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ENHANCED OPERATION OF 4-AMINOBUTYRATE SHUNT IN γ-IRRADIATED POTATO TUBERS

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Key Word Index—*Solanum tuberosum*; Solanaceae; potato tubers; metabolism; 4-aminobutyrate; γ-radiation.

Abstract—γ-Radiation at a sprout inhibiting dose of 100 Gy enhanced the operation of the 4-aminobutyrate shunt by about 3-fold 48 hr after irradiation, but there was no accumulation of the amino acid in irradiated potatoes during storage up to 6 months. γ-Radiation increased the activity of the first enzyme of the 4-aminobutyrate shunt, glutamate decarboxylase and the maximum increase of 2.6-fold was found on the fourth day after irradiation. γ-Radiation had no pronounced effect on the activity of the other two enzymes of the pathway, 4-aminobutyrate transaminase and succinate semialdehyde dehydrogenase. Based on the results obtained, a possible role for the 4-aminobutyrate shunt in potato tuber physiology was suggested.

INTRODUCTION

Many higher plant tissues contain large amounts of 4-aminobutyrate (4-AB), a non protein amino acid, in their free amino acid pool [1, 2]. A number of environmental stress conditions such as anoxia [3], low temperature [4], darkness [5], mineral deficiency [6], wounding [4], ageing [7, 8] and γ-radiation [9] are known to further elevate 4-AB levels in various plant tissues, leading to the accumulation of 4-AB. This accumulation is attributed to the increased formation [3, 7, 10] and/or a decreased utilization of 4-AB [3] in stressed plant tissues. However, the physiological significance of 4-AB accumulation in stressed plant tissues is not yet well understood.

Accumulation of 4-AB in an edible plant tissue such as potato tubers assumes additional significance as 4-AB is a well known inhibitory neurotransmitter in the human brain [11] and other roles have also been implicated recently for this compound in mammals [12]. Although the 4-AB content of potato tubers is one of the highest known among higher plants [1], the metabolism, accumulation, enzymology and role of 4-AB in this tissue has not been well studied. In addition, there are conflicting reports on the effect of γ-radiation (for sprout inhibition) on the 4-AB content of potatoes. Jaarma [9] found that irradiation of potato tubers at a sprout inhibiting dose of 100–120 Gy increased the 4-AB content and she observed that irradiated potato tubers contained double the amount of 4-AB than unirradiated potatoes, even after 6 months of storage. In contrast, Takano *et al.* [13] did not find any significant differences in 4-AB levels in control and irradiated potatoes (receiving doses of 100–120 Gy) at time intervals ranging from 1 day to 8 months after irradiation.

As a part of our detailed investigations on the metabolism, enzymology and role of 4-AB in potato tubers, we have recently reported [14] the operation of the 4-AB shunt and the presence of all the three enzymes of the 4-AB shunt in potato tubers. In a separate communication [15], we have also reported the purification and characterization in detail of the first enzyme of 4-AB shunt, GDC (glutamate decarboxylase, EC 4.1.1.15) from potato tubers. We now report here the γ-radiation induced alterations in the 4-AB shunt in potato tubers and its possible significance in the tuber physiology.

RESULTS

Formation of 4-AB

Control and irradiated potatoes were fed with uniformly labelled L-[^{14}C]-glutamate immediately after irradiation, and the distribution of radiolabel from glutamate into different compounds was determined at intervals. The results from these studies are presented in Fig. 1. L-Glutamate was predominantly converted to 4-AB, even in control dormant tubers, indicating that glutamate to 4-AB conversion was the main pathway for glutamate metabolism in potato tubers. γ-Irradiated potatoes contained less radioactivity in L-glutamate and more in 4-AB as compared to unirradiated tubers at all times. These results suggest two possibilities; one is that the radiation enhanced glutamate to 4-AB conversion and hence the higher radioactivity observed in 4-AB in irradiated tubers over that of controls. The second possibility is that the rate of glutamate to 4-AB conversion remains unaltered after irradiation but the higher radioactivity observed in irradiated tubers merely reflects a decreased utilization of 4-AB, possibly as a result of a block in 4-AB catabolism in these tubers. However, in the case of the second alternative, one should then observe the accumulation of 4-AB in irradiated tubers. So 4-AB content of control and irradiated potatoes was determined (see below).

