Modulation of L-Phenylalanine Ammonia-Lyase by Pathway Intermediates in Cell Suspension Cultures of Dwarf French Bean (*Phaseolus vulgaris* L.)

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Abstract. The increase in extractable phenylalanine ammonia-lyase (PAL; EC 4.3.1.5.) activity induced in French bean cell suspension cultures in response to treatment with autoclaved ribonuclease A was inhibited by addition of the phenylpropanoid pathway intermediates cinnamic acid, 4-coumaric acid or ferulic acid. The effectiveness of inhibition was in the order cinnamic acid > 4-coumaric acid > ferulic acid. Cinnamic acid also inhibited the PAL activity increase induced by dilution of the suspensions into an excess of fresh culture medium. Addition of low concentrations (< 10^-5 M) of the pathway intermediates to cultures at the time of application of ribonuclease gave variable responses ranging from inhibition to 30-40% stimulation of the PAL activity measured at 8 h. Following addition of pathway intermediates to cultures 4-5 h after ribonuclease treatment, rapid increases followed by equally rapid declines in PAL activity were observed. The cinnamic acid-stimulated increase in enzyme activity was unaffected by treatment with cycloheximide at a concentration which gave complete inhibition of the ribonuclease-induced response. However, cycloheximide completely abolished the subsequent decline in enzyme activity. Treatment of induced cultures with *z*-aminoxy-*ß*-phenylpropionic acid (AOPPA) resulted in increased but delayed rates of enzyme appearance when compared to controls not treated with the phenylalanine analogue. The results are discussed in relation to current views on the regulation of enzyme levels in higher plants.

Key words: Cell suspension culture – *Phaseolus* – Phenylalanine ammonia-lyase – Phenylpropanoid biosynthesis.

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Introduction

L-Phenylalanine ammonia-lyase (EC 4.3.1.5.) catalyses the deamination of L-phenylalanine to yield trans-cinnamic acid, the first reaction in the biosynthesis of a wide variety of phenylpropanoid compounds in higher plants. Transient increases in the activity of this enzyme precede or parallel the accumulation of lignin, coumarins, esters of hydroxycinnamic acids, flavonoids, isoflavonoids and pterocarps (Stafford 1974; Dixon and Bendall 1978b), and much attention has been given to the molecular mechanisms underlying such changes in PAL activity, in particular in illuminated parsley cell suspension cultures accumulating flavone glycosides (Hahlbrock and Ragg 1975; Betz et al. 1978) and in potato tuber discs accumulating chlorogenic acid (Lamb et al. 1979).

PAL activity levels may be modulated through effects on the rates of both enzyme synthesis and loss of active enzyme (Lamb et al. 1979), while in some systems activation and inactivation have been proposed as the major factors underlying PAL activity changes (Attridge and Smith 1973, Attridge et al. 1974) although this is still a matter of some debate with respect to the phytochrome-mediated induction of PAL in mustard seedlings (Tong and Schopfer 1976). It is now also appreciated that in vivo concentrations of pathway intermediates may act as indicators of, and thereby regulate, the flux through the phenylpropanoid pathway; trans-cinnamic and 4-coumaric acids have been implicated as feed-back regulators of PAL activity levels, causing inhibition of the induced enzyme increase in gherkin hypocotyls (Engelsma 1968; Johnson et al. 1975) and Jerusalem artichoke and potato tuber slices (Durst 1976; Lamb and Rubery 1976). In potato, 4-coumaric acid prevents the appearance of cinnamic acid 4-hydroxylase (EC 1.14.13.11) activity (Lamb and Rubery 1976) and
cinnamic acid, in addition to its effects on PAL, also feed-forward stimulates the appearance of hydroxycinnamoyl CoA: quinic acid hydroxycinnamoyl transferase (EC 2.3.1-), the first enzyme in the chlorogenic acid branch pathway (Lamb 1977). The effects of cinnamic acid on PAL activity levels in vivo are independent of the product inhibition of the enzyme activity in vitro (Havir and Hanson 1968; Durst 1976).

