Short communication

Lignin biosynthesis during wound healing of potato tubers in response to gamma irradiation

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Abstract

The effect of gamma irradiation on lignin biosynthesis during wound healing in potato tubers was studied by [U-14C] phenylalanine incorporation into lignin and monitoring the activities of key enzymes involved in the lignification process. There was a 40% reduction in lignin biosynthesis during wound healing in response to gamma irradiation. The level of the first enzyme involved in lignin biosynthesis, phenylalanine ammonia lyase (PAL), was five-fold higher in irradiated potatoes than in control tubers during wound healing. However the level of another key enzyme, cinnamyl alcohol dehydrogenase (CAD) was found to be 30% less in irradiated potatoes. There was no significant change in peroxidase activity and its isozyme pattern during wound healing. Differential regulation of different enzymes of lignin biosynthesis in response to gamma irradiation may be responsible for the decreased lignin biosynthesis. © 2000 Elsevier Science B.V. All rights reserved.

1. Introduction

Stimulation of lignin biosynthesis is one of the inducible defence responses in plants in response to wounding and microbial attack (Vance et al., 1980; Ride, 1983). The biochemistry of lignin biosynthesis is a complex process involving the action of several enzymes of primary phenylpropanoid metabolism as well as of lignin biosynthetic branching enzymes. Following the deamination of phenylalanine to cinnamic acid by phenylalanine ammonia-lyase (PAL), the lignin biosynthetic branching pathway is operative and the key enzymes involved are hydroxylases, O-methyl transferases, COA ligases and alcohol dehydrogenases (Kuboi and Yamada, 1978). In addition, peroxidases (EC1.11.1.7 donor:H2O2 oxidoreductase) are also involved in the last step for the polymerisation of cinnamyl alcohols to form lignin (Imberty et al., 1985). Peroxidases exhibit unique isozyme profiles in response to particular physiological situations (Lagrimini and Rothstein, 1987). Studies from several laboratories have shown that lignin biosynthesis can be monitored...
by assessing the level of these marker enzymes (Kutsuki et al., 1982).

Phenylpropanoid metabolism is highly regulated by several stress factors and environmental stimulants (Jones, 1984). The effect of gamma irradiation on phenylpropanoid metabolism and its relation to development of disease resistance in plants has been well studied (Pendharkar and Nair, 1987, 1995). One significant observation emerging from these studies is the suppression of wound periderm formation in potatoes, thereby making the irradiated potatoes more accessible to pathogens. Suppression of cinnamic acid hydroxylase (CA-4H) activity has also been related to imperfect wound periderm formation (Pendharkar and Nair, 1987). However the effect of gamma irradiation on enzymes involved in the lignin biosynthetic branching pathway is not well understood. Wound-induced peroxidase activity and isozyme patterns during lignin biosynthesis in response to gamma irradiation have also not been investigated. In the present investigation we studied further the effects on PAL, CAD and peroxidase.

2. Materials and methods

Mature potatoes (Solanum tuberosum, cv Kufri chandramuki), 3–4 weeks stored at room temperature after harvest, were obtained from the local market. Tubers free from apparent diseases and mechanical injury were selected and irradiated to 100 Gy in a package irradiator (Atomic Energy of Canada) with a Cobalt-60 source at a dose rate of 10 Gy/min and an overdose ratio of 1.4 (max/min = 1.4/1). The dosimetry was performed using the Fricke system to ensure that the tubers received a dose of 100 Gy. Irradiated and non-irradiated potatoes (60 tubers) each weighing ≈ 50–60 g were used for wound healing experiments.

In earlier investigations, potato tubers cut into two longitudinal halves have been found to provide a convenient system to study wound-induced metabolic changes (Thomas and Delincee, 1979; Thomas, 1982). Wound healing studies were carried out as described by Ramamurthy et al. (1992). Irradiated and non-irradiated tubers of uniform size were sliced in half longitudinally from bud end to stem end and the wound allowed to heal at 25°C by keeping them in trays covered with perforated polyethylene sheets. The relative humidity inside the trays was maintained at 90%. After a specified period of wound healing (up to 16 days), 1 mm thick blocks of tissue cut from below the wound surface, were used for determination of various enzyme activities and to study lignin biosynthesis.

