trans-CINNAMIC ACID AS A MEDIATOR OF THE LIGHT-STIMULATED INCREASE IN HYDROXYCINNAMOYL-CoA: QUINATE HYDROXYCINNAMOYL TRANSFERASE

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1. Introduction

The regulation of the biosynthesis of phenylpropanoid compounds from L-phenylalanine is being increasingly examined as a model system for the study of the biochemistry of higher plant photomorphogenesis [1]. A central pathway from L-phenylalanine via *trans*-cinnamic acid and *p*-coumaric acid to *p*-coumaroyl-CoA has been elucidated [2]. *p*-Coumaroyl-CoA is a key intermediate from which branch pathways originate associated with the biosynthesis of particular phenylpropanoids such as chlorogenic acid (CGA, 3′-*O*-caffeoyl-D-quinic acid), lignin and flavonoids [1–5].

In cell suspension cultures of *Petroselium hortense*, white light stimulates flavonoid biosynthesis and causes increases in the activities of the enzymes of both the central pathway and the branch pathway associated with flavonoid biosynthesis [6]. However, the central pathway enzymes*: phenylalanine ammonia-lyase (EC 4.3.1.5), cinnamic acid 4-hydroxylase (EC 1.14.13.11) and *p*-coumarate: CoA ligase (EC 6.2.1.) co-ordinately increase in activity after a lag of 1.5 h whereas the branch pathway enzymes start to increase in activity 4–6 h after the onset of illumination. This suggests a sequential mechanism whereby the light-stimulated increases in the central pathway enzyme activities cause an increase in the levels of the intermediates of the central pathway which in turn stimulate the increases in the branch pathway enzymes.

This paper demonstrates that in potato tuber discs, *trans*-cinnamic acid causes a rapid increase in the extractable activity of hydroxycinnamoyl-CoA: quinate hydroxycinnamoyl transferase (hydroxycinnamoyl transferase, EC 2.3.1.) which is the first enzyme of the branch pathway from *p*-coumaroyl-CoA leading to CGA, the major phenylpropanoid end-product in this system [3,7,8]. Evidence is presented that *trans*-cinnamic acid mediates the light-stimulated increase in hydroxycinnamoyl transferase activity.

*Abbreviations: PAL phenylalanine ammonia-lyase, CA4H cinnamic acid 4-hydroxylase, CGA chlorogenic acid

2. Materials and methods

Discs of potato (*Solanum tuberosum*) tuber parenchyme were prepared and incubated at 25°C as previously described [8]. Extracts were prepared in 0.1 M sodium phosphate buffer, pH 7.5, containing 2 mM mercaptoethanol and 1 mM EDTA and cell debris were removed by centrifugation [8]. The supernatant was gel-filtered through Sephadex G-25 and the protein-containing fractions collected and used for enzyme assays. PAL [9] and CA4H [10] were assayed by stopped spectrophotometric methods. *p*-Coumarate: CoA ligase was assayed by a continuous spectrophotometric procedure [11] using diluted enzyme samples to prevent rapid removal of the substrate ATP by endogenous apyrase [12]. Hydroxycinnamoyl-CoA: quinate hydroxycinnamoyl transferase was assayed spectrophotometrically by monitoring the production of caffeoyl-CoA at 360 nm in the back reaction using CGA and LiCoA as the substrates [13]. *p*-Coumaroyl-CoA and *p*-coumaroylquinate are preferred substrates for the reaction in the forward and back reactions respectively with V_{max} twice those for the correspond-

ing caffeoyl derivatives [13], but were not available for this study. CGA was extracted from discs and estimated following reaction with nitrous acid on an alumina column [14].

3. Results and discussion

In dormant potato tuber tissue there is relatively little extractable activity of the enzymes of the central pathway: PAL, CA4H and *p*-coumarate: CoA ligase (table 1). Following disc preparation and incubation in darkness the activities of the three enzymes increase co-ordinately after a lag of about 2 h ([8] and unpublished observations). In contrast, hydroxycinnamoyl transferase, the first branch pathway enzyme undergoes no increase in activity in darkened discs in the 8 h immediately following disc preparation (table 1).

Continuous illumination with white light has no effect on the extractable levels of CA4H and *p*-coumarate: CoA ligase activities, but increases the levels of PAL and hydroxycinnamoyl transferase (table 1). Light-stimulation of the level of PAL is well-documented in a variety of tissues [1], but has only recently been reported for hydroxycinnamoyl transferase, in buckwheat seedlings [7]. The lag before manifestation of the stimulatory effects of continuous illumination is 2 h for PAL and 5 h for hydroxycinnamoyl transferase (fig.1). Furthermore, the light-stimulated increase in CGA [8] occurs only after the increase in hydroxycinnamoyl transferase activity (fig.1).

