

of filters, it might be possible to obtain 5 $m\mu$ resolution.

SUMMARY

The design of a small compact interference-filter monochromator system for the spectral range from 365 to 800 $m\mu$ is presented, together with a theoretical discussion of optical principles. The characteristics of interference filters and supplemental gelatin and aqueous filters are given. The monochromator has a resolution of about 10 $m\mu$ throughout the visible spectrum and can produce relatively high irradiances with an incandescent lamp. Irradiances as high as 2000 $\mu\text{w}/\text{cm}^2$ can be secured with a larger unit employing a 7-kw carbon arc.

The author wishes to express appreciation to Dr. W. H. Klein and Mr. V. Elstad who made many valuable suggestions for modifications while using the monochromator system, and to Mr. D. G. Talbert who constructed the instrument and Mr. J. H. Harrison who assisted in the design of the optical system.

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THE RELATION OF OPTICAL FORM TO THE UTILIZATION OF AMINO ACIDS. I. UTILIZATION OF STEREOISOMERIC FORMS OF GLUTAMIC ACID BY CARROT ROOT DISKS¹

E. D. H. EL-SHISHINY AND M. A. NOSSEIR

BOTANY DEPARTMENT, FACULTY OF SCIENCE, ALEXANDRIA UNIVERSITY, EGYPT

According to the classical idea of Liebig the plants are able only of assimilating inorganic nitrogen. But now there is no longer reason to doubt the ability of plants to absorb and utilize organic nitrogenous compounds, since in sterile culture plants can utilize various organic nitrogenous compounds, amino acids and amides. The extensive early literature describing the utilization of organic nitrogenous compounds by plants has been reviewed by Hutchinson and Miller (7) and by Brigham (2). Several other reports concerning the availability of amino acids for plant growth, that followed these papers, were reviewed by Ghosh and Burris (6).

Very little experimental work was carried out on the relation of optical form to the utilization of amino acids in higher plants, and our knowledge in this field is very scanty. The utilization of both optical forms of aspartic and glutamic acids by pea and clover was reported by Virtanen and Linkola (15). The synthesis of both isomers of glutamine from their respec-

tive isomers of glutamic acid catalysed by a purified enzyme from pea was shown by Levintow and Meister (9, 10).

The purpose of this investigation is to compare the ability of carrot root cells to absorb and assimilate the various stereoisomeric forms of glutamic acid and to attempt some explanations of the results obtained.

MATERIAL, METHODS, AND EXPERIMENTS

The disks for this investigation were prepared from carrot roots var. Chantenay. The general procedure of preparation and pre-treatment of the disks as well as the determination of the nitrogenous fractions were as described by El-Shishiny (4). Twenty grams of disks, taken at random from a stock of disks prepared for each experiment, were used for each sample. The samples were washed for four days in aerated distilled water. The samples were then washed several times with sterilized distilled water and transferred into the sterile culture solutions, kept at 25°C ($\pm 0.1^\circ$). The samples were aerated for

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24 hours by passing CO₂-free air through each culture solution at a constant rate of four liters per hour. The air currents, after leaving the culture chambers, were passed through standard solutions of sodium hydroxide to determine the rates of CO₂ production by the different treated tissues. After 24 hours the tissue samples were drained, washed with distilled water, and analysed for the final distribution of the various nitrogenous fractions. The medium, together with the washings, was made to a convenient volume and analysed for inorganic and organic nitrogen fractions. The initial distribution of various nitrogenous fractions in the tissues was obtained by analysing two samples representing the batch of disks after the four days washing period.

Nitrogen fractions:

Protein-N = insoluble-N + nitrogen fraction precipitated with 2.5 % trichloroacetic acid.

Non-protein-N = total soluble-N - nitrogen fraction precipitated 2.5 % trichloroacetic acid.

Amino acid-N = total amino-N - amino-N equivalent to amide-N.

Rest-N = non-protein-N - (inorganic-N + total amino-N + amide-N).

Complex-N = protein-N + rest-N.

Glutamine-N = 2 × glutamine amide-N.

Asparagine-N = 2 × asparagine amide-N.

