The Metabolism of Glutamic Acid in Germinating Green Gram Seeds

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(*Received 5 May* 1955)

During germination of green gram seeds (*Phaseolus radiatus*), glutamic acid differs from the other amino acids by its rapid degradation (Sivaramakrishnan & Sarma, 1954*a*). It is converted into asparagine during germination (Sivaramakrishnan & Sarma, 1954*b*). This conversion takes place in Bengal gram (*Cicer arietinum*) and horse gram (*Dolichus biflorus*) seeds and may be presumed to be common to all legume seeds, supplying in part the energy requirements of their germination (Sivaramakrishnan, 1955). But a rough approximation revealed that, at the most, only a portion of the metabolized glutamic acid could have been converted into asparagine, suggesting the formation of other products. It has recently been shown that glutamic acid is converted into arginine and proline in *Escherichia coli* (Abelson, Bolton, Britten, Cowie & Roberts, 1953), and in rats (Salbach, Koeppe & Rose, 1951). The questions whether a similar conversion into arginine and proline takes place in germinating seeds also, and, if so, how far this conversion is quantitatively significant in the metabolism of glutamic acid, were deemed worth investigating. Hence a study of the metabolism of glutamic acid, using uniformly ^{14}C-labelled glutamic acid and involving the isolation of the four amino acids, glutamic acid, aspartic acid, arginine and proline, was conducted, and the details of the results obtained are presented here. These investigations on the catabolism of glutamic acid revealed that the rate of degradation of glutamic acid is far greater than was presumed earlier. They also suggested a simultaneous synthesis of glutamic acid during germination, and also that carbon dioxide is probably the major end-product of glutamic acid degradation.

EXPERIMENTAL

Materials

The green gram seeds were obtained from the Government Pulses Specialist, Coimbatore. They were carefully hand-picked, only the well-formed ones with a healthy green colour being chosen, and the odd-sized or defective ones discarded. Seeds thus chosen invariably weighed 40 ± 5 mg. each; and at least 98–99% of these germinated.

L-Glutamic acid hydrochloride (4·6 mg., 0·1 mc), and D-glucose (1·3 mg., 0·1 mc), both uniformly labelled with ^{14}C, were obtained from the Radiochemical Centre, Amersham, England.

Methods

Catabolism of glutamic acid. All the apparatus and solutions used in germinations were previously sterilized by autoclaving at 15 lb./sq.in. pressure (120°) for 15 min.

Seeds (20 g.), divided into four equal portions, were used for the germination. The seeds were sterilized by soaking for 1–2 min. in 0·1% $HgCl_2$, followed immediately by repeated washings with sterile distilled water. Under these conditions, no detrimental effects due to contact with $HgCl_2$ could be observed in the germination. Each portion was transferred aseptically into a sterile 11 cm. Petri dish containing a sterilized filter circle. The medium in each dish consisted of 0·23 mg. ^{14}C-labelled glutamic acid hydrochloride with an activity equal to $7{\cdot}19 \times 10^6$ counts/min. in sterile water. The volume of the medium was adjusted with sterile water to 24 ml., which was just enough for 72 hr. germination. Germinations were carried out in diffuse light in a sterile chamber at room temp., which varied between 28 and 31°. Seedlings grown under these conditions, if crushed and plated out in a nutrient medium, were always found to be free from any bacterial contamination.

At the end of the germination, the seedlings and Petri dishes with filter-papers were washed separately, and the radioactivity in the collected washings was determined. This gave a measure of the maximum amount of the radioactive compound left behind.

The seedlings, containing approximately 17 g. dry solids and 85 ml. water, were ground to a fine paste, mixed with 125 ml. 10N-HCl (final vol. 210 ml. 6N-HCl) and refluxed for 22 hr., the solution being kept just boiling. Excess of HCl was then removed by vacuum distillation, the final concentrate was diluted with water and cooled in the ice chest to precipitate out the humin completely. The humin was filtered off and the hydrolysate made up to 200 ml. A sample of this solution (20 ml.) was removed for the estimation of the four amino acids, as well as the total radioactivity after suitable dilution. Arginine was estimated colorimetrically according to Macpherson (1946), glutamic acid manometrically by enzymic decarboxylation

with *Clostridium welchii* S.R. 12 (Meister, Sober & Tice, 1951), and aspartic acid and proline were determined by microbiological assay with *Leuconostoc mesenteroides* P 60 [aspartic acid according to Hac & Snell (1945) and proline according to Sauberlich & Baumann (1949)].

From the remaining hydrolysate (180 ml.) the amino acids were isolated in the following order: arginine as flavianate, glutamic and aspartic acids as their barium salts, and proline as rhodanilate. In the isolation of arginine monoflavianate, the details given by Vickery (1940) were closely followed. The monoflavianate crystals were repeatedly recrystallized, until they showed a brilliant golden-yellow lustre and constant radioactivity. Glutamic and aspartic acids were separated together as their barium salts (Foremann, 1914) and purified by a reprecipitation. The two amino acids were then separated as glutamic acid hydrochloride and copper aspartate according to conventional procedures given by Block & Bolling (1945). Proline was isolated as the rhodanilate according to Bergmann (1935). All these amino acid derivatives were purified by several recrystallizations or reprecipitations until they showed constant radioactivity. Radioactivity measurements were carried out with a windowless gas-flow counter, using a Tracerlab SC-1C autoscaler and a SC-4 Eagle Preset Counter, with a probable statistical error in counting not exceeding 2%. All samples (0·5–1·0 mg.) were counted as uniform thin layers in stainless-steel planchets of diam. 2·4 cm. No corrections were made for self-absorption.

