THE RELATION OF OPTICAL FROM TO THE UTILIZATION OF AMINO ACIDS

III - Utilization of Stereoisomeric forms of Alanine by Carrot Root Disks

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INTRODUCTION

It was generally believed that the D-amino acids do not occur naturally and that the synthetic compounds of the D-series, if introduced into living matter, were metabolically inert. But now there is on longer reason to doubt the ability of plants to absorb and utilize various isomers of amino acids. The numerous studies, that have been made, of the use of amino acids as nitrogen sources for growing plants, embryos, plant tissues, and the limited nuuber of investigations of the utilization of stereoisomeric varieties of amino asids by higher plants were referred to in the first and second paper of this series by El-Shishiny and Nosseir (5 and 6). In these papers, it was also found that the L-forms of aspartic and glutamic acids are absorbed much faster than the D-forms, either given alone or with ammonium sulphate to carrot disks. The L-forms of hoth acids were well utilized and also stimulated the utilization of ammonium nitrogen, but the D-enantiomorphs accumulated in the cells.

In recent investigations, a number of features between plant species in the utilization of enantiomorphs as in the utilization of different amino acids have been discussed. Thus Nitsch and Nitsch (16) found that not onlyD-alanine, D-aspartic or glutamic acid cannot serve as sole sources of nitrogen for Helianthus tuberosus tissues, whereas the L-forms can, but also inhibitory effects on the growth was shown by the acid when supplied simultaneously with nitrate.

Again a number of features of the process of uptake of several amino acids by carrot tissues have been described by Birt and Hird (2). Thus it has been shown that the D-and L-isomers of many amino acids are taken up against a concentration gradient and that such uptake is inhibited by certain metabolic inhibitors. Moreover, the D-forms of alanine, pyenyl-alanine, leucine, isoleucine, valine, methionine and histidine were fully recoverable from the tissues after uptake. With the exception of glutamic and aspartic acid, alanine, phenyl-alanine, there was little metabolic destruction of the amino acids used.

Miettinen (15) reported that DL-glycine, serine, valine, leucine were assimilated by pea plants in the same way as DL-alanine and effectively utilized for the synthesis of protein. However, the D-enantiomorph of alanine was proved to be toxic for the pea plant whereas the L-form was beneficial.

The study of the metabolism of stereoisomers of amino acids in living organisms has increased our knowledge about the range and nature of oxidative and synthetic reactions available to the body. Moreover, biological differences between these two groups of stereoisomerides illustrate the marked specificity which specializes reactions of living matter. The aim of the present study is to investigate the ability of carrot root disks to absorb and utilize stereoisomeric forms of alanine, namely L- and D-forms.

MATERIAL, METHODS, AND EXPERIMENTS

The disks for this investigation were prepared from carrot roots variety « Chantenay ». The general procedure of preparation and pretreatment of the disks as well as the determination of the nitrogenous fractions are as previously described by El-Shishiny (4) and El-Shishiny & Nosseir (5 and 6). Twenty grams of disks (about 40 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 regenerated aerated distilled water. The samples were then washed with sterilized distilled water and transferred into the sterile culture solutions, kept at 25°C. ($\pm$ 0.1), where they were aerated for 24 hours by passing a current of CO_2 - free air through each culture chamber at a constant rate of 4 liters per hour. The air currents, after leaving the culture chambers, were passed through standard solutions of sodium hydroxide to determine the rate of CO_2 produced by the differently treated tissues. After 24 hours, the tissues were drained, washed with distilled water, and analysed for the final distribution of the various nitrogenous fractions. The medium, together with the washing, were made up to a convenient volume and analysed for inorganic and organic nitrogen fractions. The initial distribution of various nitrogenous fractions in the tissues were obtained by analysing two samples representing the batch of disks after the 4 days washing period.