Experiment I was designed to investigate the uptake and utilization of L-glutamic acids when supplied alone and in combination with ammonium sulphate to carrot root disks. Twelve samples after being washed for 4 days, were transferred into culture vessels, each containing 350 ml of sterile culture solution, according to the following scheme: Samples 1 and 2, distilled water; 3 and 4, 0.0025 M ammonium sulphate; 5 and 6, 0.0025 M L-glutamate; 7 and 8, 0.0025 M L-glutamate + 0.0025 M ammonium sul-

TABLE I

AMINO ACID AND AMMONIUM NITROGEN ABSORBED AND ASSIMILATED BY CARROT ROOT DISKS DURING 24 HOURS FROM DIFFERENT CULTURE SOLUTIONS

CULTURE MEDIA	MG N*/100 GM FRESH WT OF TISSUE			
	ABSORBED		ASSIMILATED	
	NH ₄ - N	AMINO ACID-N	NH ₄ - N	AMINO ACID-N
Distilled water	0.00	0.00	-0.32	-0.41
0.0025 M (NH ₄) ₂ SO ₄	45.33	0.00	34.77	1.37
0.0025 M L-Glutamate	0.00	38.41	-0.65	27.10
0.0025 M (NH ₄) ₂ SO ₄ + 0.0025 M L-Glu- tamate	68.14	38.90	57.52	36.93
0.0025 M D-Glutamate	0.00	13.59	-0.32	3.55
0.0025 M (NH ₄) ₂ SO ₄ + 0.0025 M D-Glu- tamate	23.57	14.65	8.12	5.60

* Means of duplicate samples.

TABLE II

AMINO ACID AND AMMONIUM NITROGEN ABSORBED AND ASSIMILATED BY CARROT ROOT DISKS DURING 24 HOURS FROM DIFFERENT CULTURE SOLUTIONS

CULTURE MEDIA **	MG N*/100 GM FRESH WT OF TISSUE			
	ABSORBED		ASSIMILATED	
	NH ₄ - N	AMINO ACID-N	NH ₄ - N	AMINO ACID-N
0.0025 M (NH ₄) ₂ SO ₄	40.11	0.00	29.88	2.57
0.0025 M L-Glutamate	58.71	28.76	48.60	29.41
0.005 M L-Glutamate	70.90	46.07	60.52	47.19
0.0025 M L- + 0.0025 M D-Glutamate	44.00	38.42	28.35	34.83
0.0025 M L- + 0.005 M D-Glutamate	42.20	44.10	26.87	37.60
0.005 M DL-Glutamate	40.42	43.67	24.45	39.20

* Means of duplicate samples.

** All media contain 0.0025 M (NH₄)₂SO₄.

phate; 9 and 10, 0.0025 M D-glutamate; 11 and 12, 0.0025 M D-glutamate + 0.0025 M ammonium sulphate. The results obtained from this experiment were recorded in table I and III.

Experiment II was designed to investigate the effect of D-glutamate on the uptake and utilization of the other enantiomorph when supplied simultaneously to the tissues. Twelve samples, after being washed for 4 days, were transferred into culture chambers, each containing 350 ml of sterile culture solution, according to the following scheme: Samples 1 and 2, 0.0025 M ammonium sulphate; 3 and 4, 0.0025 M ammonium sulphate + 0.0025 M L-glutamate; 5 and 6, 0.0025 M ammonium sulphate + 0.005 M L-glutamate; 7 and 8, 0.0025 M ammonium sulphate + 0.0025 M L-glutamate + 0.0025 M D-glutamate; 9 and 10, 0.0025 M ammonium sulphate + 0.0025 M L-glutamate + 0.005 M D-glutamate; 11 and 12, 0.0025 M ammonium sulphate + 0.005 M DL-glutamate. The results obtained from this experiment were recorded in tables II and IV.

L-, D-, and DL-Glutamic acid of CEP grade were purchased from the California Foundation for Biochemical Research. The other chemicals used were of analytical grade produced by Merck and Company, Inc. The amino acids were neutralized to pH 7 with KOH before supplying them to the tissues.