The incorporation of [U-14C] phenylalanine (specific activity 1.8 × 10¹⁰ Bq/mmol; purchased from the Board of Radiation and Isotope Technology, Mumbai, India), into lignin and the isolation of lignin, were carried out as described by Pendharkar and Nair (1995). In a typical experiment 3.7 × 10⁶ Bq of [U-14C] phenylalanine was fed to the wound surface and the wound was allowed to heal. At regular intervals the wound periderm was removed (5 g) and homogenised in acetone (– 30°C). The insoluble residue was prepared and hydrolysed as described by Pendharkar and Nair (1995) and an aliquot of 100 μl was used to estimate the radioactivity incorporated into lignin. The incorporation of [U-14C] phenylalanine into lignin was monitored from three replicates.

Phenylalanine ammonia lyase (PAL) was extracted from 5 g tissue by using 10 ml borate buffer (0.1 M, pH 8.8) as described by Ussuf and Nair (1980) and assayed according to Zucker (1965) by measuring the absorbance of transcinnamic acid at 290 nm. Activity was expressed as micromoles of transcinnamic acid formed/h/mg protein. Protein content was determined by the method of Bradford (1976) using bovine serum albumine (BSA) as a standard. Three tissue replicates were extracted and each was assayed three times.

Cinnamic acid dehydrogenase (CAD) enzyme was extracted from 5 g tissue by using 10 ml Tris/HCl buffer (0.2 M, pH 7.5) as described by Mansell et al. (1974). For assaying the CAD activity, the formation of coniferyl aldehyde from coniferyl alcohol was monitored spectrophotometrically by measuring the increase in absorbance at 400 nm for 3 min. Enzyme activity was expressed as pkat/mg protein. One katal (kat)
is the amount of enzyme which converts 1 mol substrate/s. Three replicates were extracted and each was assayed three times.

Peroxidase was extracted from 50 g tissue with 100 ml of 0.5 M sodium phosphate buffer (pH 6.0), containing 0.8 M KCl and 2 gm Polyclar AT as described by Lee (1973). The purification was carried out by ammonium sulphate fractionation (30–90%) and subsequent affinity chromatography on Con (A) Sepharose. This purified enzyme was used for determination of peroxidase activity.

Peroxidase activity was measured using guaiacol as the hydrogen donor as described by Thomas et al. (1982). The reaction was initiated by the addition of hydrogen peroxide. The amount of tetraguaiacol produced was monitored by measuring the absorbance at 470 nm for 2 min. Peroxidase activity was expressed as variation of absorbance/min/mg/protein. The electrophoretic profiles of the Con A Sepharose-purified potato peroxidase was carried out by SDS-PAGE according to Laemmli (1970).

Isoelectric focussing and activity staining of potato peroxidase was performed as described by Thomas and Delincee (1979) using pH 2-11 ‘Servalyt’ carrier ampholytes (Serva, Heidelberg, Germany) and Sephadex G-75 superfine (Pharmacia, Uppsala, Sweden) on a 20 × 20 cm glass plates, the separation distance being 15 cm. Peroxidase activity was detected by the paper print technique using 1% urea-peroxide and 1% o-phenylene diamine as described by Delincee and Radola (1972).

3. Results and discussion

Incorporation of [U-14C]-phenylalanine into lignin as a function of the wound healing period is shown in Fig. 1. Maximum incorporation of [U-14C] phenylalanine into lignin was observed on the tenth day of wound healing and thereafter it remained constant in control potatoes. The pattern in irradiated potatoes was similar, although there was a 40% reduction in incorporation, indicating an impairment in lignin biosynthesis as a result of gamma irradiation.

The constitutive expression of PAL activity in intact potato tuber was low and this level remained unaltered during the period of storage (Fig. 2(a)). However, PAL activity markedly increased during wound healing over the first 8 days in both control and irradiated potatoes and thereafter decreased slightly. The increase in PAL activity in irradiated potatoes was four- to five-fold higher than that in control potatoes.