Nitrogen fractions :

Protein — N	= insoluble — N + nitrogen fraction precipitated by 2.5 per cent trichloroacetic acid.
Non — protein — N	= total soluble — N minus nitrogen fraction precipitated by 2.5 per cent trichloroacetic acid.
Amino acid — N	= total amino — N minus amino — N equivalent to total amide — N.
Rest — N	= non — protein — N minus (inorganic — N + total amino — N + total amide — N).
Complex — N	= protein — N + rest — N.
Glutamine — N	= glutamine amide — N × 2.
Asparagine — N	= asparagine amide — N × 2.

Experiment 1. was designed to study the uptake and utilization of L- and D-alanine when supplied alone or in combination with ammonium chloride to carrot root disks. Therefore duplicate samples after being washed for 4 days in distilled water, were transferred into culture vessels each containing 350 ml. of the following culture solutions : Distilled water; ammonium chloride; L — alanine; L — alanine + ammonium chloride; D — alanine; D — alanine + ammontum chloride. The concentration of alanine and NH_4Cl in the solutions was 0.005 M. Experiment II was designed to investigate the effect of various concentrations of L — alanine, when supplied alone or in combination with NH_4Cl, on the nitrogen metabolism of carrot root disks. For this purpose, duplicate samples were cultured in the following solutions : Distilled water; ammonium chloride; 0.0025 M L — alanine; 0.005 M L — alanine; 0.01 M L — alanine; NH_4Cl + 0.0025 M L — alanine; NH_4Cl + 0.005 M L — alanine; NH_4Cl + 0.01 M L — alanine. The concentration of NH_4Cl in the solutions was always 0.005 M.

The chemicals used in this investigation were of analytical grade produced by Merck & Co. Inc.

RESULTS AND DISCUSSION

Uptake and Utilization of L — and D — alanine :

It appears from the results depicted in table (1) that carrot root disks were able to absorb both stereoisomeric forms of alanine from their respective solutions. The absolute amount of amino — N absorbed from L — alanine was, in all experiments, greater than that absorbed from D — alanine under similar conditions. The presence of ammonium chloride in the external medium was without any significant effect on the uptake of both isomers of

TABLE 1

Amino acid and ammonium nitrogen absorbed and utilized by carrot root discs during 24 hours from different culture solutions. Mg. nitrogen (means of duplicate samples) per 100 g. fresh weight of tissue. The amounts utilized are also given as percentages of the total amounts available in the tissues, ie. initially present plus absorbed

Culture media	Absorbed		Available		Found		Utilized			
	NH_4-N	NH_2-acid-N	NH_4-N	NH_2-acid-N	HN_4-N	NH_2-acid-N	NH_4-N		NH_2-acid-N	
							mg.	%	mg.	%
Distilled water	0.00	0.00	0.00	12.94	0.00	5.49	0.00	—	7.45	57.57
0.005 M NH_4Cl	53.69	0.00	53.69	12.94	14.70	10.88	38.99	72.61	2.06	15.92
0.005 M L-alanine	0.00	52.91	0.00	65.85	0.71	27.96	−0.71	—	37.89	57.54
0.005 M NH_4Cl + 0.005 M L-alanine	39.12	53.66	39.12	66.60	81.68	31.89	17.44	44.58	34.71	52.11
0.005 M D-alanine	0.00	44.93	0.00	57.87	0.72	49.04	−0.72	—	8.83	15.26
0.005 M NH_4Cl + 0.005 M D-alanine	28.53	43.61	28.33	56.55	20.55	47.77	7.78	27.46	8.78	15.52

alanine. Differences in the rates of absorption of the isomers from the media may be the result of differences in their rates of penetration into the cell. It might be suggested that there is an active process operating. This process may involve association with a carrier at the surface and movement of the complex, followed by dissociation, in the internal vacuole of the cells. L — and D — alanine seem to be taken up by the same mechanism as suggested by Birt & Hird (3). But the affinity of L — alanine for the carrier system appears to be greater than that of the D — isomer. Since the cell membranes consist of optically active material, differences in the penetrability of stereoisomers according to this suggestion might be possible.

