A Simple Method for the Determination of the Relative Concentration of Total Amino Acids in Juice Expressed from Sugar Beet Plant Tissues

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Introduction

Earlier investigations have shown that the concentration of certain amino acids is greatly altered in the leaves of sugar beet plants infected with beet yellows virus (2)[2] and also with beet western yellows virus (1). In some tests, the concentration of aspartic acid and of glutamic acid was reduced as much as 70% in the leaves of plants showing the chronic stage of beet yellows. The concentration of certain other amino acids was increased and the concentration of still other amino acids remained unchanged. It became evident that a method for the determination of the concentration of total amino acids, relative to the concentration of certain individual amino acids, in the different tissues of healthy and yellows-infected beet plants, would be highly desirable. A simple method was developed which measures the intensity of the color produced by the reaction of the amino acids present in the tissue extract with ninhydrin. The method has been useful in determining the changes in the concentration of the total amino acids in relation to certain individual amino acids in leaves of healthy and beet yellows-infected plants.

Interest expressed in the method has prompted the present report.

Method

The ninhydrin reaction and the elution technique are not new. Both are common knowledge to investigators using paper chromatography (3). The concentration of total amino acids is expressed in terms of concentration of glutamic acid. The reaction is carried out on filter paper strips subjected to the same conditions, while the color is developing, as the papergrams used in the determination of certain individual amino acids, especially those of aspartic acid, glutamic acid and glutamine. The color is eluted from the paper and the intensity determined colormeterically.

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[2] Numbers in parentheses refer to literature cited.

Apparatus.—Photoelectric colorimeter with a No. 54 filter.

Reagents.—Glutamic acid standard solution (200 mg diluted to 100 ml with water).

Ninhydrin.—(3 g in 500 ml of redistilled ethanol).

Extraction solvent.—(ethanol 50%).

Paper.—Whatman No. 3MM. Cut into strips 5 inches wide and draw light pencil-lines ½ and 2½ inches from one edge. Make narrow strips ½ by 2½ inches long, left attached to the sheet at the 2½-inch line, by removing ⅛-inch segments across the paper. Taper each strip from about one inch, leaving about ⅛-inch of the paper in the center at the bottom (see Figure 1).

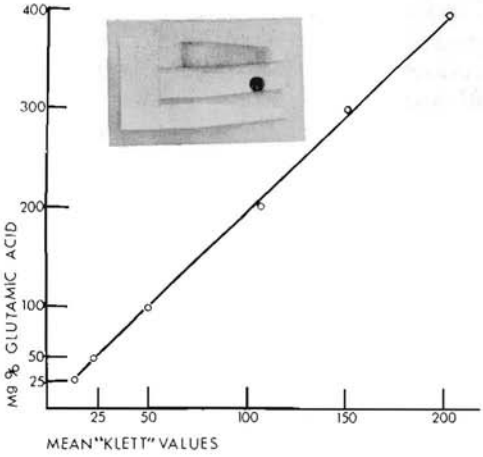

Figure 1.—A typical calibration curve.

Sample treatment.—Thaw, mix and centrifuge tissue extracts.

Tissue extracts used in test.—Leaf, petiole, crown and root tissue was taken from one sugar beet plant lifted from the field at about the middle of the growing season. The tissue was quick-frozen before the juice was expressed. The tissue extracts were stored at 0°F until used.

Procedure.—Place 5λ of the glutamic acid standard solution on strips at the ½-inch line. To other strips, apply a sufficient volume of the sample so that the colorimeter readings will fall in the same general range as that of the standard solution. Dilute the samples if necessary. Spread the samples over the strips by applying distilled water to the tip at a rate of approximately 16 drops per minute. From 6 to 8 drops are necessary. Do not wet the paper with water above the 2½-inch line. Hold the strips in a horizontal position while the drops of water are being applied. The samples can be spread uniformly over the strips by proper adjustment of the number and size of drops, their

rate of application, and the angle at which the paper is held. Spreading of the sample will allow sufficient ninhydrin to come into contact with the amino acids to produce the maximum color intensity. Dry the papers for ½ hour at 45°C then dip into ninhydrin reagent. Allow the strips to stand at room temperature until the free ethanol has evaporated. Place the papers in an oven maintained at 45°C and 50% relative humidity for 3 hours. Hold the oven to the above conditions by forcing preconditioned air through the oven during the 3-hour period. Cut the strips off at the 2½-inch line, fold and drop them, ends down, into calibrated Klett tubes containing .5 ml of the extraction solvent. Extract the color from the strips with frequent shaking for a period of ½-hour. Remove the strips and read the color intensity in the colorimeter using a No. 54 filter. Make corrections for the blank.

Results

Tests were conducted to determine whether or not glutamic acid would serve as a reliable standard of reference when different amounts are used. A series of dilutions of glutamic acid was made. The color intensity was determined by the above method using 4-5λ aliquots of each concentration. The mean colorimeter readings were plotted against the concentration of glutamic acid and a regression line drawn. A typical test is shown in Figure 1. The correlation coefficient, 0.999, is highly significant. In three other tests, the correlation coefficients were, 0.998, 0.996 and 0.992 highly significant, also.

