TRANSAMINASE ACTIVITY IN PLANTS

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Summary

1. Transaminase activity (alanine → glutamic, aspartic → glutamic, glutamic → alanine) has been detected in a number of pulses.

2. An easy and specific method for the determination of transaminase activity using circular paper chromatography is described.

3. The optimum pH for alanine → glutamic acid transaminase is found to be 7·4.

4. Germination increases the enzyme activity (alanine → glutamic).

5. Inhibition of the transaminase activity by pterygospermin has been reported for the first time and the bearing of this result on the antibiotic action of pterygospermin is indicated.

Ever since the discovery of transamination reaction (Braunstein and Kritzmann, 1937; Needham, 1930) a large body of data has been accumulated regarding the specificity, distribution and kinetics of transaminases. Braunstein and Kritzmann (1937) have reported that several natural and racemic amino acids acted as amino group donors in the presence of α-ketoglutaric acid and glutamic aminophorase present in animal tissues. After extensive studies on animal tissues Cohen (1939) demonstrated that marked transamination occurred only with alanine, glutamic and aspartic acids and their corresponding keto acids. Green, *et al.* (1945) showed that purified preparations of glutamic-oxalacetic and glutamic-pyruvic transaminases possess a high degree of specificity.

On the other hand, Tannenbaum and Shemin (1950) have found from their isotopic studies that leucine takes part in transamination reaction when incubated with α-ketoglutaric acid and an enzyme preparation from pig heart muscle. Hird and Rowsell (1950) observed that phenylalanine, tyrosine and alanine were formed from the corresponding keto acids and glutamic acid in the presence of insoluble particles of rat liver.

More recently, Cammarata and Cohen (1950) have obtained evidence using tissue extracts to show that 20 different amino acids are able to form glutamic acid from α-ketoglutaric acid.

The lack of agreement among the results of previous investigators indicated above can be attributed partly to the inadequacy and unspecific character of the analytical methods employed.

The investigations on transaminating systems in plants were, however, less intensive. Virtanen and Laine (1938) demonstrated that the system, α-amino-dicarboxylic acid + pyruvic acid ⇌ α-ketodicarboxylic acid + alanine, occurs also in green plants and root nodules. Later it was found (Virtanen, Lane, 1941; Rautanen, 1946) that transamination occurs only with certain aliphatic α-keto acids. When the aromatic α-ketoacids formed one component, no transamination occurred with α-amino dicarboxylic acids. These workers concluded that the scope of transamination in plants is limited and that the pathway for the formation of aromatic amino acids is obscure.

The presence of transaminase systems in plants has also been reported by other workers (Kritzmann 1939; Von Euler, *et al.*, 1938; Cedrangolo and Carandante, 1940).

Albaum and Cohen (1943) have studied transamination in germinating oat seedlings. The reaction glutamic acid + Oxalacetic acid ⇌ α-ketoglutaric acid + aspartic acid, proceeded at a very rapid rate, and the reverse reaction at about $\frac{1}{3}$ the forward rate. They have shown a close relationship between transaminase activity and protein synthesis existed in plants.

Recently, Leonard and Burris (1947) in a detailed study have reported high transaminase activity in various plants and plant tissues.

In view of the fact that the germinating seedling is a seat of vigorous metabolic activity, where proteins from the cotyledons are constantly broken down and the amino acids formed taken up by the growing plant for the *de novo* synthesis of proteins, it was decided to conduct a detailed study of transaminating systems in seeds, their scope and exact function in protein synthesis in plants.

In this paper are presented the results of a preliminary study of transaminase reactions in green gram (*Phaseolus radiatus*).

METHODS

In addition to the manometric techniques (Ames, *et al.*, 1947) several methods have been proposed recently. Green, Leloir and Nocito (1945) suggested a spectrophotometric assay for transaminase activity which was later elaborated by Cammarata and Cohen (1951).

In the manometric method employed by Cohen (1950) in his earlier studies, a glutamic acid decarboxylase preparation from *Clostridium welchii*

was used for the determination of glutamic acid formed by transamination reaction. This method led to some erroneous conclusions, the cause of which was later traced to the presence of glutamic-oxalacetic transaminase activity at pH 5·0 in the glutamic decarboxylase preparation. The strain of *Clostridium welchii* used in these studies was also found to be capable of decarboxylating aspartic acid. This clearly shows that the manometric methods should be employed with caution.

The method reported by Tulpule and Patwardhan (1952) is based on the conversion of α-ketoglutaric acid and pyruvic acid into the respective dinitrophenylhydrazones and estimating them colorimetrically after paper chromatographic separation (ascending technique). Unless great care is taken during the several extraction processes, errors due to losses are liable to creep in. In addition, the method is somewhat tedious.

In recent times, paper chromatography has been applied for the detection of transamination reactions (Fincham, 1951; Feldman and Gunsalus, 1951). Awapara and Seale (1952) studied the distribution of transaminases in rat organs quantitatively by unidimensional paper chromatography.