Formation of carbon dioxide from glutamic acid. A large Pyrex desiccator was sterilized by smearing the inside with formalin and heating in the air-oven at 110° to remove the formalin. After cooling, a sterile 11 cm. Petri dish with a filter circle was introduced and the upper half removed. Sterilized seeds (5 g.) were allowed to germinate in this open dish inside the closed desiccator for 72 hr. The medium contained 0·0257 mg. of labelled glutamic acid hydrochloride with an activity equal to $8{\cdot}039 \times 10^5$ counts/min. in 24 ml. sterile water. The CO_2 produced was absorbed in 50 ml. N-NaOH and precipitated from the hot solution as $BaCO_3$ by the addition of $BaCl_2$. The solution was filtered through an AG-4 sintered crucible, the precipitate washed thoroughly, and then dried to constant weight by heating. The total radioactivity in the $BaCO_3$ corresponds to the activity lost during germination.

The seedlings and the Petri dish, which contained a small amount of unabsorbed solution, were washed separately and the radioactivity in the combined washings was determined. The total radioactivity in the seedlings was also determined, though approximately, by grinding the seedlings with water in a Waring Blendor and measuring the activity in a very small portion of the macerate.

Synthesis of glutamic acid during germination. As the levels of many of the amino acids tested did not vary to any considerable extent during germination (Sivaramakrishnan & Sarma, 1954*a*), carbohydrates were considered possible sources of the synthesized glutamic acid and the formation of glutamic acid from glucose was investigated. The experiment consisted in allowing seeds to germinate in a medium containing ^{14}C-labelled glucose, and determining the radioactivity of glutamic acid hydrochloride isolated from the seedlings. The experimental details were the same as those given under 'Catabolism of glutamic acid', except that 0·0325 mg. of glucose with an activity equal to $30{\cdot}425 \times 10^5$ counts/min. was used in each dish instead of glutamic acid. Only aspartic and glutamic acid were isolated in this experiment.

RESULTS AND DISCUSSION

In Tables 1 and 2 are presented the results of the distribution of radioactivity from [^{14}C]glutamic acid after 72 hr. germination. It will be seen (Table 1) that the radioactivity retained by glutamic acid after 72 hr. is only about 5% of that supplied. Thus, during 72 hr. germination, as much as 95% of the radioactive compound has undergone degradation. This clearly points to an extremely rapid catabolism of glutamic acid in germinating seeds.

Table 1. *Distribution of radioactivity in various amino acids in green gram seedlings after germination in the presence of* ^{14}C*-glutamic acid*

For experimental details see text.

Constituent	Total radioactivity (counts/min.)	Percentage of added radioactivity
Radioactive glutamic acid added	$28{\cdot}8 \times 10^6$	100
Total hydrolysate	$3{\cdot}95 \times 10^6$	13·7
Glutamic acid	$1{\cdot}40 \times 10^6$	4·86
Aspartic acid	$1{\cdot}32 \times 10^6$	4·58
Arginine	$6{\cdot}25 \times 10^4$	—
Proline	$3{\cdot}05 \times 10^4$	—
Arginine + proline	$9{\cdot}30 \times 10^4$	0·325
Residual activity in Petri dish	$0{\cdot}286 \times 10^6$	0·995

On the other hand, determinations of glutamic acid content in the ungerminated seeds (204·2 mg./5 g. of seeds), and in the seedlings after 72 hr. germination (132·3 mg./5 g. seeds) indicate a net fall in the glutamic acid level of only about 35% during the same period (Sivaramakrishnan & Sarma, 1954*a*). In sharp contrast to aspartic acid and asparagine, whose levels vary quite considerably, the level of glutamic acid at the end of 72 hr. germination is constant to within 5%, so that the net fall in glutamic acid is always equal to $35 \pm 2{\cdot}5$%. Whereas the radioactivity lost by the glutamic acid gives an estimate of its catabolism, the manometric determinations give only the balance between its degradation and synthesis during that period. Applying this, it will be seen that, while there is about 95% degradation of glutamic acid during 72 hr., there must have been a synthesis of glutamic acid to the extent of about 60% during the same period since the net fall in glutamic acid content is only 35%. Evidence for the synthesis of glutamic acid is presented later on. Isotopic investigations, in combination with

periodical manometric determinations, thus reveal that glutamic acid is in a highly metabolically active state in germinating seeds.

