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ALTERATIONS IN *SOLANUM TUBEROSUM* POLYPHENOL OXIDASE ACTIVITY INDUCED BY GAMMA IRRADIATION

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Key Word Index—*Solanum tuberosum*, Solanaceae, potato tuber, gamma irradiation, catecholase, cresolase

Abstract—On gamma irradiation of potato tubers at sprout-inhibiting dose (10 krad) the cresolase activity showed a 45% increase while catecholase was reduced by 25%. This reduced the ratio of catecholase to cresolase from 11–12 in unirradiated to 5–6 in irradiated potatoes. Chlorogenic acid oxidation was enhanced by about 25% on irradiation. The increase in the oxidation of *p*-cresol corresponded with the production of diphenolic compounds. The process of activation of cresolase was slow, reversible and oxygen dependent. A comparative study of the isoenzyme pattern suggested that this activation was due to conformational change, rather than synthesis of new protein.

INTRODUCTION

GAMMA irradiation at high levels is known to produce physiological changes in fruits and vegetables, which at times results in browning. In banana, doses above 50 krad caused considerable radiation injury and the fruits became black on storage. This colour change was correlated with an increase in polyphenol oxidase (*o*-Diphenol-O_2 oxido reductase) activity.[1] In certain varieties of potato tuber, irradiation even at sprout-inhibiting dose levels (5–15 krad) has been reported to induce browning.[2] There have been conflicting observations on the changes in activities of the enzyme responsible for browning.[3–7] Thus, Ogawa and Uritani[2] have shown a transient increase in diphenolase activity in tubers whereas Cheng and Henderson[8] observed a decrease in diphenolase activity at this dose level. The present investigation was therefore undertaken to make a critical assessment of the effect of gamma irradiation on polyphenol oxidase in certain varieties of potatoes grown in India. The varieties used in the present study were grown in Punjab (Chandramukhi) and Maharashtra. These tubers on irradiation at the sprout-inhibiting dose of 10 krad did not show any signs of browning.

RESULTS

In potatoes most of the polyphenol oxidase activity was located in the peel tissue.[9] The studies reported here were conducted with the enzyme isolated from skin tissue only. The

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[2] OGAWA, M and URITANI, I (1970) *Agr Biol Chem* **34,** 870
[3] SUSSMAN, A S (1953) *J Cell Comp Physiol* **42,** 273
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[6] COLTER, D J. and SAWYER, R L (1961) *Am. Potato J* **38,** 58
[7] OGAWA, M, HYODO, H and URITANI, I (1969) *Agr Biol Chem (Tokyo)* **33,** 1220
[8] CHEUNG, K W K and HENDERSON, H M (1972) *Phytochemistry* **11,** 1255
[9] PATIL, S S and ZUCKER, M (1965) *J Biol Chem* **240,** 3938

two substrates employed for this study were *p*-cresol for monophenolase and catechol for diphenolase, these being the standard substrates generally used for determination of polyphenol oxidase activity Polyphenol oxidase is a complex enzyme having multiple forms, each being substrate-specific and functioning independently.[10]

When the enzyme was isolated from peelings from unirradiated tubers the ratio of catecholase to cresolase was about 11 1 Using the enzyme isolated in the same manner from tubers previously exposed to 10 krad of gamma rays, the ratio was about 5·5 1. The dose used was very specific in bringing about the change Maximum reduction in this ratio occurred at 10 krad with a linear increase with further increments of the dose up to 500 krad when the ratio was again about 11:1. Among different substrates tested for activity with the enzyme preparation from the irradiated sample a marked increase in the oxidation of *p*-cresol and chlorogenic acid was noted, whereas catecholase activity was reduced considerably (Table 1).

TABLE 1 A COMPARISON OF ACTIVITY OF PHENOLASE COMPLEX ISOLATED FROM UNIRRADIATED AND IRRADIATED POTATOES TOWARDS DIFFERENT SUBSTRATES

Substrate	Sp act μmol O_2 consumed/min/mg protein	
	Control	Irradiated
p-Cresol	29 5	43 0
Tyrosine	1 2	1 4
Catechol	326 0	253 0
Chlorogenic acid	462 0	560 0
Caffeic acid	279 0	326 0
DOPA	67 0	56·0
Tyramine	17 5	23 2
Catechin	140 0	185 0

The respective substrates were added to the reaction mixture at a final concentration 5×10^{-4} M Details of the procedure are described under Experimental

Cresolase and catecholase activities of the control samples were unchanged after a week's storage. On the other hand, a 1·5-fold increase in cresolase was observed within 24 hr after irradiation However, this activity dropped to 25% of the normal level in 7 days. Catecholase was very sensitive to gamma irradiation, the fall in activity within 24 hr being 25%, and activity continued to fall progressively to less than 10% of the original value in 7 days. The ratio of catecholase to cresolase therefore decreased rapidly in the first 24 hr after irradiation, attaining half the control value, and this was followed by an increase in the ratio which reached the original value of 11·1 within a week

