentrations with the two treatments are quantitative due to retarded development of the 10 C treated seeds, then 2-day-old 23 C axes should have concentrations of amino acids nearly equal to those of the 5-day-old 10 C treated axes. Indeed, nearly equal concentrations of asparagine, glutamate, glycine, alanine, GABA, ethanolamine, lysine, histidine, tryptophan, and arginine are seen (Figs. 2B and 3B). This indicated that the processes involved in controlling concentrations of these amino acids may have only been slowed by low temperature. On the other hand, much higher concentrations of aspartate, threonine, glutamine, serine, β-alanine, and ornithine and much lower concentrations of methionine sulfoxides, proline, valine, isoleucine, leucine, tyrosine, and phenylalanine were in the 2-day 23 C axes as compared to the 5-day 10 C axes, indicating that differences in pool size of these amino acids were not linearly related to germination rates with the two temperature treatments. In cotyledons of the 5-day 10 C treatment (Fig. 3A) almost all free amino acids were in much lower concentration than in the 2-day 23 C treatment (Fig. 2A), again indicating that low temperature affects amino acid pool sizes by other than just slowing germination.

Enzyme Activities. In that ratios of aspartate and glutamate were near 1.0 in most of the data presented here (Figs. 2 and 3) and the equilibrium constant for the GOT reaction is near 1.0 (18), it appeared that this enzyme could account for the relative concentrations of aspartate and glutamate found in these studies. Figure 5 indicates that the levels of GOT activity in the 23 C treatment are much higher in the axes and noticeably higher in the cotyledons than in the 10 C treatment. Even when one considers the levels of GOT activity at 10 C (Fig. 6) it seems that there would be adequate activity with both treatments to keep concentrations of glutamate and aspartate in equilibrium as long as turnover rates are not high for these two amino acids. The E_a values for GOT with the two treatments were nearly equal at 8.6 kcal/mol between 20 and 30 C above the inflections at 8 C. The Arrhenius plots for GOT activity shown here, however, are for axis supernatant fractions and may not reflect the physical properties of mitochondrial GOT. At 2 days the percentages of GOT activity found in 10 and 23 C cotyledon mitochondrial fractions were 4.5 and 4.8%, respectively, whereas the percentages were 8.7 and 16.2%, respectively, in the axes. At 5 days the percentages of GOT found in mitochondrial fractions of cotyledons from the 10 and 23 C treatments were 4.8 and 7.8%, respectively, and the axis mitochondrial percentages were 5.0 and 22.8%, respectively. These data indicate that formation or activation of mitochondrial GOT is slowed in cotyledons and almost completely inhibited in axes by low temperature.

Figure 4 indicates that [^{14}C]aspartate is converted to glutamate, in significant amounts, within a short period in both soybean cotyledons and axes grown at optimal germination temperature indicating that aspartate and glutamate may be rapidly interconverted. In fact, levels of labeled glutamate were higher than those for labeled asparagine in axes after 3 hr (Fig. 4B). Other free amino acids which contained less label after the addition of [^{14}C]

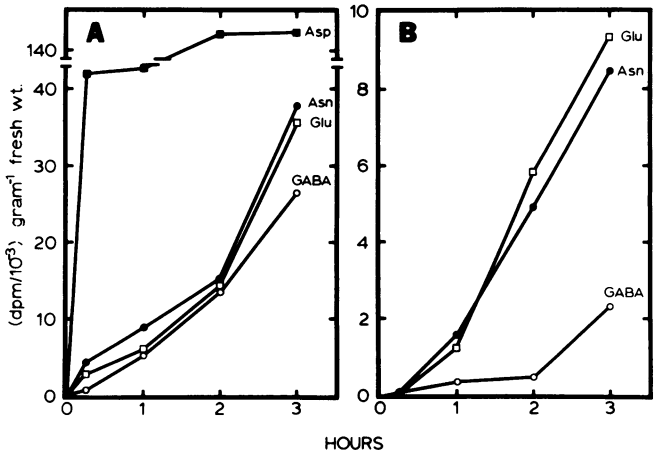

FIG. 4. Production of free amino acids from [^{14}C]aspartate in germinating *G. max* cotyledons (A) and axes (B). Seedlings were 5 days old and grown at 23 C.

aspartate to cotyledons were GABA, serine, glutamine, and proline.

