Elicitor-Induced Changes of Enzyme Activities Related to Isoflavone and Pterocarpan Accumulation in Chickpea (*Cicer arietinum* L.) Cell Suspension Cultures

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The extractable activities of thirteen enzymes of primary and secondary metabolism have been measured in chickpea (*Cicer arietinum* L.) cell suspension cultures after treatment with an elicitor from the fungus *Ascochyta rabiei* (Pass.) Lab. The cell culture, derived from the *A. rabiei* resistant cultivar ILC 3279, constitutively accumulated the isoflavones biochanin A and formononetin together with their 7-O-glucosides and the 7-O-glucoside-6″-malonates. After elicitor application the cells rapidly form the pterocarpan phytoalexins medicarpin and maackiain.

Among the enzymes of primary metabolism only the glucose 6-phosphate dehydrogenase exhibited a significant increase in activity with a maximum four hours after application of the elicitor. In phenylpropane metabolism the activities of phenylalanine ammonia lyase and chalcone synthase were enhanced by the elicitor and exhibited highest levels after four hours. In contrast the chalcone isomerase activity was not influenced by the elicitor. A substantial enhancement occurred with the isoflavone 7-O-glucosyltransferase activity eight hours after elicitor application.

The results suggest that in this cell culture the elicitor-induced biosynthesis of pterocarpan phytoalexins was accompanied with a rapid and transient increase of those enzyme activities which are located at branching points of related pathways, *i.e.* pentose phosphate cycle, general phenylpropane metabolism, flavonoid formation and isoflavone conjugation.

Introduction

Chickpea (*Cicer arietinum* L.) is an important grain legume of dryland agriculture in many countries of Asia and Africa [1]. The main phenolics in chickpea are the two isoflavones biochanin A and formononetin [2] which predominantly accumulate as the 7-O-glucoside-6″-malonates [3]. The enzymes, catalyzing the four reactions involved in the metabolism of isoflavone conjugates [4] are isoflavone 7-O-glucosyltransferase (IGT), isoflavone 7-O-glucoside-6″-O-malonyltransferase, isoflavone 7-O-glucoside-6″-O-malonate malonylesterase (IME) and specific β-glucosidases which have all been characterized [5–8].

The most important fungal disease of chickpea is Ascochyta blight, which is caused by the deuteromycete *Ascochyta rabiei* (Pass.) Lab. (syn. *Mycosphaerella rabiei* Kovachevski) [1]. After infection with *A. rabiei* the pterocarpan phytoalexins medicarpin and maackiain accumulated in the resistant chickpea cultivar ILC 3279 to high levels whereas the susceptible cultivar ILC 1929 produced only small amounts of these compounds [9].

In our investigations to elucidate the cultivar specific differences in fungal resistance and in the ability to form phytoalexins we have been using cell suspension cultures of these chickpea cultivars. Both cell culture lines constitutively accumulate the isoflavones, the glucoside- and the malonylglucoside conjugates [10] in a pattern as known from the plants [9]. Furthermore, the cell cultures show pronounced cultivar-specific differences in the elicitor-induced formation of phytoalexins [10]. Only the culture ILC 3279 was shown to accumulate great amounts of phytoalexins after elicitor treatment.

Medicarpin and maackiain biosynthesis proceeds *via* the general phenylpropane pathway with formononetin as a central intermediate [11]. The cell

Abbreviations: ANA-esterase, α-naphthylacetate esterase; BGM, biochanin A 7-O-glucoside-6″-O-malonate; CHI, chalcone isomerase; CHS, chalcone synthase; FGM, formononetin 7-O-glucoside-6″-O-malonate; Glc6PDH, glucose 6-phosphate dehydrogenase; HPLC, high pressure liquid chromatography; ICDH/NAD, isocitrate dehydrogenase, NAD-dependent; ICDH/NADP, isocitrate dehydrogenase, NADP-dependent; IGT, UDP-glucose isoflavone 7-O-glucosyltransferase; ILC, international line of chickpea; IME, isoflavone 7-O-glucoside-6″-O-malonate malonylesterase; Maa, maackiain; Med, medicarpin; PAL, phenylalanine ammonia lyase.