Table (2) indicates that there was no material change in the amount of amino — N removed by carrot root disks when L — alanine concentration, either supplied alone or in combination with ammonium chloride, was doubled or increased 4 — fold. The suggested carrier system involved in the uptake, in this case, might have been saturated at low concentration.

The uptake of alanine was accompained by an increase in the respiration rate of carrot root disks. This result might indicate the dependence of uptake on respiratory energy. Similar observations were arrived at by Webster (20) and Birt & Hird (2) for the uptake of stereoisomers of alanine and other amino acids.

Carrot root disks showed a marked ability to utilize L — alanine since the deficit in amino acid — N is equivalent to 71.6% of the absorbed L — alanine, which corresponds to 57.54% of the initially present plus the absorbed amino acid — N from L — alanine media. Addition of equimolecular concentration of ammonium chloride to 0.005 M L — alanine in the external medium was of no significant effect on the rate of amino acid — N utilization. No remarkable changes in the rates of amino acid — N utilization were also obtained either by decreasing L — alanine concentration to 0.0025 M or by increasing it to 0.01 M, whether supplied alone or simultaneously with NH_4Cl, in the external media, table (2). The utilized amino acid — N seems to be at the expense of the absorbed L — alanine since the amounts of amino acid — N absorbed were more or less equivalent and this might account for the equivalent levels in amino acid — N in tissues cultured in L — alanine solutions, (tables 3 and 4). The utilization of alanine by other plant tissues was also reported by several investigators (8, 9, 10, 14, 15, 16, 17 & 18).

On the contrary, the results obtained from the present investigation suggested, however, that D — alanine, absorbed by carrot root disks, might have behaved as inert metabolite, since the increase of amino acid — N level in the tisues was almost equal to the absolute amount absorbed, compare

TABLE 2

Amino acid and ammonium nitrogen absorbed and utilized by carrot root discs during 24 hours from different culture solutions. Mg. nitrogen (means of duplicate samples) per 100 g. fresh weight of tisues. The amounts utilized are also given as percentages of the total amount available in the tissues, ie. initially present plus absorbed.

Culture media	Absorbed		Available		Found		Utilized			
	NH_4-N	NH_2-acid-N	NH_4-N	NH_2-acid N	NH_4-H	NH_2-acid-M	NH_4 N		NH_2-acid-N	
							mg.	%	mg.	%
Distilled water	0.00	0.00	0.37	11.56	0.37	7.08	0.00	—	4.38	38.76
0.005 M NH_4Cl	43.83	0.00	44.20	11.56	9.04	9.55	35.16	79.54	2.01	17.39
0.00.5 M L-alanine	0.00	52.36	0.37	64.42	0.38	17.53	−0.01	—	46.89	72.80
0.005 M L-alanine	0.00	55.34	0.37	67.40	0.38	18.68	−0.01	—	48.72	72.28
0.01 M L-alanine	0.00	54.50	0.37	66.06	0·76	18.28	−0.39	—	48.78	72.33
0.0025 M L-alanine + 0.005 M NH_4Cl	23.70	53.89	24.07	65.45	16.48	17.74	7.59	31.54	47.71	72.90
0.005 M L-alanine + 0.005 M NH_4Cl	23.80	55.25	24.17	66.81	16.14	19.69	8.03	33.23	47.12	70.52
0.01 M L-alanine + 0.005 M NA_4Cl	20.76	56.40	21.13	67.96	16.14	19.79	4.99	23.61	48.17	70.36

44.93 mg. absorbed from D — alanine with 43.55 mg. increase, and 43.61 mg. absorbed from D — alanine plus NH_4Cl with 42.28 mg. increase, tables (1 & 3). This can, also, be demonstrated by comparing the amounts utilized, calculated as percentages of the available amino acid — N, with the amounts of the corresponding control tissues cultured in distilled water or in ammonium chloride, table (1). From this comparison, it could be, also, concluded that the small amounts of amino acid — N that had disappeared from tissues cultured in D — alanine solutions might be at the expense of the L — from originally present in the cells.