These temporal relationships suggest that the light-mediated increase in PAL activity relative to CA4H and *p*-coumarate: CoA ligase increases the concentrations of the central pathway intermediates which in turn cause the increase in hydroxycinnamoyl transferase activity. Indeed, exogenous *trans*-cinnamic acid (2.0 mM, pH 7.0) produces a rapid increase in hydroxycinnamoyl transferase activity in discs incubated in darkness. Maximum activity is reached

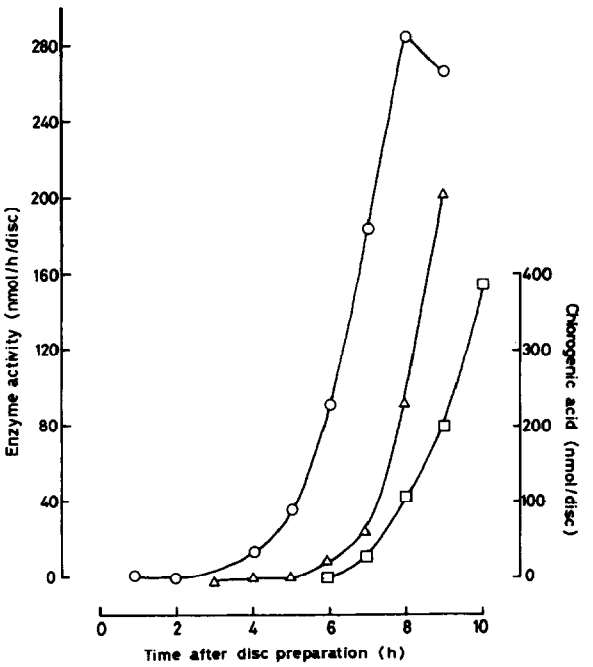

Fig.1. Time-course of the stimulatory effect of illumination on the level of PAL activity (—○—), hydroxycinnamoyl transferase activity (—△—) and CGA (—□—). Results are derived from simultaneous estimations on equivalent discs incubated continuously in white light or darkness.

Table 1
The levels of activity of enzymes of CGA biosynthesis in potato tuber tissue

Enzyme	Activity (nmol/h/disc)			
	Freshly prepared discs	Discs incubated for 8 h		
		In darkness	In light	Light stimulation
PAL	52	322	511	+ 189
CA4H	21	87	75	−12
p-Coumarate: CoA ligase	27	308	313	+ 5
Hydroxycinnamoyl transferase	130	136	264	+ 128

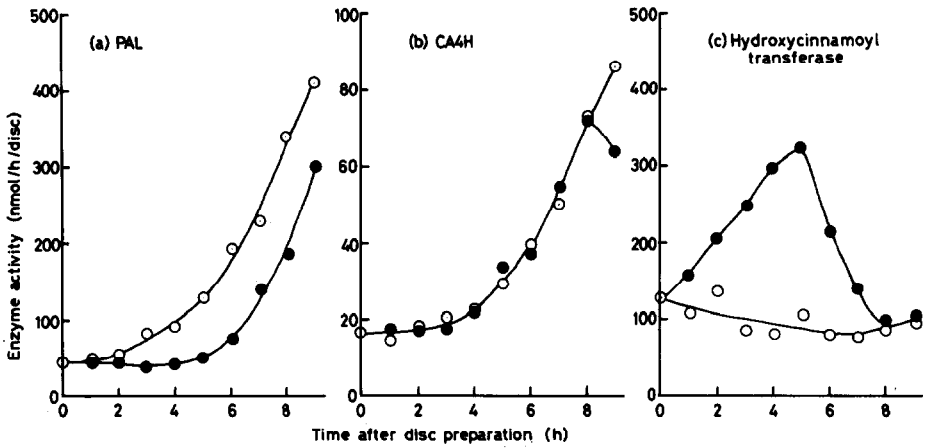

Fig.2. Time-course of the effect of exogenous *trans*-cinnamic acid (2 mM, pH 7.0) on the levels of activity of (a) PAL, (b) CA4H and (c) hydroxycinnamoyl transferase. Discs were incubated continuously in darkness on water (–○–) or *trans*-cinnamic acid (–●–).

5 h after disc preparation and then falls rapidly to reach basal levels 4 h later (fig.2). In contrast, *trans*-cinnamic acid inhibits the initial increase in PAL activity and has no effect on CA4H activity except at a late stage when high levels of *p*-coumarate will have accumulated [15]. This suggests that *trans*-cinnamic acid per se is the active agent because if *p*-coumaric acid or some later intermediate were responsible, then simultaneous decrease in the level of both PAL and CA4H would be expected [15]. Accordingly, exogenous *p*-coumaric, caffeic, quinic, ferulic or chlorogenic acids do not stimulate the level of hydroxycinnamoyl transferase. *trans*-Cinnamic acid has no stimulatory effect on the activity of hydroxycinnamoyl transferase in vitro.

trans-Cinnamic acid plays a key role in the regulation of phenylpropanoid biosynthesis by (a) sensitive product inhibition of PAL [16] (b) feed-back coarse control of the level of PAL activity [15] and (c) feed-forward coarse control of hydroxycinnamoyl transferase. Feed-forward coarse control does not seem previously to have been detected in phenylpropanoid biosynthesis. In potato tuber discs PAL is the primary site of photocontrol [8] and it is suggested that *trans*-cinnamic acid transmits the signal to hydroxycinnamoyl transferase, a secondary site for photocontrol, specific to CGA biosynthesis. Thus, light-stimulation of CGA accumulation does not occur until increased levels of hydroxycinnamoyl transferase are apparent, and the rapidity of the changes in the activity of the enzyme following *trans*-cinnamic acid treatment is consistent with the enzyme undergoing rapid turnover as might be expected for a regulatory enzyme [17].

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