In the present investigation, the transaminase activity of seeds was followed using circular paper chromatographic technique briefly outlined in an earlier communication from this laboratory (Giri, *et al.*, 1952). This method is specific, easy and facile, singularly free from the sources of error which vitiate the other methods and at the same time capable of giving remarkably reproducible results.

Preparation of enzyme extracts.—The enzyme was prepared by extracting the powder of resting seeds with water (1 : 4 w/v) for one hour in a refrigerator. The Extract (covered with toluene) was exhaustively dialysed against distilled water for 24–48 hours at 0–5° C. The dialysed extract was made up to double the original volume and centrifuged. The cloudy supernatant was used in these experiments. The residue after centrifugation was devoid of activity. The enzyme preparation retained its activity at 0–5° C. at least for one week.

Determination of Activity.—The reaction mixtures usually consisted of the following:

0·1 c.c. of M/15 l-amino acid
0·1 c.c. of M/15 α-ketoglutaric acid or pyruvic acid (neutralized with NaOH)
1·0 c.c. of M/15 phosphate buffer pH 7·4
0·8 c.c. of Enzyme preparation

Total vol. 2·0 c.c.

The reaction mixtures were incubated for about 5 min. at 35° C. before the addition of enzyme.

At the end of 2 hours, the reaction was stopped by the addition of an equal volume of warm ethanol. The protein precipitated was centrifuged off and the clear supernatant used for chromatography. Aliquots of 20 μ l. were spotted on the circumference of a circle (4 cm. diam.) drawn with a pencil from the centre of a Whatman No. 1 filter-paper (24 cm. diam.) and the chromatogram developed as described earlier (Giri, *et al.*, 1952 *b*) using butanol: acetic acid: water as solvent. Simultaneously amino acid standards were run on the same paper; multiple development technique was invariably used in view of the clearer separation achieved. After air-drying, the papers were uniformly sprayed with 0·5% ninhydrin solution in 95% acetone. The dried papers were then heated at 65° C. for exactly 30 min. The colour development was found to be complete in about 10 min. in most cases but as a measure of safety 30 mins. heating was employed. The bands corresponding to the amino acids in question were cut off carefully and the colour extracted in a test tube with 4 c.c. of 75% alcohol containing 0·2 mg. of $CuSO_4$, 5 H_2O. A blank correction was applied by cutting out an approximately equal area of the uncoloured region in the paper. Readings were taken using a Klett-Summerson photoelectric colorimeter with green filter 540 $m\mu$ (Giri, *et al.*, 1952 *a*).

The enzyme activity is expressed as milligrams of glutamic acid formed in the reaction mixture unless otherwise stated.

When crude extracts were incubated with α-ketoglutaric acid some glutamic acid was always formed. This is due to the presence of Amino donors in the extract. The formation of glutamic acid was negligible when the dialysed extracts were incubated with α-ketoglutarate. In all these experiments, the controls with (α-ketoglutarate + extract) and (amino donor + extract) were carried out.

RESULTS AND DISCUSSION

Recovery experiments were conducted to test the accuracy of the method. To 0·4 c.c. of an aqueous extract of *Phaseolus radiatus* (1 : 4 w/v), various amounts of glutamic acid were added and the total volume was made upto 1·0 c.c. The amount of glutamic acid in the extract and in the mixture was estimated and the percentage recovery calculated. The results presented in Table I show that the recovery is quite satisfactory.

In view of the conflicting statements in literature regarding the specificity of the enzyme and the scope of the transamination reaction, various

TABLE I. *Recovery of Glutamic Acid*

Glutamic acid in the extract (found) in mg.	Glutamic acid (added) in mg.	Glutamic acid in the mixture (found) in mg.	Glutamic acid recovered in mg.	% Recovery
0·48	1·0	1·55	1·07	107
0·5	2·0	2·56	2·06	103
0·5	4·0	4·8	4·3	107

amino acids were incubated with α-ketoglutaric acid or pyruvate and the enzyme preparation. Suitable controls were also run. It was found that only aspartic acid, alanine and glutamic acid took part in transamination reactions in the presence of green gram extract.

Time-Course of Reaction.—The course of the reaction was followed with time. The reaction mixtures were set up as before (total volume 5 c.c.). At various intervals aliquots of the reaction mixture were transferred into a test tube containing an equal volume of warm ethanol. Tables II *a*, *b*, *c* show the results.

