Proc. Indian natn. Sci. Acad. **B48** No. 1 pp 103–108 (1982)

Transaminase Activity and Free Amino Acids in Germinating *Vigna unguiculata* (Cowpea) Seeds

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(Received 28 April 1981; after revision 20 November 1981)

Both aspartate aminotransferase and alanine aminotransferase showed changes in activity during germination. The activity of alanine aminotransferase was higher at all stages of germination. The ratio between the two activities did not remain a constant.
The total free amino acids registered a progressive increase during germination, reaching a peak value in 48 hr and then falling abruptly. The spectrum of the free amino acids differed during the different stages of germination. The findings have been discussed in relation to the roles of the aminotransferases and free amino acids during seed germination.

Key Words: *Vigna unguiculata* **(Cowpea), Aminotransferases, Amino acids, Germination**

Introduction

Mobilization of cotyledonary reserve proteins is a major event during the germination of legume seeds (Beevers 1968, Pusztai & Duncan 1971, Ericson & Chrispeels 1973 and Maye & Poljakoff-Mayber 1975). The amino acids generated by proteolysis of reserve proteins are translocated to and utilized by the growing embryonic axis (Beevers & Guernsey 1966), or used for the *de novo* synthesis of enzyme proteins in the cotyledons (Young & Varner 1959 and Young et al. 1960). Amino acids formation and transformation, therefore, constitute an important aspect of the metabolism of germinating legume seeds. An important reaction involving amino acids in plants is transamination. Using preparations from seedlings, a number of amino acids have been shown to be transaminated in the presence of keto acids (Wilson et al. 1954 and Forest & Wightman 1972).

Transaminases have been studied in relation to germination in a number of seeds (Wong & Cossins 1966, 1969, Forest & Wightman 1971 and Ghildiyal & Sinha, 1971). In the present investigation, changes in aspartate aminotransferase

(EC 2.6.1.1.) and alanine aminotransferase (EC 2.6.1.2.) activities were followed in the seeds of *Vigna unguiculata* to elucidate their roles during germination. Simultaneously, the free amino acids were determined in germinating seeds.

Material and Methods

Vigna unguiculata (L.) walp. (var. culture II) seeds were procured from the Rice Research Station, Pattambi, Kerala. After surface sterilization with 0.10% (w/v) $HgCl_2$ solution and thorough washing with sterile distilled water, the seeds were germinated in the dark at 26+1°C on filter paper discs in sterilized petri dishes. Sterile distilled water was provided during germination. Transaminase activities and free amino acids were determined on ungerminated and 12-, 24-, 48- and 72-hr germinated seeds. Analyses were carried out on whole seeds and seedlings after seed coat removal. A minimum of two samples were analyzed in each case. Mean value of two separate experiments are reported.

Ten percent homogenates, for enzyme assays, were made by grinding the tissue with acid—washed sand in ice-cold phosphate buffer (10 mM, *p*H 7.0) in a chilled glass mortar with pestle. The slurry was squeezed through a layer of muslin and the filtrate used as the source of enzyme. Alanine aminotransferase and aspartate aminotransferase activities were determined according to Tonhazy et al. (1950), as described by Sanwal et al. (1964); incubations were carried out at 37°C for 30 min. Pyruvate was estimated according to Friedman and Haugen, (1943). No pyridoxal phosphate was added to the assay system since the crude enzyme preparation apparently contained sufficient coenzyme; according to Wightman and Forest (1978) plant aminotransferases have a much tighter binding between the protein and coenzyme. The concentration of endogenous amino acids (and possibly keto acids) present in the assay systems was negligible in comparison to the high concentration of amino acids and keto acid used as substrates and as such would not contribute appreciably to endogenous activity. The control assays showed only very little or negligible colour.

Protein was determined in the homogenates according to Lowry et al. (1951) with modification (Khanna et al. 1969). Ethanol-soluble amino acids, extracted from seeds/seedlings were separated on paper, chromatographically, after initial treatment with Dowex 50 W–X 8 (Kliewer 1964) according to Smith et al. (1967) and determined according to Giri et al. (1952).

One unit of activity corresponds to the formation of 1μ mole of keto acid per minute at 37°C under the experimental conditions. Specific activity is expressed as units per mg protein.

Results and Discussion

The changes in dry weight, protein and aminotransferase activities during the germination of *Vigna unguiculata* seeds are reported in table 1.

The dry weight showed an initial decrease during 12 hr germination; thereafter, no appreciable change was registered till the end of 48 hr, when further decrease was noted. Protein per seedling showed only marginal decrease during 24 hr germination. The protein value in the 48 hr sample registered an increase. Since there was a marked enhancement in the value of total free amino acids in this sample (as explained below), an increase in protein was not expected. The seedling at this stage was ground with ease, presumably contributing to minimum protein being retained

Table 1 *Changes in Dry Solids, Protein and Alanine Aminotransferase and Aspartate Aminotransferase Activities in Germinating* Vigna unguiculata *Seeds*

Germination (hr)	Dry weight (%)	Protein (mg/seed, seedling)	Activity (Units/seed, seedling)		Specific activity (Units/mg protein)	
			Alanine aminotransferase	Aspartate aminotransferase	Alanine aminotransferase	Aspartate aminotransferase
0	90.21	9.91	0.40	0.16	0.04	0.02
12	45.71	8.67	1.37	0.36	0.16	0.04
24	41.58	8.68	0.72	0.13	0.08	0.02
48	28.45	12.07	1.31	0.41	0.11	0.04
72	19.95	6.75	0.70	0.20	0.11	0.03

The values reported are the mean of two separate determinations. The analytical details are as reported in Materials and Methods

with the debris fraction on muslin. Subsequently, there was a marked decrease in protein at 72 hr. Similar results were obtained by Prisco et al. (1975) in *V. sinensis* seeds, where depletion of protein in cotyledons was observed at the end of 48 hr germination.