Quantitative changes in amino acid content

Glutamate, glutamine and 4-AB content of control and γ-irradiated potato tubers during storage after irradiation is shown in Table 1. There was no significant difference in

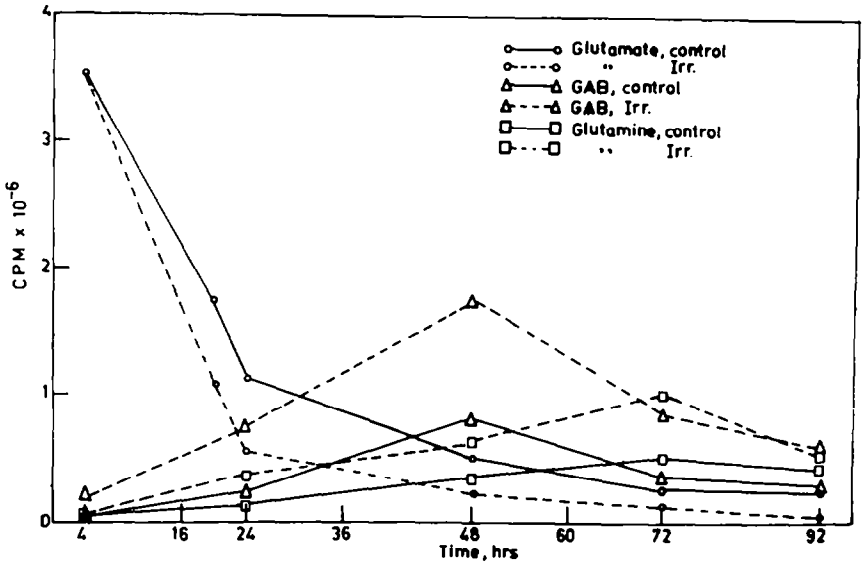

Fig. 1. Radioactive distribution of L-[U-^{14}C]-glutamate into different compounds in control and γ-irradiated potato tubers.

the 4-AB content of control and irradiated potatoes as determined by a more sensitive and highly specific enzymatic method. There was no accumulation of 4-AB in irradiated potato tubers at all time intervals studied, and in fact, a slight decrease was observed on some occasions. However, a significant decrease in glutamate level accompanied by an increase in glutamine level was found in irradiated tubers indicating that glutamate to glutamine conversion was also enhanced by γ-radiation, possibly by activating glutamine synthetase. These results, together with the data from [^{14}C]-glutamate feeding studies, strongly suggested that both processes, formation of 4-AB from glutamate and its further breakdown, were enhanced by irradiation, explaining the unaltered 4-AB levels observed after irradiation.

4-AB catabolism

Unirradiated and irradiated potatoes were fed with uniformly labelled [^{14}C]-4-AB immediately after irradiation and samples were analysed for radiolabel distribution in various compounds at different time intervals in an attempt to understand the catabolic pathway of 4-AB in potatoes. The results are presented in Fig. 2. About 85–90% of the total radioactivity fed as [^{14}C]-4AB was recovered in CO_2 plus alcohol soluble fraction, and most

Table 1. 4-AB, glutamate and glutamine content* of control and γ-irradiated potato tubers during storage

Time after irradiation	4-AB		Glutamate		Glutamine	
	Control	Irradiated	Control	Irradiated	Control	Irradiated
4 hr	3.23 ± 0.17	3.2 ± 0.12	6.39 ± 0.3	6.09 ± 0.19	11.77 ± 0.8	14.13 ± 0.19
16	3.25 ± 0.065	3.4 ± 0.17	6.36 ± 0.2	5.86 ± 0.11	10.84 ± 0.3	13.8 ± 0.12
24	3.11 ± 0.18	3.2 ± 0.11	6.42 ± 0.05	5.21 ± 0.09	11.06 ± 0.12	13.65 ± 0.18
36	3.5 ± 0.31	3.65 ± 0.17	5.97 ± 0.26	4.9 ± 0.13	11.26 ± 0.1	15.84 ± 0.35
48	3.8 ± 0.09	3.18 ± 0.09	6.49 ± 0.1	4.75 ± 0.06	11.2 ± 0.04	13.7 ± 0.25
72	3.8 ± 0.2	3.6 ± 0.12	6.02 ± 0.34	4.53 ± 0.3	11.24 ± 0.2	14.66 ± 0.15
96	3.85 ± 0.1	3.13 ± 0.14	6.23 ± 0.29	4.15 ± 0.2	11.5 ± 0.15	13.8 ± 0.8
120	3.83 ± 0.4	3.38 ± 0.21	6.48 ± 0.46	4.1 ± 0.04	11.76 ± 0.11	13.2 ± 0.63
7 days	3.32 ± 0.2	3.48 ± 0.13	6.32 ± 0.27	4.38 ± 0.1	12.9 ± 0.25	15.54 ± 0.14
15	3.43 ± 0.11	3.8 ± 0.19	6.39 ± 0.16	4.36 ± 0.08	13.54 ± 0.1	14.63 ± 0.48
30	2.72 ± 0.43	3.25 ± 0.09	6.66 ± 0.12	5.32 ± 0.4	14.04 ± 0.18	17.12 ± 0.16
60	4.2 ± 0.25	3.4 ± 0.13	6.75 ± 0.03	6.5 ± 0.16	13.62 ± 0.4	13.43 ± 0.22
90	4.05 ± 0.18	3.7 ± 0.04	6.87 ± 0.16	6.44 ± 0.18	12.74 ± 0.1	11.85 ± 0.15
120	—†	4.4 ± 0.16	—	6.84 ± 0.14	—	11.23 ± 0.15
180	—	4.6 ± .3	—	6.26 ± .07	—	11.16 ± 0.14