The colorimeter readings for the glutamic acid standard solution were found to remain remarkably constant from day to day. The standard solution was run on 12 different occasions over a period of 6 months. The mean for the 24 determinations was 74.3. The standard deviation was 2.00. It is recommended, however, that the standard solution and a blank be run with each set of determinations.

To test the precision of measurements by the above method, the concentration of total amino acids was determined in the four tissue-extracts taken from one sugar beet plant. The results are shown in Table 1.

Spectrophotometeric analysis showed that a maximum absorbance occurred at the usual wave length of 570 mμ (for blue-violet color) and at a wave length of 410 mμ for both the tissue extracts and the glutamic acid standard solution. At a wave length of 350 mμ, used for orange-yellow color, the absorbance was only 35% of that at 410 mμ. At wave length 440 mμ (sometimes used) absorbance was only 8% of the absorbance recorded at 410

Table 1.—Concentration of total ninhydrin-reacting amino acids (calculated as glutamic acid) in the expressed juice from different tissues of a healthy sugar beet plant.

	Glutamic acid 200 mg %	Expressed juice from			
		Roots	Crowns	Leaves	Peticles
Aliquot (μl)	5	2.5	2.5	5	5
Reps. no.	9	9	9	9	9
Mean "Klett" reading	74.9	140.2	79.3	75.1	48.3
Std. dev.	1.36	3.70	2.52	0.74	0.79
Std. error	0.45	1.23	0.84	0.25	0.26
S. E. in % of Mean	0.60	0.88	1.06	0.33	0.54
Conc. total amino acids calc. as glumatic acid (mg %)		748	423	203	128

Conversion factor used: 200/74.9.

mμ. The slight shift in the wave length at which maximum absorbance occurred may be due to a combination of the reagents used and the conditions under which the color was developed.

In separate tests the total amino acid values, determined by using the photoelectric colorimeter with a No. 54 filter, were compared with the values obtained by using a spectrophotometer set at 570 mμ and at 410 mμ. Table 2, shows the ratios of the concentration of total amino acids, determined by absorbance at the two wave lengths, to values obtained using the colorimeter and a No. 54 filter. It is evident that the relative concentration of the total amino acids in the different tissues of the beet plant can be determined with a highly satisfactory degree of precision by taking the readings, using either wave length or with a photoelectric colorimeter using a No. 54 filter.

It is well known that compounds other than amino acids are present in plant tissue that react positively with ninhydrin. In sugar beet plants, the concentrations of these compounds, and the amino acids, are greatly affected by the nutritional level of the plants and by environmental factors. In some exploratory tests, it was necessary to apply at least 10λ to 2-dimensional

Table 2.—Ratios of concentration of total amino acids[1] determined by absorbance at two wave lengths to values determined by the photoelectric colorimeter ("Klett") using a No. 54 filter.

Exp. juice from	Ratio 570 mμ / "Klett"			Ratio 410 mμ / "Klett"			Ratio 410 mμ / 570 mμ		
	X 100		Mean	X 100		Mean	X 100		Mean
Test	1	2		1	2		1	2	
Leaf	96	102	99	101	107	104	105	105	105
Petiole	94	98	96	102	103	102	107	105	106
Crown	103	98	100	106	98	102	103	100	102
Root	105	96	102	108	101	104	103	105	104

[1] Calculated as glumatic acid.

papers before faint spots appeared that could not be identified as one of the normally occurring amino acids. In all probability, the relatively small volumes of tissue extracts required by this method (2.5 to 5 μl) was such that the color produced by the compounds, other than amino acids reacting with ninhydrin, was insignificant. It is also possible that those amino acids present in very low concentrations may not have been included in the determinations. This could be one disadvantage of the method.

The method has several distinct advantages. Small volumes of extracts may be used without further treatment. The color for total amino acids on the paper strips and for certain individual amino acids on papergrams can be developed at the same time under the same conditions. The method eliminates the rinsing and transfer of the samples to the absorption cell, which is necessary when a spectrophotometer is used. As many as 80 samples have been run in duplicate in a day by one technician.

The concentration of total amino acids in the mature leaves of healthy sugar beet plants was found to be approximately 1.6 times greater than that in the petioles while the concentration in the crowns was about double that in the leaves. The concentration of total amino acids in the roots was approximately double that in the crowns and nearly four times that in the leaves.

Summary

A simple colorimeteric method for the determination of the relative concentration of total amino acids (calculated as glutamic acid) in different plant tissue extracts is described. The reaction of the amino acids with ninhydrin is carried out on small strips of filter paper under the same controlled conditions used in this laboratory for the determination of individual amino acids by paper chromatography. The method has given excellent agreement with aliquots of the standard solution and with juice extracted from the different tissues of sugar beet plants.

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