Both aspartate and alanine aminotransferase showed changes during germination. Expressed per seed/seedling, the latter activity increases over 3-fold and the former over 2-fold in 12 hr. There was a drop in 24 hr, followed by a rise in 48 hr and then a drop in 72 hr sample. Expressed as specific activity, the pattern of changes during the initial 48 hr was the same as above, but there was practically no change in 72 hr sample. At all stages during germination, alanine aminotransferase activity exceeded aspartate aminotransferase. This was in contrast to the two aminotransferase activities reported in many leguminous seeds, but was similar to the pea seed aminotransferases (Smith & Williams 1951). Specific aminotransferases have been reported (Ellis & Davies 1961). Since the ratio of the two activities did not remain constant, it appeared that distinct enzymes in cowpea catalyzed the two aminotransferase reactions.

Changes in free amino acids are reported in table 2.

The values for total free amino acids (sum of individual amino acids recovered from the chromatograms) showed an increase in 12 hr, a doubling in 24 hr, a further doubling in 48 hr, followed by a halving in 72 hr. Proteolysis of the reserve proteins seemed to be dominant during the first two days; later, utilization outstripped the release of amino acids in free condition. A number of amino acids were present in the ungerminated seeds, serine, alanine, tryptophan, glycine, aspartic acid, glutamic acid and γ-aminobutyric acid predominating. The presence of tryptophan, an essential amino acid, was especially significant. Lysine, proline, valine/methionine and asparagine were present only as traces and histidine could not be detected. With progressive germination, there were changes in the content of free amino acids; the pattern of variation was not the same for the different amino acids.

Table 2 *Free Amino Acids in Germinating* Vigna unguiculata *Seeds*

Amino acid	Germination periood (hr)				
	0	12	24	48	72
	Content, μ mol/seed, seedling)				
Ala	0.43	0.48	1.15	1.92	1.84
Arg	0.11	0.14	0.32	0.77	0.23
Asn	t	0.12	1.19	0.96	0.46
Asp	0.15	0.04	0.41	0.13	0.21
GABA	0.16	0.05	0.10	2.13	t
Gly	0.18	0.24	0.31	0.46	t
Glu	0.14	0.44	1.00	0.65	1.06
His	n.d.	0.12	n.d.	0.83	n.d.
Leu(s)	0.05	0.06	0.14	1.13	0.36
Lys	t	0.06	0.26	0.39	0.16
Pro	t	0.13	0.33	0.78	t
Ser	0.53	0.07	0.30	0.50	0.32
Thr	0.08	0.03	0.16	0.39	t
Try	0.20	0.57	0.28	0.96	t
Tyr	0.02	t	0.11	0.31	t
Val + Met	t	0.31	0.29	0.88	1.62
Total	2.05	2.86	6.35	13.19	6.26

Tha extraction and estimation of amino acids are given under Materials and Methods. The values reported are the mean of two separate determination

t, trace amount; *n.d.*, not detected

As has beens uggested (Derbyshire et al. 1976), this could be attributed to the variation in the free amino acid composition of storage protein. Also, the free amino acids released from the storage proteins would get interconverted enzymatically to produce new protein amino acids and non-protein amino acids. Aminotransferase is one reaction involved. At the end of 48 hr, marked increases were noticed in most of the amino acids except asparegine aspartic acid and glutamic acid. Acid amides are known to accumulate in etiolated seedlings. In the present case, asparagine was the only amide present, glutamine being absent at all stages. In the 72-hr sample there was a decrease in most of the amino acids. A significant exception was the marked increase in the combined value of valine and methionine, both essential amino acids. Aspartic and glutamic acid increased in amounts. The level of alanine remained essentially unaltered. Cysteine/cystine and glutamine could not be detected either in the dry seed nor at any stage of germination. No direct correlation existed between the aminotransferase activities and the corresponding free amino acid contents. Similar results were reported by Forest and Wightman (1971) in *Phaseolus vulgaris* seedlings.

An obvious role of the amino acids is for the synthesis of specific proteins in the embryonic axis and may originate either during proteolysis of the reserve proteins in the cotyledons, or from appropriate aminotransferase reactions. In addition, the amino acids might serve as respiratory substrates after deamination. The marked decrease in the free amino acids during 48–72 hr, with simultaneous decrease in total protein in seedling, was suggestive of amino acids being increasingly utilized for respiratory purposes. The reversibility of transaminases would enable the amino acids to be converted to keto acids. In conjunction with glutamate dehydrogenase and malate dehydrogenase, transaminases can channel amino acids into Krebs cycle to serve as respiratory substrates. Among the amino acids, the utilization during 48–72 hr seemed to be selective; thus, γ-aminobutyric acid, the most abundant component in the 48 hr sample was detected only in traces in the 72 hr sample.

Acknowledgement

We are grateful to Professor P S Krishnan, FNA, for his critical suggestions in the preparation of the manuscript.

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