The above results seem to be substantial to the findings of Birt & Hird (2) who showed that the D — form of alanine and other amino acids were fully recoverable from carrot root slices after uptake. In this respect, carrot root disks seem to behave like Lupinus albus seedlings (14), Helianthus tuberosus tissues (16) and pea plants (15). It can be stated that the accumulation of the absorbed D — alanine by carrot root disks, in the present investigation, might indicate that the prevailing enzyme system is specific to the L — form.

Effect of L — and D — alanine on the Uptake and Utilization of Ammonium — N :

Carrot root disks cultured in 0.005 M NH_4Cl alone, tables (1 & 2) absorbed from 40 — 53 per cent of the supplied ammonium — N. The rate of ammonium — N uptake was depressed when equimolecular concentration of L — or D — alanine was present simultansously with ammonium chloride, but the depression caused by D — alanine was much greater than that caused by the L — enantiomorph, table (1). When the concentration of L — alanine in the culture solution was decreased to 0.0025 M or increased to 0.01 M., the rate of ammonium — N uptake was also depressed but to a greater extent with 0.01 M concentration.

It was also noted that when ammonium chloride and alanine were supplied together, they were absorbed simultaneously, bnt amino acid — N in much higher preference to ammonium — N. Results obtained by other investigators showed that such preference in the absorption of ammonium and amino acid — N varies greatly for various amino acids and also for various plants (5, 9 & 18).

Tables (1 & 2), again, show that amounts equivalent to about 72 and 80 per cent of the ammonium — N absorbed by carrot tissues cultured in 0.005 M single salt solution of ammonium chloride were transformed into organic — N. L — alanine depressed ammonium — N assimilation and this depression was greatly pronounced by 0.01 M concentration, table (2). When L — alanine was replaced by D — alanine of the same concentration, the inhibiting effect of the D — isomer was greater than that of the L — isomer. The inhibiting effect of L — or D — alanine was greater on the assimilation than on the absorption of ammonium — N. Consequently, the ammonium — N levels in tissues cultured in solutions containing L — or D — alanine were always higher than the level of ammonium — N in tissues cultured in ammonium chloride alone.

Effect of L — and D — alanine on the Distribution of the Various Nitrogenous Fractions :

The increase in the levels of amide — N and complex — N in carrot tissues cultured in solutions containing L — alanine alone or with NH_4Cl, over those of control tissues in distilled water and ammonium chloride respectively, tables (3 and 4), suggested the utilization of L — alanine in the formation of these fractions. Thus in absence of ammonia, it seems probable that one part of L — alanine absorbed was deaminated (1 and 2) and that a part of the ammonia produced was involved in amidation of glutamic acid (11, 12 & 19) originally present, or given by amination of α — ketoglutaric acid (7) using another part of the liberated ammonia, or given by transamination of second part of L — alanine with α — ketoglutaric acid (13). The α — ketoglutaric acid might be initially found in the tissues or metabolically formed through respiratory cycle. Similarly, the slight increase in asparagine — N in tissues cultured in L — alanine solutions alone, table (4), might be at the expense of a third part of alanine which has undergone deamination and the subsequent amidation of aspartic acid originally present, or produced by amination of its non-nitrogenous presursor (7), or by transamination of a fourth part of alanine with oxalacetic acid initially found or metabolically produced. The existence of enzyme system responsible for the coupling of ammonia and aspartic acid was shown by Webster & Varner (22) in lupine and wheat germ tissues. The pyruvic acid produced through deamination or transamination of alanine might have furnished a substrate for respiration or might be involved in other metabolic reactions.

The results of the present investigation indicated that ammonia was removed as fast as it was released from alanine deamination since the tissues did not show any significant change in the levels of ammonium nitrogen. Moreover, the tissues cultured in L — alanine solutions, table (4), showed greater increase in the complex — N, mainly protein, than in the amides. This might indicate the incorporation of L — alanine in protein formation. The incorporation of L — alanine — 1 — C^{14} into the proteins of plant tissue homogenates was proved by Webster (21). Also, Miettinen (15) showed that L — alanine was effectivey utilized for the synthesis of protein by pea plants.