The levels of GDH activity found in cotyledons and axes with the two treatments at 2 days (Fig. 5) were similar to those in our previous study (10). At 5 days GDH activity in the 23 C treated cotyledons was much lower than in the 10 C treatments, whereas in axes the reverse was true. Increases in GDH activity occurred between 2 and 5 days in both treatments in the case of axes.

In eukaryotic organisms GDH is localized either in mitochondria or chloroplasts (7, 8); hence, the percentage of total GDH activity recovered in mitochondria in these nonchlorophyllous tissues may be used as an indication of mitochondrial integrity. Studies with peas have shown that mitochondrial intactness is correlated positively with germination and imbibition time (31). The percentages of GDH activity recovered in the 23 C mitochondrial pellets for both axes and cotyledons were much higher (100 and 42%, respectively) than in the previous study at 2 days (10), indicating that the extraction medium employed in this study was more suitable for isolating mitochondria. At 2 days axes and cotyledons from the 10 C treatment had, respectively, 39.7 and 20.6% of their GDH activity in mitochondrial pellets, indicating a much lesser degree of integrity than in the 23 C treatment. At 5 days the 23 C axes and cotyledons had 100 and 38.2% of their GDH activity, respectively, in mitochondrial pellets, while the same tissues from the 10 C treatment contained 44.1 and 26.8%, respectively. These data indicate that only small changes in mitochondrial intactness occurred between 2 and 5 days with the two treatments.

Figure 6 shows that in both treatments at 5 days, axis GDH Arrhenius plots were triphasic as compared to biphasic plots at 2 days (10). Inflections in GDH Arrhenius plots occurred at 21.1 and 7.0 C in the plots made with the 23 C axis preparations. Axis GDH Arrhenius plots had different E_a values with the two treatments, as was seen in our previous study (10). Values of 5.7 and 3.8 kcal/mol between 30 and 40 C and 15.1 and 8.0 kcal/mol between 10 and 20 C were obtained for the 23 and 10 C preparations, respectively.

Cotyledon GS activities were higher in the 23 C treated seeds at both 2 and 5 days although the activities of the 10 and 23 C treatments increased significantly from 2 to 5 days (Fig. 5). In axes at 2 days, GS activities were nearly equal on a fresh weight and axis basis for both treatments. At 5 days GS activities from the 10 C treatment were only 27.6% of those of the 23 C treatment on an axis basis; however, again activities of both treatments had increased significantly over 2-day levels indicating either activation or synthesis of GS during this period. The Arrhenius plots of GS activity (Fig. 6) are quite unusual with breaks or inflections at 25.5