The activation of cresolase due to irradiation took place within 24 hr of irradiation. The increased oxygen uptake with *p*-cresol as substrate was coupled with hydroxylation of the ring, thus the formation of diphenols was faster and the amount formed was greater in the irradiated samples. The maximum denoting the net amount of diphenol present shifted from 10 min in control to 15 min in irradiated samples. It is not clear how gamma irradiation could cause a transient change in the activity of the phenolase complex. The part played by oxygen in this transformation was assessed by irradiating potatoes in a nitrogen atmosphere. When potatoes were completely deprived of oxygen, both during irradiation and storage there was no change in these activities and the ratio remained at 11 1 But

[10] MASON, H S (1965) *Ann Rev Biochem* **34**, 595

after irradiation in N_2 and subsequent storage in air the characteristic change took place, reducing the ratio to 6·5:1 (Table 2). It seems that oxygen has a definite function in this activation, although the mechanism by which it acts is unknown. The K_m for *p*-cresol was 350 and 625 μM and for oxygen 560 and 180 μM for control and irradiated extracts respectively.

TABLE 2 ROLE OF OXYGEN IN ALTERING PHENOLASE ACTIVITY OF IRRADIATED POTATOES

Sample and description of treatment given	Sp act μmol O_2 consumed/min/mg protein Cresolase	Catecholase	Ratio Catecholase/cresolase
1 Control potatoes no treatment	23 8	278	11 7
2 Control potatoes Deprived of air and stored in pre-purified N_2	23 0	258	11 2
3 Potatoes irradiated at 10 krad in air and stored in air	36 0	202	5 6
4 Potatoes deprived of air Irradiated in N_2 and stored in pre-purified N_2	22 9	252	11 0
5 Potatoes deprived of air Irradiated in N_2 and then stored in air	34 0	221	6 5

The storage time was 24 hr and temperature was 28–30°. Details of the procedures are stated under Experimental

The transient activation of cresolase after irradiation may be due to modification in the activities of isoenzymes of the phenolase complex. This possibility was ascertained by separating the isoenzymes by disc gel electrophoresis. For this purpose the enzyme protein was further purified by ammonium sulfate fractionation Electrophoresis of the 25 to 50% ammonium sulfate fraction from control and irradiated potatoes showed eight protein bands. Cresolase activity was present in bands 1, 2, 3 in control potatoes, while this activity was found in bands 1, (neg.) 2, 3, 5 and 8 (pos.) in irradiated samples, indicating an induction of cresolase activity in bands 5 and 8. Apart from this, a distinct increase in the cresolase activity of band 1 and 2 on irradiation was observed. Catecholase activity was present in all the eight bands in both control and irradiated samples. However, a marked reduction of this activity was found in all but bands 1 and 2. Chlorogenic acid oxidation on the other hand showed an increase as a result of irradiation in bands 1 and 8.

DISCUSSION

The multiplicity of potato polyphenol oxidase has been demonstrated by Constantinides and Bedford,[11] who showed at least 11 isoenzymes. It is apparent that gamma irradiation induced only a change in the relative activities of the isoenzymes and does not affect the pattern of separation Some important observations emerging from these studies are the

[11] CONSTANTINIDES, S M and BEDFORD, C L (1967) *J Food Sci* **32**, 446

induction of cresolase activity, the reversibility of this activation, and the oxygen dependency. Such a specific activation has not been observed hitherto in any treatments which are known to alter the activities of the phenolase complex.[12-16] Most of these activation processes are known to be irreversible. Swain *et al.*[17] propose that this type of activation may be either due to partial denaturation, which in its early stages may be reversible or due to conformational changes in the enzyme. Activation of grape catechol oxidase upon either acid shock or urea treatment is attributed to conformational changes of the enzyme molecules which are very rapid and reversible [18] A similar mechanism is envisaged in the transient activation of cresolase by gamma irradiation although the activation was not rapid. The function of oxygen in this process has yet to be ascertained. A reduction of diphenolase activity at sprout-inhibiting dose level is reported by Cheung and Henderson[8] who correlated the decrease with the change in the activities of isoenzymes. The results obtained in this study also suggest that there is no net synthesis of any protein which can be related to the increase in cresolase activity.

EXPERIMENTAL

Potato tubers used for the experiment were 2–3 weeks old after harvest They were stored at 0–4° in the dark Potatoes were allowed to attain room temp before being irradiated in a gamma cell-220 at a dose rate of 110 krad per hr The tubers received a 10 krad dose They were stored at 25° (25–28°) and relative humidity 50–60%.