RESULTS AND DISCUSSION

UPTAKE AND ASSIMILATION OF L- AND D-GLUTAMIC ACIDS: It appears from the results depicted in table I that carrot root disks have absorbed far more L- than D-glutamate-N from their respective solutions. The absolute amount of amino-N absorbed from L-glutamate was about three times larger than that absorbed from D-glutamate culture solutions. Glutamate uptake was accompanied with higher respiration rates. This seemed to indicate the dependence of glutamate uptake upon respiratory energy as suggested by Webster (17). But differences in the degree of

absorption of the two isomers may be the result of differences in their rates of penetration into the cell. It may be suggested that the amino acid first combines with some cellular constituent at the surface and this complex is transported into the cell. Since the membrane consists of optically active material, differences in penetrability of stereoisomers according to this line might be possible.

Carrot root disks showed marked ability to utilize L-glutamic acid since the deficit in the balance of amino acid-N was equivalent to 70.6% of the absorbed amino-N from the L-glutamate medium, table I. The utilization of glutamic acid by other plant tissues was also reported by other investigators (1, 3, 5, 12, 13, 14, 15 and 17). The presence of ammonium sulphate with L-glutamate in the culture solution promoted the utilization of the L-glutamate. The stimulation here was the result of the increased ammonium-N level in the cells, since the amount of amino-N absorbed from L-glutamate was equal to that absorbed from L-glutamate-ammonium sulphate medium. But the increase of L-glutamate concentration in the L-glutamate-ammonium sulphate medium; from 0.0025 M to 0.005 M, promoted both the absorption and assimilation of amino acid-N to the same extent, table II.

On the contrary, there was no convincing evidence that carrot root disks were able to utilize D-glutamic acid. The deficits in the balance of amino acid-N in carrot disks cultured in solutions of D-glutamate alone or with ammonium sulphate were very small. However, when D-glutamate was added to the L-glutamate-ammonium sulphate medium, or when DL-glutamate was used simultaneously with ammonium sulphate, table II, more amino acid-N was utilized. But judging by the results obtained from experiment I, table I, and the demonstrated synergistic effects of mixtures of stereoisomers of amino acids by Macht (11), this increase might be on the expense of the L-form absorbed and that originally present in the cells. And since the method used for the determination of amino acid-N did not differentiate between the two isomers,

the above suggestion needs confirmation, and therefore awaits further investigation. Results obtained by other investigators showed that the ability to utilize D-glutamic acid varies greatly for the various plants. Virtanen and Linkola (15) reported that pea and clover utilize both forms of glutamic acid, while Levintow and Meister (10) demonstrated that the rate of glutamine synthesis from D-glutamate is about 40% of the rate of the L-isomer catalysed by an enzyme preparation from peas.

EFFECT OF L- AND D-GLUTAMATE ON THE UPTAKE AND UTILIZATION OF AMMONIUM-N: As shown in tables I and II, ammonium and L-glutamate, when present together in the outer solution, were absorbed simultaneously by carrot root disks, but ammonium in preference to glutamate.

It was also shown that L-glutamate, when present simultaneously with ammonium sulphate in the culture solution, enhanced greatly the total uptake of ammonium-N. On the contrary D-glutamate, under similar conditions, exerted a marked depressing effect on the uptake of ammonium-N. Moreover when the concentration of L-glutamate, in the L-glutamate-ammonium sulphate medium, was doubled the total uptake of ammonium-N was increased by 76.8%. But when the increase in glutamate concentration, in the L-glutamate-ammonium sulphate medium, was due to the antipode, the stimulating effect of L-glutamate on ammonium-N uptake was neutralized by the inhibiting effect of the antipode. The total amount of ammonium-N absorbed was more or less equivalent to the amount absorbed from the control solution of ammonium sulphate alone.

L-glutamate stimulated ammonium assimilation which appeared to be the direct effect of the increased absorption of ammonium-N, since the absolute increase in ammonium assimilation due to L-glutamate was equivalent to the absolute increase in ammonium-N absorption. This result accounts for the equivalent levels in ammonium-N in tissues cultured in ammonium sulphate and in ammonium-sulphate-L-glu-

TABLE III
EFFECT OF CULTURING CARROT ROOT DISKS IN SOLUTIONS OF 0.0025 M L- OR D-GLUTAMIC ACID ALONE OR IN COMBINATION WITH 0.0025 M AMMONIUM SULFATE