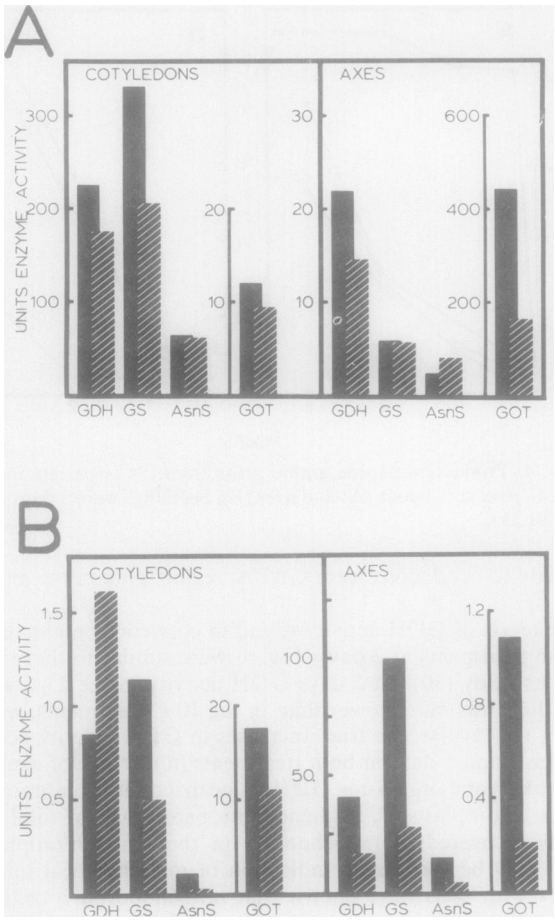

FIG. 5. Activities of glutamate dehydrogenase (GDH), glutamate oxaloacetate transaminase (GOT), glutamine synthetase (GS), and asparagine synthetase (AsnS) during *G. max* germination after 2 (A) and 5 (B) days at 10 C (▨) and 23 C (▬). Cotyledon enzyme activities are expressed as nmol (GDH, GS, AsnS) and μmol (GOT) NADH oxidized (GDH, GOT) or product formed min^{-1} g^{-1} fresh wt or 0.1 g^{-1} fresh wt (GDH) for 2-day-old seedlings (A). Axis activities are expressed as nmol NADH oxidized (GDH, GOT) or product formed (GS, AsnS) min^{-1} $axis^{-1}$ for 2-day-old seedlings (A). Five-day-old cotyledon (B) activities are all expressed on a nmol min^{-1} g^{-1} fresh wt basis whereas axis activities at 5 days are either on a nmol min^{-1} $axis^{-1}$ (GDH, GS, AsnS) or μmol min^{-1} $axis^{-1}$ basis. GDH and GOT activities include the activities of both supernatant (15,000*g*) and mitochondrial (15,000*g*) fractions, whereas GS and AsnS activities are for supernatant fractions only.

C, and E_a values which are very low (2.0 and 1.2 kcal/mol above and below the break for 23 C GS; 2.8 kcal/mol and a negative value above and below the break for 10 C GS).

Cotyledon AsnS activities (Fig. 5) were about the same for both treatments at 2 days. At 5 days the 23 C treatment had 308% higher AsnS activity than the 10 C treatment in cotyledons. The difference between treatments at 5 days was due to an increase (59%) in AsnS activity between 2 and 5 days with the 23 C treatment and a steep decrease (−61%) in activity with the 10 C treatment during the same period. AsnS Arrhenius plots were not reproducible due to very low activities. AsnS axis activities were actually higher on an axis basis with the 10 C treatments at 2 days (Fig. 5); at 5 days the 23 C treatment was 265% higher than the 10 C treatment in AsnS activity.

There is some question as to the validity of the hydroxamate assay for AsnS activity in higher plants. While this assay is apparently quantitatively valid for organisms which utilize NH_4^+ for amidation of aspartate (30), the use of NH_4^+ rather than glutamine as an amide source in soybeans results in only 26% as much AsnS activity (34). This means that AsnS data presented here are lower than and only a reflection of maximal activities. However, comparisons between the two treatments can be qualitatively made. In contrast, the hydroxamate assay for GS is known to be both an excellent quantitative and qualitative assay in legumes (26).

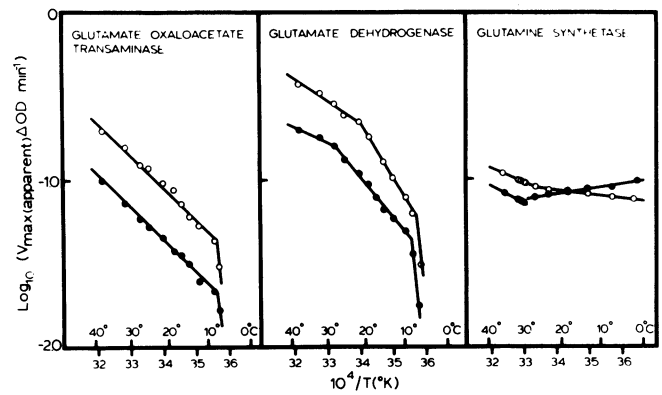

FIG. 6. Arrhenius plots of GOT, GDH, and GS from 5-day-old *G. max* seedlings germinated at 23 C (○) and 10 C (●). GDH plots are for axis mitchondrial pellets (15,000*g*), whereas GOT and GS plots are for supernatant fractions (15,000*g*). GOT and GS activities are 0.1× and 10× actual activities, respectively.