Evidence of a physiological role for pathway intermediate modulation of PAL comes from experiments in which endogenous cinnamic acid concentrations are either increased as a result of inhibition of cinnamic acid 4-hydroxylase activity (Durst 1976) or lowered via in vivo inhibition of PAL activity (Amrhein 1979). Such treatments result in either inhibition or stimulation of extractable PAL activity respectively. The stimulation of PAL observed following excision and floating on H2O of hypocotyl segments (Engelsma 1968, 1979) or dilution of cell suspension cultures into excess medium (Hahlbrock and Wellmann 1973) may also be the result of lowered intracellular concentrations of pathway intermediates. In the present paper we report the effects of pathway intermediates on PAL activity in French bean cell suspension cultures, following induction of the enzyme by dilution of the cultures or treatment with denatured ribonuclease A, an inducer of isoflavone and pterocarpan accumulation (Dixon and Bendall 1978a) preceded by de novo synthesis of PAL (Lamb and Dixon 1978). It is shown that inhibition of extractable enzyme activity by exogenously supplied pathway intermediates is a very rapid process which may, however, be preceded by equally rapid, transient increases in enzyme activity.

Materials and Methods

Cell suspension cultures of French bean cultivar Canadian Wonder were initiated and maintained by regular subculture at 14-d intervals in a modified Schenk and Hildebrandt medium as previously described (Dixon and Fuller 1976). All cultures used in the following experiments were in exponential growth phase (6–7 days after subculture). PAL induction in response to 0.5 mg ml−1 autoclaved bovine pancreatic ribonuclease A (Sigma Chemical Co.) was measured in 10 ml batches of culture in sterile 25 ml conical flasks incubated as described elsewhere (Dixon and Bendall 1978a).

Cinnamic, 4-coumaric and ferulic acids (Sigma Chemical Co.) were each re-crystallised three times from aqueous ethanol prior to use.

Cells for enzyme assay were harvested by suction filtration on sintered glass filters, transferred to small stoppered vials and stored frozen at −70°C until required. Extracts were prepared in 50 mM Tris-HCl, pH 8.5, containing 7.1 mM 2-mercaptoethanol, with the inclusion of 1/10th the weight of cells of insoluble polyvinylpolypyrrolidone (Av. M. wt. 360,000). Cell debris was removed by centrifugation at 20,000 g for 30 min. Supernatants were assayed for PAL by a spectrophotometric procedure (Lamb et al. 1979). In all the experiments described, extracts from cells treated with AOPPA or from cells treated with concentrations of cinnamic, 4-coumaric or ferulic acids greater than 4·10−4 M were passed through a column of Sephadex G-15 (4.5·1.5 cm) prior to assay. Protein was determined by a modification of the method of Lowry et al. (Leggett-Bailey 1962).

Results

Effects of Cinnamic Acid on PAL Activity Induced by Dilution. PAL activity increased as a result of transfer of French bean cell suspension cultures to fresh culture medium (Fig. 1). Initial increases in enzyme activity were measured at intervals during an 8 h period subsequent to transfer; previous work had indicated that PAL activity in the bean cultures reached a maximum at around 8 h when induced by a fungal elicitor preparation (Dixon and Lamb 1979) and at 8–12 h when induced by denatured ribonuclease (Dixon and Bendall 1978a and below). In the present work the rate and extent of PAL appearance increased with increasing dilution of the cultures. However, dilution into fresh medium containing trans-cinnamic acid (10−3 M) completely prevented PAL appearance; extractable enzyme activity decreased to virtually zero by 2 h after transfer. No increases in activity were observed if cells were diluted 50-fold into media in which cultures had previously been growing (conditioned media).

Effects of Pathway Intermediates on Ribonuclease-induced PAL Activity. Six-fold increases in PAL activity were observed in the bean suspensions 8 h after treatment with 0.5 mg ml−1 autoclaved ribonuclease