NITROGEN FRACTIONS	CONTROLS *		CHANGES IN N FRACTIONS DURING 24 HRS			
	H ₂ O	(NH ₄) ₂ SO ₄ **	H ₂ O**		(NH ₄) ₂ SO ₄ †	
			L-GLUTAMATE	D-GLUTAMATE	L-GLUTAMATE	D-GLUTAMATE
	<i>mg N/100 gm initial fresh wt of tissue ††</i>					
Ammonia-N	0.32	+ 10.24	+ 0.33	0.00	+ 0.06	+ 4.89
Glutamine-N	1.91	+ 15.82	+ 6.49	+ 1.96	+ 42.51	- 11.68
Asparagine-N	0.32	+ 4.96	+ 4.52	+ 2.26	+ 7.08	+ 0.13
Amino acid-N	8.67	- 1.78	+ 10.90	+ 9.63	+ 3.34	+ 10.42
Rest-N	5.52	+ 3.38	- 2.89	- 2.90	- 0.49	- 5.45
Protein-N	66.78	+ 11.80	+ 16.95	+ 2.61	+ 8.72	- 6.32

* Control tissues are cultured in distilled water and in 0.0025 M ammonium sulfate.

** Compared with control in water.

† Compared with control in ammonium sulfate.

†† Means of duplicate samples.

TABLE IV
EFFECT OF CULTURING CARROT ROOT DISKS IN SOLUTIONS OF 0.0025 M AMMONIUM SULFATE ALONE OR IN COMBINATION WITH L-, D-, OR DL-GLUTAMIC ACID

NITROGEN FRACTIONS	CONTROL IN (NH ₄) ₂ SO ₄	CHANGES IN THE N FRACTIONS DURING 24 HRS *				
		0.0025 M L-GLUTAMATE	0.005 M L-GLUTAMATE	0.0025 M L- AND 0.0025 M D-GLUTAMATE	0.0025 M L- AND 0.005 M D-GLUTAMATE	0.005 M DL-GLUTAMATE
<i>mg N/100 gm initial fresh wt of tissue **</i>						
Ammonia-N	10.99	- 0.12	+ 0.15	+ 5.42	+ 5.10	+ 5.74
Glutamine-N	19.33	+ 34.65	+ 54.39	+ 24.91	+ 22.82	+ 25.19
Asparagine-N	6.06	+ 2.19	+ 7.01	+ 2.72	+ 3.52	+ 2.80
Amino acid-N	5.31	+ 1.92	+ 1.45	+ 6.16	+ 9.07	+ 7.04
Rest-N	7.00	+ 5.50	+ 11.65	+ 5.16	+ 3.67	+ 5.16
Protein-N	63.79	+ 2.65	+ 0.01	- 4.66	+ 1.03	- 1.21

* Compared with control in ammonium sulfate.

** Means of duplicate samples.

tamate media, tables III and IV. On the contrary, D-glutamate expressed greater depressing effects on the assimilation than on the absorption of ammonium-N by carrot root disks, table I. Even when D-glutamate was supplied simultaneously with L-glutamate and ammonium sulphate, the absolute amounts of ammonium-N assimilated were less than that with ammonium sulphate alone. Consequently the ammonium-N levels in tissues cultured in solutions containing D-glutamate were always higher than the level of ammonium-N in tissues cultured in ammonium sulphate alone.

EFFECT OF L- AND D-GLUTAMIC ACID ON THE DISTRIBUTION OF THE VARIOUS NITROGENOUS FRACTIONS: The increase in the levels of amide-N and complex-N in tissues cultured in solutions containing L-glutamate alone or with ammonium sulphate, over those of control tissues in distilled water and ammonium sulphate respectively, tables III and IV, suggested the utilization of L-glutamate in the formation of these fractions. In the absence of ammonia, it seems probable that one part of L-glutamate absorbed was deaminated and that the ammonia produced was involved in amidation of the remaining part as suggested by Krebs (8) and Webster (16). The results of the present investigation indicated that ammonia was removed as fast as it was released since the tissues did not show any significant change in the levels of ammonium-N. Moreover the tissues cultured in L-glutamate alone table III, showed greater increase in the protein than in the amides. This might indicate the incorporation of glutamic acid in protein formation. The incorporation of a significant amount of C¹⁴ of L-glutamate in the tissue proteins of carrot and other plant tissues was proved by Webster (17).