DISCUSSION

Previous studies have shown that in soybean germination and seedling growth the hydrolysis of storage protein may be very orderly with the various storage proteins degraded in sequence (4). This would suggest that the proportions and concentrations of various free amino acids would change during germination due to variations in the amino acid compositions of such storage proteins as globulin 7S and 11S components (5). The two major amino acids found in soybean storage proteins are aspartate and glutamate (5, 25); hence, it could be expected that these two amino acids and their derivatives would be a major proportion of free amino acids during germination no matter what storage protein was the source. We observed this (Figs. 2 and 3) as others have (16). Our determinations of high concentrations of free asparagine and glutamine are also consistent with previous soybean (16) and legume (2) germination studies. High concentrations of asparagine and glutamine have also been noted in phloem exudate of *Lupinus alba* (1, 32), shoots of *Pisum arvense* (27), germinating cottonseed (3), and roots, shoots, and bleeding sap of tomato (19, 20). These data would indicate that asparagine and glutamine are major transport amino acids in many higher plants. This study also demonstrates that concentrations and percentages of asparagine and glutamine are greatly reduced by low temperature (Figs. 2 and 3). The mechanism or mechanisms involved in reducing the amounts of asparagine and glutamine with low temperature could include an alteration of transport; however, our data would not support this proposition in that maintaining the seedlings at 10 C rather than 23 C results in lower concentrations of asparagine and glutamine in both cotyledons (source) and axes (sink), except in the case of glutamine in 2-day-old cotyledons (Fig. 2B). In all cases the precursors of these amides, aspartate and glutamate, were in higher concentrations in the cold-treated seedlings. Indeed GS and AsnS activities were higher in the 23 C treated seedlings except in the case of 2-day-old axes (Fig. 5). If the Arrhenius plots of GS activities (Fig. 6) represent near physiological activities at optimal and low temperatures, the values in Figure 5 would be a close approximation for maximal levels of GS activity with the two treatments. ATP levels may also severely limit the production

of asparagine and glutamine with cold treatment. Others have shown that ATP is limiting in cold-treated chilling-sensitive plants such as cotton (33). This may be due to an inactivation of the ATP synthesis system (22). Our previous study (10) suggests that this may be the case in that mitochondrial respiration is severely restricted in soybeans at low temperature. Differences in levels of GS and AsnS activity at 5 days (Fig. 5) with the addition of ATP may have been due to differences in rates of enzyme synthesis. Low temperature is known to inhibit protein synthesis in wheat seedlings (35).

Our findings of large quantities of peptides in 23 C treated axes (Table I) are not surprising. Dipeptides have been shown to occur in substantial concentrations in soybeans germinated at optimal temperature (15, 16). Also, a peptide transport system has been demonstrated in germinating barley embryos and it has been suggested that peptide transport would be more efficient than amino acid transport (12). Our data do not necessarily support this concept. There were not substantial quantities of peptides in cotyledons (source) when there were high levels in axes (sink), whereas known transport amino acids were high in both tissues of the 23 C treatment at 2 days (Fig. 2).

Studies with cotyledonless lupine embryos indicate that high levels of added asparagine will increase GDH activity dramatically, while having little or no effect on GOT activity (29). Our GDH data would suggest that this could be the case in soybean axes, in that there appears to be a correlation between high asparagine concentrations (Figs. 2B and 3B) and high GDH activity (Fig. 5). In that axes are a sink for asparagine, it might be presumed that asparagine would be deamidated and/or deaminated to a large degree in axes, producing free NH_4^+ which is known to induce GDH activity in soybean tissues (7). Although the role of GDH in glutamate production has been questioned (23), it could function in producing α-KG for energy production during germination of species such as *G. max* which contain large amounts of stored glutamate.

Macnicol (21) has indicated that aspartate and glutamate may be interconverted in *Pisum* by their deamination, the addition and substraction of a carbon in the tricarboxylic acid cycle to produce α-KG and OAA to produce glutamate and aspartate, respectively. We support this hypothesis in that we found glutamate to be rapidly labeled with the addition of [^{14}C]aspartate to soybean cotyledons (Fig. 4). As we observed in soybeans, studies with pea seedlings have shown that glutamate is the most prevalent labeled protein amino acid with the addition of [^{14}C]aspartate to cotyledons (17). Our data would also support the role of GOT in the regulation of aspartate and glutamate metabolism in soybeans in that we found high levels of GOT in all tissues (Fig. 5), and nearly equal concentrations of aspartate and glutamate in all but one free amino acid sample (Figs. 2 and 3).

Our Arrhenius plots of GDH activity from 5-day-old soybean axes (Fig. 6) are very similar to those of *Vigna* succinate oxidase (28) in that they both consist of three phases. These data could indicate that GDH is associated with membrane lipids in that fully developed membrane systems in chilling-sensitive plants also have three phases. These membrane-lipid phases have been implicated in determining the number of phases and inflections in Arrhenius plots of enzymes (28). Arrhenius plots for GOT were biphasic (Fig. 6) and were not different from plots for other cytosol enzymes in our previous tudy. These data indicate that E_as at low temperature could have a pronounced effect on both GDH and GOT activity. This in turn could affect ratios of glutamate and aspartate and the rate of glutamate production or degradation in soybean tissues at low temperature. NADP-isocitrate dehydrogenase, which produces α-KG and is greatly reduced in activity by low temperature (10), could also affect glutamate metabolism.

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