Enzyme activity was measured by the initial rate of O_2 consumption in the case of diphenolase and a maximum constant rate of O_2 uptake after the initial lag period in the case of monophenolase The O_2 uptake was determined using a Clark electrode in a 3 ml cuvette at 30° The reaction mixture consisted of 1·5 μmol of substrate, 2 mg of enzyme protein and freshly aerated 0·02 M sodium phosphate buffer pH 7 to a final vol of 3 ml, and was stirred constantly The O_2 uptake was determined from the slope of the tracings The sp act is expressed as μmol O_2 consumed per min per mg protein The protein was determined by the Biuret procedure [19] The diphenols formed in the reaction mixture were determined according to the method of Nair and Vaidyanathan [20] For the determination of the K_m for *p*-cresol, O_2 was at 240 μM and when the O_2 concentration was varied *p*-cresol was fixed at 1×10^{-3} M O_2 concentration was varied by bubbling O_2-free N_2 through the reaction mixture prior to the addition of substrate, until the required O_2 concentration was reached, as determined by the O_2 electrode The reading was confirmed again immediately after addition of the substrate, and before the reaction was initiated

Method of irradiation and incubation under N_2 atmosphere Potato tubers were placed in a desiccator and connected to a vacuum pump and kept *in vacuo* (720 mm Hg) for 10 min Then pre-purified N_2 was passed into the desiccator The process was repeated 3× The samples were quickly removed from the desiccator and packed in aluminium pouches under pre-purified N_2 in a vacuum packaging machine The pouches were irradiated at 10 krad One of these bags was left unirradiated as a control sample under N_2 Of the two pouches irradiated one was opened and incubated in air for 24 hr The control and irradiated pouches were kept in N_2 for 24 hr Enzyme was made from the peelings of these samples and the irradiated potatoes

Acetone powder All enzyme preparations were carried out at 0° to 4° unless otherwise stated The potatoes were peeled uniformly using a mechanised peeler The peels were immediately frozen at −30° overnight The frozen peel was homogenized in a pre-cooled Waring Blendor with chilled acetone at −30° in the ratio 1·5 for 2 min The slurry was filtered and the wet cake was blended again with acetone, and this was repeated Finally the cake was dried free of acetone at 0–4° This powder can be stored for several weeks at −30° without loss of enzyme activity

Enzyme preparation The enzyme was prepared according to the method of Patil and Zucker[9] with some modifications Acetone powder (20 g) was stirred with 300 ml of cold 0·02 M acetate buffer pH 5·7 (buffer A) for 45 min The residue was removed by filtration through a double layer of cheese cloth The filtrate was then centrifuged at 17000 *g* for 10 min To 290 ml of clear supernatant 435 ml of chilled acetone was added and the ppt was

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quickly separated by centrifugation. The ppt was dissolved in 150 ml of buffer A The undissolved portion was removed by centrifugation at 17 000 *g* for 10 min To 150 ml supernatant 150 ml chilled acetone was added and the ppt separated by centrifugation at 17 000 *g* for 10 min and dissolved in 20 ml of buffer A This was used as enzyme preparation for assays For electrophoresis the enzyme was further fractionated with $(NH_4)_2SO_4$ and the ppt in this case was dissolved in 0 02 M sodium phosphate buffer pH 7 2 (buffer B) To 20 ml of acetone fraction in buffer B, 2 88 g of solid $(NH_4)_2SO_4$ was stirred in and the ppt obtained was removed by centrifugation To the supernatant an additional 3 86 g $(NH_4)_2SO_4$ was added after 10 min, the ppt was collected by centrifugation and dissolved in 10 ml of buffer B This soln was dialysed for 6 hr against the same buffer with a buffer change (3 1) every 2 hr This fraction which contained all monophenolase, catecholase and chlorogenic acid oxidase activity was used for electrophoresis

Gel electrophoresis A 7% polyacrylamide gel was used at 0–4° The 25–55% $(NH_4)_2SO_4$ fraction was dissolved in buffer B to a protein concentration of 6 mg/ml This soln was dialysed and the final protein concentration was adjusted to 5 2 mg/ml 0 5 ml of this soln was mixed with 0 5 ml of 40% sucrose and 0 1 ml of this soln was taken for electrophoresis The electrophoresis was carried out in Tris-glycine buffer pH 8 3 Current was 1 mA per tube for first 15 min and then 5 mA per tube for 3 hr The gel after electrophoresis was cut into slices of 2 mm thickness and each slice was minced with 1 ml buffer The activity of these slices were determined colorimetrically [21] To 1 ml of eluate 2 ml of substrate buffer mixture (1 7 ml buffer B plus 0 3 ml 5×10^{-3} M substrate) was added and incubated at 30° for 30 min The optical density at 420 nm was measured and plotted against fraction number The intensity produced was proportional to the activity on the band The protein bands were detected by staining with 1% Amido black dye in 7% HOAc

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