TABLE 3

Effect of culturing carrot root disks in solutions of 0.005 M L — or D — alanine alone in combination with ammonium chloride. The figures are in mg. N (means of duplicate samples) per 100 g. fresh weight tissue

Nitrogen fractions	Controls*		Changes in N fractions during 24 hours			
			H_2O XX		NH_4Cl X	
	H_2O	NH_4Cl XX	L-alanine	D-alanine	L-alanine	D-alanine
Ammonium-N	0 00	+14.70	+ 0.71	+ 0.72	+ 6.93	+ 5.85
Glutamine-N	8.12	+ 7.64	+10.31	+ 2.71	+ 6.90	− 1.34
Asparagine-N	2.54	+ 1.76	− 0.41	− 1.10	+ 0.60	+ 8.02
Amino acid N	5.49	+ 5.39	+22.47	+43.55	+21.01	+36.89
Rest-N	23.11	+20.29	+13.52	− 2.10	− 1.63	−17.83
Protein-N	70.51	+ 4.34	+ 8.23	+ 0.84	+ 5.03	− 3.54

* Control tissues are cultured in distilled water or 0.005 MNH_4Cl.

X Compared with control in NH_4 Cl.

XX Compared with control in water.

TABLE 4

Effect of culturing carrot root discs in solutions of L — alanine alone or in combination with ammonium chloride. The figures are given in mg. — N (means of duplicate samples) per 100 g. fresh weight of tissue.

Mitrogen fractions	Controls*		Changes in N fractions during 24 hours					
			H_2O XX			NH_4Cl X		
	H_2O	NH_4Cl XX	0.0025 M L-alanine	0 005 M L-alanine	0.01 M L-alanine	0.0025 M L-alanine	0,005 M L-alanine	0.01 M L-alanine
Ammonia-N	0.37	+ 8.67	+ 0.01	+ 0.01	+ 0.39	+ 7.44	+ 7.10	+ 7.10
Glutamine-N	2.26	+ 9.78	+11.30	+11.02	+17.97	+ 6.46	+ 5.32	+ 5.34
Asparagine-N	1.46	+ 4.06	+ 3.47	+ 3.58	+ 8.18	+ 0.32	+ 0 16	+ 0.92
Amino acid-N	7.03	+ 2.47	+10.45	+11.60	+11.20	+ 8.19	+10.14	+10.24
Rest-N	5.63	+ 2.83	+ 3.35	+ 4.24	– 4.08	– 3.70	– 1.57	– 2.37
Protein-N	63.04	+15.66	+23.50	+24 00	+20.82	+14.16	+14.52	+13.00

* Control tissues are cultured in distilled water and in 0.00M NH_4Cl.

X Compared with control in NH_4Cl.

XX Compared with control in water.

On the other hand, carrot root disks cultured in D — alanine, table (3), showed a smaller increase in amide — N X 2. This increase might he at the expense of complex — N which showed a more or less equal decrease. Addition of D — alanine to ammonium chloride caused a marked decrease in the glutamine - and complex — N fractions probably due to the inhibiting effect of this isomer on ammonium — N absorption and assimilation. But addition of L — alanine to ammonium chloride, although inhibited ammonium — N absorption and assimilation, yet it caused considerable increases in the organic — N fractions, reasonably, at the expense of the utilizable L — alanine.

SUMMARY

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

1. L — alanine was absorbed at a faster rate than D — enantiomorph.

2. Carrot root disks showed a marked ability to utilize L — alanine, but the D — isomer absorbed accumulated as such in the cells.

3. L — and D — alanine depressed the rate of ammonium uptake and assimilation; the depression caused by the D — form was more greater than that of the L — form. With both isomers, the depression on ammonium assimilation was more pronounced than on absorption.

4. L — alanine caused a greater increase in the protein than in amides, forming liberally glutamine; asparagine was formed, however, at a lower rate. On the contrary, D — alanine inhibited glutamine and complex — N synthesis.

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