The activity retained in the hydrolysate amounts only to 13% of the activity supplied, of which 4·9% is due to glutamic acid itself. Hence only 8·8% out of 95% metabolized is accounted for by products in the hydrolysate. The remainder, which constitutes the major portion of the glutamic acid metabolized (about 86%), must have been lost either in the course of germination or during the preparation of the hydrolysate. Utilization of glutamic acid for respiration through its keto analogue, α-oxoglutaric acid, and the Krebs cycle, is highly probable, and in this case the radioactivity would be lost as carbon dioxide. Chibnall (1939) has pointed out the possibility of protein being used for respiration as well as for amide formation, since the amounts of other carbon compounds were not sufficient for both. In view of its close association with α-oxoglutaric acid through glutamic dehydrogenase, which is known to be present in these seedlings (Damodaran & Nair, 1938), and its high metabolic activity, glutamic acid would appear to be the most probable respiratory substrate among the protein constituents. That it may be so used will be evident from the data presented in Table 2, where it will be seen that as much as 52% of the total radioactivity supplied as glutamic acid is recovered in the respired carbon dioxide.

Table 2. *Formation of radioactive carbon dioxide during germination of green gram seeds in the presence of* ^{14}C-*glutamic acid*

For experimental details see text.

Constituent	Total radioactivity (10^5 counts/min.)	Percentage of added radioactivity
Radioactive glutamic acid added	8·04	100
Carbon dioxide (as $BaCO_3$)	4·185	52·05
Seedlings	2·31	28·8
Hydrolysate	0·805	10·0
Washings, including unabsorbed solution	0·750	9·33
Total activity accounted for	7·25	90·1

Germinating seedlings also contain a wide variety of highly active glutamic transaminases (Stumpf, 1951; Albaum & Cohen, 1943), and it is very probable that, through the mediation of these enzymes, glutamic acid plays a central role in the synthesis of various amino acids in the growing plant. The rapid degradation of glutamic acid observed in the present investigations favours such an active participation of glutamic acid in amino acid synthesis. These reactions also lead to the conversion of glutamic acid into α-oxoglutaric acid, which on subsequent oxidation through the Krebs cycle gives rise to carbon dioxide. This sequence of reactions thus accounts for the radioactivity in the carbon dioxide.

A comparison of the total activity in the seedlings with that in the hydrolysate (Table 2) shows that appreciable activity is lost as volatile degradation products during the preparation of the hydrolysate.

The radioactivity present in aspartic acid (Table 1) is similar to that of glutamic acid, and is a major portion of the activity in the hydrolysate. Aspartic acid is thus an important metabolite of glutamic acid. Arginine and proline also contain activity. Thus, the conversion of glutamic acid into arginine and into proline, noted in other species (Abelson *et al.* 1953; Salbach *et al.* 1951), takes place in germinating seedlings also. But the total amount of radioactivity present in these two compounds is only 0·33% of the total activity supplied. As such, these conversions do not seem to represent major products of glutamic acid metabolism.

Table 3. *Incorporation of radioactivity into glutamic acid during germination of green gram seeds in the presence of* ^{14}C-*glucose*

For experimental details see text.

Constituent	Radioactivity (10^5 counts/min.
Radioactive glucose supplied in the medium	122·0
Total hydrolysate	5·95
Glutamic acid	0·336
Aspartic acid	1·98
Washings of seedlings and Petri dishes	Negligible
Specific activity of glutamic acid	7·44*
Specific activity of aspartic acid	24·2*

* Counts/min./μmole.

The results obtained with ^{14}C-labelled glucose are presented in Table 3. As with glutamic acid, the activity retained by the hydrolysate is very low and amounts to only 5%. The rest is lost either as carbon dioxide or as volatile degradation products. The glutamic acid isolated was found to be radioactive, and the radioactivity incorporated into it, though somewhat less, is quite comparable with that of aspartic acid, which is the amino acid known to accumulate in significant amounts during germination of legume seeds. This finding, coupled with the fact that the observed radioactivity is that retained by the amino acid despite the more dominant catabolism, indicates that the synthesis

of glutamic acid from glucose, and hence from carbohydrates, may be appreciable.

Aspartic acid accounts for about one-third of the activity in the hydrolysate. The specific activity in aspartic acid is considerably greater than that of glutamic acid. This suggests that, though a small amount of aspartic acid may be synthesized from glucose through glutamic acid, the major portion is formed directly.

SUMMARY

1. The metabolism of uniformly ^{14}C-labelled glutamic acid during the germination of green gram seeds has been investigated. A very rapid catabolism has been noted, along with strong presumptive evidence for the synthesis of glutamic acid during germination.

2. The major portion of the radioactivity of the glutamic acid metabolized is recovered in the carbon dioxide given off during germination.

3. Aspartic acid and asparagine are important products of glutamic acid degradation.

4. A slight conversion of glutamic acid into arginine and into proline has been noted.

5. By the use of ^{14}C-labelled glucose, evidence for the synthesis of glutamic acid from carbohydrates during germination has been noted.

6. The rapid degradation and the extensive synthesis of glutamic acid during germination clearly indicate its high metabolic activity in germinating seeds.

One of us (V.M.S.) is indebted to the Government of India for the award of a Senior Research Scholarship during the tenure of which these investigations were carried out.

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