TABLE II (*a*). *Time-Course of Reaction*
(*Alanine → Glutamic Acid Transaminase*)

Amino acid	Time in hours							
	0	½	1	2	4	8	11	20
Glutamic acid formed in mg.	0·5	0·9	1·2	1·4	1·45	1·6	1·6	2·0

TABLE II (*b*). (*Aspartic Acid → Glutamic Acid Transaminase*)

Amino acid	Time in hours				
	0	1	2	4	24
Aspartic acid in mg. ..	2·35	2·1	2·05	1·95	1·8
Glutamic acid in mg. ..	0·2	0·5	0·7	0·8	0·95

TABLE II (c). (*Glutamic Acid → Alanine Transaminase*)

Amino acid	Time in hours				
	0	1	2	4	24
Glutamic acid in mg. ..	2·5	2	1·8	1·7	1·6
Alanine in mg. ..	..	0·4	0·5	0·6	0·75

Effect of *pH*.—The effect of pH on the enzyme catalysing the reaction l-alanine + α-ketoglutarate → glutamic acid + pyruvate is shown in Table III. The optimum pH was found to be 7·4 with a relatively sharp decline on either side. The optimum pH was found to be the same as for the alanine-glutamic transaminase from pig heart muscle as reported by Green, *et al.* (1945).

TABLE III. (*Effect of pH on the Alanine → Glutamic Transaminase*)

pH	Glutamic acid formed in 2 hrs. (in micrograms)
3·9	0
5·8	405
6·2	450
7·4	562
8·0	315
8·5	0

Comparative Study of the Transaminase Activity of Various Pulses.—A preliminary survey for the presence of alanine-glutamic transaminase was made in some of the common Indian pulses. The results are given in Table IV.

The results clearly show that *Vigna catiang* is the richest source of the enzyme catalysing the interaction between alanine and α-ketoglutaric acid. Most of the quantitative data reported in this paper were however obtained using green gram as the source, as the above experiment was done only later.

TABLE IV. *Transaminase Activity of Pulses*
(Alanine → Glutamic Transaminase)

Pulses	Glutamic acid formed in 45 min. (in micrograms)
Bengal gram (*Cicer arietinum*)	86
Green gram (*Phaseolus radiatus*)	228
Cow pea (*Vigna catiang*)	342
Field bean (*Dolichos lablab*)	(negligible)

Effect of Germination on the Alanine-Glutamic Acid Transaminase Activity of Green Gram.—The effect of germination was studied in the case of green gram. The resting seeds were first washed with ethanol to avoid bacterial contamination and then soaked in running water for 12 hours. The soaked seeds were spread between the folds of moist filter-paper and germinated for 24 hours. The germinated seeds were mashed with water (1 : 4 on the basis of dry wt.) and after keeping for 1 hour it was centrifuged. The supernatant was dialysed for 48 hours in cellophane bags at 0°–5° C. The resting seed powder was also treated in the same manner and the enzyme activities compared. The reaction mixtures were the same as before. The results are given in Table V.

TABLE V. *Effect of Germination on Transaminase Activity*

	Glutamic acid (in micrograms)		
	0 hr.	2 hrs.	Activity in 2 hrs.
Resting seeds	118	330	112
Germinated seeds	212	472	260

The above results show that germination increases transaminase activity in green gram.

Inhibition of Activity of Transaminases by Pterygospermin.—During the tudy of the mechanism of antimicrobial action of pterygospermin, an

antibiotic present in the roots of *Moringæ pterygosperma*, Kurup, Narasimha Rao and Venkataraman in these laboratories have observed that pterygospermin exerts a strong inhibitary action on the microbial transaminase systems (glutamic →alanine) such as those present in *Penicillium chrysogenum* ϕ176 and also in *Micrococcus pyogenes* var *aureus*.

We have quantitatively studied the action of this antibiotic on the plant transaminases. It is found that alanine ⇌ glutamic acid transaminase from green gram is also strongly inhibited by pterygospermin in low concentrations (1/20,000).

Pending a fuller discussion of the significance of these observations it may be mentioned that as transaminase reaction occupies a central position in protein synthesis and also in view of the reported high transaminase activity (Lichstein and Cohen, 1945) in micro-organisms, it is possible that the action of pterygospermin and other antibiotics is in some way intimately connected with this enzyme function and thereby interfering with protein synthesis, so necessary for growth. Considering from a different angle also, this finding is of significance. It is clear from Table VI that pterygospermin (P.S.)

TABLE VI. *Inhibition of Transaminase Activity by Pterygospermin*

Reaction	Alanine→Glutamic acid			Glutamic→Alanine acid			Aspartic→Glutamic acid		
	Control	+P.S.	% Inhibition	Control	+P.S.	% Inhibition	Control	+P.S.	% Inhibition
Amino acid formed at the end of 3 hours in micrograms	208	83	60	80	39	49	168	160	4·8

at the concentration employed (1/20,000) inhibits the enzyme catalysing the reaction alanine ⇌ glutamic acid, whereas the inhibition of the activity of the aspartic ⇌ glutamic enzyme by the antibiotic is negligible. This specificity of inhibition observed by us for the first time may be of value in studying a particular reaction, *viz.*, aspartic-glutamic by excluding the other reactions and investigating the fate of one keto acid and the corresponding amino-acid alone. Investigations on these lines are in progress.

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