* Expressed as μmol/g fr. wt. Mean value ± standard error of four determinations.

† All the control tubers sprouted by this time.

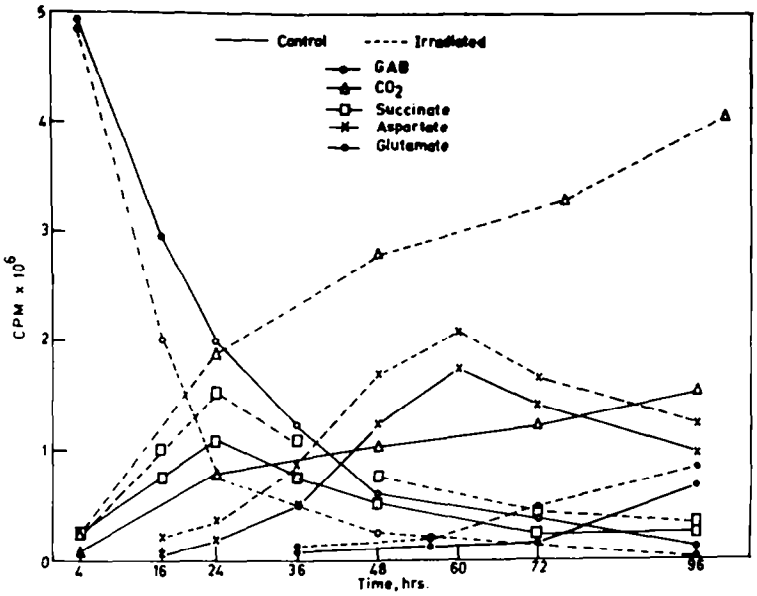

Fig. 2. Radioactive distribution of [U-C^{14}]-4-AB into different compounds in control and γ-irradiated potato tubers.

of the label was found in the compounds given in Fig. 2. As reported earlier [14], the pattern of radioactive distribution in different compounds was consistent with the contention that the 4-AB shunt was operative in potato tubers. That is, 4-AB is formed from L-glutamate by the action of GDC and is further metabolized to succinate via succinate semialdehyde by the enzymes, 4-AB-T (4-AB transaminase, EC 2.6.1.19) and SSADH (succinate semialdehyde dehydrogenase, EC 1.2.1.24). It can be further seen from the Fig. 2 that γ-radiation has enhanced the 4-AB breakdown and about a two-fold increase was found by 24 hr after irradiation. A faster decrease in radiolabel in 4-AB in irradiated tubers was accompanied by faster increase in $^{14}CO_2$ liberated, indicating that 4-AB catabolism was activated all the way up to CO_2 level by irradiation.

4-AB shunt enzymes

In order to find out whether the enhanced 4-AB shunt operative in irradiated potato tubers was due to the effect of γ-radiation on 4-AB shunt enzymes, the activities of the three enzymes of the shunt were measured in cell-free extracts of control and irradiated potato tubers. γ-Radiation was found to increase GDC activity of potato tubers and a maximum increase of about 2.6-fold (Fig. 3) was found on the fourth day after irradiation. On the other hand, there was no significant effect of γ-radiation on 4-AB-T and SSADH activities in potato tubers, although 4-AB-T displayed a marginal increase (10–20%) after irradiation (data not shown).