On the other hand, tissues cultured in D-glutamate alone, table III, showed an increase of 4.22 mg amide-N × 2 and a deficit in the balance of amino acid-N of 3.55 mg per 100 gm fresh weight of carrot disks. And since the increase in protein-N balances the decrease in rest-N, it might be concluded that D-glutamate was

involved in the formation of amides. But the increase in amide-N might be on the expense of the natural form originally present, since addition of D-glutamate to ammonium sulphate caused a marked decrease in the amide-N × 2 and complex-N below that of control disks cultured in ammonium sulphate alone. The latter conclusion was again substantiated by the results of experiment II, table IV. These results indicated that D-glutamate exerted a depressing effect on the formation of amides from L-glutamate and ammonia. The degree of inhibition of the glutamine synthesizing system in carrot root by D-glutamate was less than that reported by Krebs (8) for that of guinea pig kidney.

SUMMARY

The uptake and utilization of the different stereoisomers of glutamic acid by carrot root disks were studied.

1. L-glutamate was absorbed at a much faster rate than the D-enantiomorph.
2. Carrot root disks showed a marked ability to utilize L-glutamate especially when supplied in combination with ammonium sulfate. On the other hand, there is no evidence of the utilization of D-glutamate which seems to accumulate in the cells.
3. L-glutamate stimulated the rate of ammonium uptake and assimilation in contrast to the depressing effect of D-glutamate on both uptake and assimilation of ammonium-N.
4. Addition of D-glutamate to L-glutamate neutralized the stimulating effect of the latter on the uptake and utilization of ammonium-N.

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THE INFLUENCE OF BORON ON STARCH PHOSPHORYLASE AND ITS SIGNIFICANCE IN TRANSLOCATION OF SUGARS IN PLANTS^{1,2}

W. M. DUGGER, JR., T. E. HUMPHREYS AND BARBARA CALHOUN
DEPARTMENT OF BOTANY, FLORIDA AGRICULTURAL EXPERIMENT STATION,
UNIVERSITY OF FLORIDA, GAINESVILLE, FLORIDA

An increase in the translocation of sugars in plants caused by boron has been demonstrated by Gauch and Dugger (6) and Sisler et al (26). Mitchell et al (22) obtained additional evidence that boron increases translocation of sugars and indirectly the translocation of growth-modifying substances. Previous work by Mitchell and Brown (21) and others showed that translocation of growth-modifying substances from leaves to other plant parts was dependent upon the translocation of sugars.

The hypothesis presented by Gauch and Dugger (6) to explain the influence of boron on translocation is based on: 1) the formation of borate-sugar complex; and 2) the movement of the complex through cellular membranes more rapidly than non-borate sugar molecules. Of the several possible explanations for the way boron could cause an increase in sugar translocation, these investigators favored the one which associated the borate ion with the cellular membrane. In this position the borate ion reacts chemically with the sugar molecule, and the resulting complex is transported across the membrane. A second reaction on the inside of the membrane releases the

sugar into the cell. Because of the rapid utilization of labeled sugars by living cells, these workers did not investigate this "carrier" concept as has been done in mineral element absorption studies (4).

Plants grown in a medium deficient in boron have been shown to accumulate carbohydrates, particularly starch, in the leaves (7). Because of this effect and the studies which show that boron increases translocation, it was decided to study the influence of boron on starch formation in vivo and in vitro.

In vitro studies by Winfield (29) showed a slight inhibition of starch phosphorylase by high concentrations of boron. He concluded that boron reacted with sugars in the same way as phosphorus; and because of the small boron:phosphorus ratio in plants, he did not believe that boron influenced the starch \rightleftharpoons glucose-1-phosphate equilibrium. Torsell (27), however, found no effect of 10^{-3} M arylboric acids on starch phosphorylase.

The effect of boron on the starch phosphorylase reaction has been reinvestigated in view of the fact that boron may possibly complex glucose-1-phosphate and thus prevent its conversion to starch. In addition the influence of boron on the hexokinase reaction has also been investigated. The results of these studies are presented in this paper.

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