The results suggest that as a result of activation of PAL by gamma irradiation, the initial metabolic pathway for lignin biosynthesis might be activated. However, results from the incorpo-

![Fig. 1. [U-14C]-Phenylalanine into lignin. The vertical error bars represent SE of the mean.](image-url)
ration of [U-14C]-phenylalanine into lignin have shown that lignin biosynthesis was reduced. To understand the mechanism involved in this paradoxical situation, further studies were carried out to determine the effect of gamma irradiation on another key enzyme, CAD, involved in lignin biosynthesis.

Induction of CAD activity was comparatively slow during the first 6–8 days after wounding and thereafter activity increased as wound healing progressed (Fig. 2(b)). Maximum activity was reached in 14 days. Unlike PAL activity, the level of CAD activity from irradiated potato tubers during wound healing was 30% less than that of control tubers. There was no alteration in the constitutive level of CAD activity in unwounded control tubers.

Thus the activity of CAD, a specific enzyme involved in lignification, showed a positive correlation with PAL activity and lignin biosynthesis in control potatoes during wound healing. However, the higher levels of PAL activity in irradiated tubers was not accompanied by a parallel increase in CAD activity. One possible reason for this could be the ineffective utilization of transcinamic acid by gamma-irradiated tuber tissues, a view also expressed in a study on biochemical aspects of gamma-irradiated potatoes by Pendharkar and Nair (1987). Another possibility could be the preferential channelling of transcinamic acid by secondary pathways to flavonoids and anthocyanins. Thus irradiation resulted in the impairment of CAD activity which limits the supply of lignin monomers required for polymerisation.

The enzyme involved in the polymerisation of phenylpropanoid units into complex lignin polymers is mediated by peroxidase. Induction of peroxidase activity was monitored using the purified
peroxidase enzyme. A 45-fold purification with 62% recovery was achieved by using the purification procedure. The electrophoretic separation of purified peroxidase enzyme on 10% SDS-PAGE showed a major band having a molecular mass of 45 000 (data not shown). Peroxidase from both control and irradiated potatoes exhibited the same electrophoretic profile during wound healing. Induction of peroxidase in both control and irradiated tissues was slow during the initial period of wound healing (Fig. 2(c)) The activity reached a maximum in about 10 days and then remained constant. Even though the level of extractable peroxidase was found to be slightly higher in control potatoes, no significant difference in peroxidase activity was observed as a result of irradiation during wound healing.

In order to find out whether there are any major changes in the isozymes of peroxidase formed during wound healing in control and irradiated potatoes, isoelectric focusing coupled with activity staining was carried out. The isoelectric pattern of peroxidase from unwounded and wounded tissue of both control and irradiated potatoes after staining with o-phenylene-diamine is shown in Fig. 3. During wound healing several isozymes with a wide range of PIs were induced in control and irradiated potatoes. However, the isozyme pattern in control and irradiated potatoes did not show any significant differences.

Peroxidase has been implicated in lignification and suberisation during wound healing (Lagrimini and Rothstein, 1987; Roberts et al., 1988). The peroxidase activity reached a maximum in about 8–12 days when wound healing was almost completed, further confirming the involvement of peroxidase in the wound healing process. The lack of any differences in peroxidase and the isozyme patterns suggests that peroxidase was not affected by gamma irradiation. Although the isozyme pattern remained the same, the possibility of inactivation of a specific peroxidase isozyme which contributes only a very small portion of the total peroxidase involved in the lignification process, cannot be ruled out.

Our results suggest that irradiation interferes differentially with the various enzymes involved in lignin biosynthesis. The induction of PAL activity was several fold higher in irradiated potatoes. However, no correlation with lignin deposition was observed. The induction of CAD activity was significantly lower in irradiated potatoes and these data correlated with decreased lignin accumulation. Studies from our laboratory have also shown that irradiation impaired the induction of cinnamic acid-4-hydroxylase (CA-4H), the second enzyme of phenylpropanoid pathway (Pendharkar and Nair, 1987). Reduced accumulation of lignin, accompanied by the impairment in wound periderm formation as a result of gamma irradiation, may influence the postharvest physiology of potatoes and may affect the long term storage stability of irradiated potatoes.

References