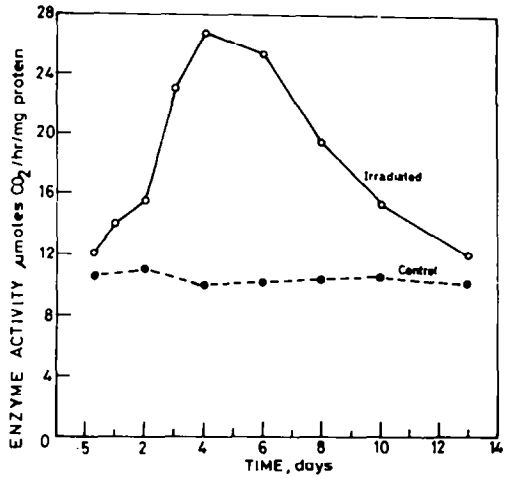

Fig. 3. Glutamate decarboxylase activity in control and γ-irradiated potato tubers during storage.

DISCUSSION

γ-Irradiation of potato tubers at a sprout inhibiting dose of 100 Gy accelerated the 4-AB shunt pathway by about 3-fold, but there was no significant accumulation of 4-AB in irradiated potatoes during storage. Our results on the 4-AB content of irradiated potatoes during storage are in good agreement with the work reported by Takano *et al.* [13] but differ from the work of Jaarma [9]. Since the latter worker's claim of 4-AB accumulation in irradiated potatoes was solely based on the data obtained with one dimensional paper chromatograms of potato crude extracts without any preliminary purification step, these results may be vitiated by other interfering substances present in the crude extracts.

The increased rate of 4-AB formation from L-glutamate in irradiated potatoes *in vivo* was well correlated with the increased GDC activity as measured in crude potato extracts *in vitro*. The mechanism(s) by which γ-radiation increases GDC activity is not yet fully understood and regulation of GDC activity in potato tubers is complex [15]. However, there was no correlation between the increased breakdown of 4-AB as reflected by *in vivo* [^{14}C]-4-AB incorporation studies and the activities of the

catabolic enzymes, 4-AB-T and SSADH in potato crude extracts *in vitro*. There could be several reasons for this. Since GDC is known to be the key enzyme of the 4-AB shunt [16], steady state levels of 4-AB in potato tubers might be controlled by GDC rather than by 4-AB-T. It is also possible that the existing 4-AB-T activity in un-irradiated tubers is more than sufficient to channel additional 4-AB formed after irradiation. It is interesting to note that both GDC and 4-AB-T have pH optima of 5.8 [3, 15] and 9.0 [3] respectively, values which differ from the potato tuber cellular pH of 6.8. So the actual enzyme activities *in vivo* will be determined by the pH of the microenvironment surrounding the enzyme active sites. In any case, failure to detect free succinate semialdehyde in potato tubers [14] coupled with the fact that potato SSADH has an extremely low K_m (5–10 μM) for succinate semialdehyde [unpublished data] suggests that a further increase of SSADH would not be required to convert any extra succinate semialdehyde formed after irradiation.

4-AB metabolism in γ-radiation stressed potato tubers differs from other plant tissues under various stress conditions [3–9] in one significant respect. Most other stressed plant tissues passively accumulate 4-AB as a result of an increase in 4-AB formation and/or a decrease in its utilization. Increased 4-AB formation in stressed tissues is attributed either to the increased availability of L-glutamate, the substrate for 4-AB forming enzyme [6, 8, 17] or an increase in GDC activity [3, 4, 7] or both. A decrease in cellular pH known to occur under stress conditions (especially anaerobiosis) [10], is more favourable for GDC and unfavourable for 4-AB-T action, thus further elevating 4-AB levels and lowering its utilization. In addition, the last reaction of the 4-AB shunt catalysed by SSADH is oxygen sensitive and hence is blocked under anaerobic conditions. In contrast, γ-radiation accelerated both processes, 4-AB formation and its further catabolism in potato tubers and there was no net 4-AB accumulation in potatoes after irradiation. γ-Radiation is known to deplete glutamate levels [9, 18] and increase cellular pH [19] as a result of a decrease in citrate levels in potato tubers, both factors contributing to the prevention of 4-AB accumulation.

Potatoes, immediately after irradiation, are in a transiently activated metabolic state which persists for 2–3 days before the tubers go into a perpetual dormant state [18, 20, 21]. One of the most pronounced and immediate effects of radiation on potato tubers is a large increase in respiration, and 2–5-fold increases have been reported by different workers [18, 22, 23]. Enhanced operation of the 4-AB shunt in irradiated potato tubers at a time when the tuber itself is in a highly active metabolic state indicates a dynamic role for the 4-AB shunt in the tuber physiology. One possible role that could be envisaged is that of channeling the additional 2-oxoglutarate formed as a result of increased respiration after irradiation of potatoes. Presence of high 4-AB-2-oxoglutarate transaminase and SSADH in potato tubers (unlike other plant tissues [24–26]) and their localization in mitochondria [4, 14, 20] where the tricarboxylic acid cycle occurs, also support this contention. Thus the carbon skeletons of 4-AB could be actively contributing to the energy generating processes in the cell. Balaz *et al.* [21] have reported that 4-AB flux represents 8% of the total tricarboxylic acid cycle in guinea pig brain slices but no similar information is available from plant sources. However, the presence of considerable amounts of 4-AB shunt enzymes in the post-mitochondrial supernatants of crude potato extracts [4, 14, 20] indicates that 4-AB has some other, as yet undefined functions in the potato tuber.

EXPERIMENTAL

Plant material. Potato tubers (*Solanum tuberosum* L., Kufri chandramukhi cultivar) were obtained from the local market within 1 month after harvest and were stored at room temp.

Chemicals. L-[U-^{14}C]-Glutamate (130 mCi/mmol) and [U-^{14}C]-4-AB (213 mCi/mmol) were obtained from the Isotope Group, BARC (Bombay). Hyamine hydroxide was the product of New England Nuclear. Gabase and fine chemicals were from Sigma. All other reagents were of highest purity.

γ-Irradiation. γ-Irradiation of potato tubers at a dose rate of 35 Gy/min was done in a Gamma Cell 220 (Atomic Energy of Canada Ltd) in air and at room temp. and tubers received a sprout inhibiting dose of 100 ± 20 Gy.

Storage studies. Healthy and well cured potatoes of uniform size were selected and divided into two lots. One lot was γ-irradiated, whereas the other lot served as control. Both lots, 30 kg in each, were stored in well ventillated hard board boxes at room temp. (25–28°) and a relative humidity of 60–65%. The samples were inspected from time to time and the tubers showing a tendency to sprout or rot were immediately removed. Samples were taken out at different time intervals for the determination of amino acid content.

Determination of amino acid content. Sliced potato tuber tissue (100 g) was extracted with ice cold 0.6 M $HClO_4$ in a Waring blender (3 × 200 ml). After removal of acid insoluble material by centrifugation (15 000 *g*, 20 min, 0–2°), the amino acids in the acid extract were separated by pH adjustment to 7 with 1 M KOH, removal of $KClO_4$ by centrifugation and ion exchange chromatography on Dowex-50 (H^+form). Dowex-50 eluates, after removal of alkali and concn, were used for the determination of amino acids. 4-AB was determined enzymatically using a commercial Gabase preparation [28]. Since automatic amino acid analyser using citrate buffers could not separate the dicarboxylic amino acids from their amides, glutamate and glutamine content of Dowex-50 eluates was determined by making use of two principles [29]. The dicarboxylic amino acids were adsorbed on acid treated alumina (neutral alumina, Brookman activity 1 from National Chemical Laboratory, Pune was stirred in 1 M HCl for 1 min and then washed with H_2O until free of acid) and were eluted with 1 M HCl. The soln from which the dicarboxylic amino acids have been removed was subjected to acid hydrolysis (100°, 1 hr in 2 M HCl) to convert amides into dicarboxylic amino acids and the hydrolysates were rechromatographed exactly as before. The method consistently gave greater than 98% recovery of standard glutamate applied and the presence of large amounts of amides did not interfere in the separation. Glutamate present in the alumina column eluates was determined in the presence of aspartate by the colorimetric method of ref. [30]. This method, although less sensitive (in the range of 1–8 μmol), is highly specific for glutamate and aspartate, even at 10 times higher conc, did not interfere.

Labelled studies with radioactive glutamate or 4-AB, preparation of cell free extracts and 4-AB shunt enzyme assays were carried out exactly as before [14].

Protein estimation. Protein content of cell free extracts was determined by the method of ref. [31].

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