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culture ILC 3279 offers a good object to study the metabolic reactions of the cells and the selectivity of the elicitor action with regard to isoflavone and pterocarpan biosynthesis. Investigations on other cell culture systems have shown that fungal elicitors lead to an induction of phytoalexin biosynthetic enzymes, but that they also influence various other areas of cellular metabolism such as ethylene formation or modifications in the cell wall structure [12–14].

This paper demonstrates the effect of *A. rabiei* elicitor on the activities of thirteen enzymes of primary and secondary metabolism and on the phosphate concentration in the nutrient medium in this chickpea cell culture. Furthermore, the changes of various enzyme activities during the growth cycle of the cell culture are also shown.

Materials and Methods

Chemicals

4-coumaroyl-CoA was synthesized as described [15]. 2-[^{14}C]malonyl CoA (2.24 GBq/mmol) was obtained from Amersham Buchler (Netherlands) and diluted with unlabeled malonyl-CoA (Sigma, München, F.R.G.) to a final specific radioactivity of 1.11 GBq/mmol.

Biochanin A 7-O-glucoside-6″-O-malonate was isolated from roots of chickpea and purified as previously described [3]. Biochanin A 7-O-glucoside and isoliquiritigenin (4,2′,4′-trihydroxychalcone) were obtained from the collection of the institute.

Glucose 6-phosphate, fructose 1,6-bisphosphate, glycerol 3-phosphate dehydrogenase/triosephosphateisomerase, NADH and cytochrome *c* were obtained from Boehringer (Mannheim, F.R.G.). UDP-glucose, isocitrate (D/L) and α-naphthylacetate were purchased from Sigma (München, F.R.G.) and NADP, NAD and Dowex 1 × 2 were obtained from Serva (Heidelberg, F.R.G.). Guajacol was purchased from Schuchardt (München, F.R.G.), biochanin A from EGA-Chemie (Steinheim, F.R.G.) and naringenin from Roth (Karlsruhe, F.R.G.).

All other chemicals were obtained from Merck (Darmstadt, F.R.G.).

Cell cultures

The cell cultures of *Cicer arietinum* ILC 3279 were grown on modified PRL-4c-medium [16] as described [10]. 2.5 g cells were transfered into new medium every 7 days. The suspension cultures were propagated in the dark at 25 °C in 200 ml flasks containing 40 ml medium on a rotary shaker at 120 rpm.

Elicitor preparation

The strain of *Ascochyta rabiei* used for elicitor preparation was obtained from Centralbureau voor Schimmelcultures, Baarn, Netherlands (CBS-number: 53.465) and cultured as described earlier [17]. The elicitor was prepared from the mycelium of the fungus after growth for 4 to 5 days according to Kessmann and Barz [18].

Treatment of cell cultures with elicitor

A sample of 5 mg elicitor was dissolved in 1 ml water. This solution was autoclaved and added to the cell suspension culture (40 ml) under sterile conditions. The controls obtained 1 ml autoclaved water.

Preparation of enzyme extracts

All extracts were prepared with fresh cells which were separated from the medium by filtration. Aldolase (EC 4.1.2.7), Glc6PDH (EC 1.1.1.49), ICDH/NADP (EC 1.1.1.42), peroxidase (EC 1.11.1.7), ANA-esterases, CHI (EC 5.5.1.6), PAL (EC 4.3.1.5) and glucosidase (EC 3.2.1.21) have been measured in a crude extract which was prepared as follows: 4 g cells together with 2 g Dowex 1 × 2 (phosphate) and 8 ml buffer (100 mM K_2HPO_4/KH_2PO_4, pH 8.0 containing 1.4 mM mercaptoethanol) were homogenized with an ultraturrax (Janke and Kunkel, Staufen i. Br., F.R.G.) 3 × 30 sec. After centrifugation for 15 min at 26,000 × *g* the supernatant was filtered through glasswool and directly used for enzyme assays.

IME, IGT and CHS (EC 2.3.1.74) were measured after ammoniumsulfate precipitation from the crude extract. Solid $(NH_4)_2SO_4$ was added within 15 min, the solution was stirred for another 15 min and then centrifugated for 30 min at 43,000 × *g*. The precipitate was dissolved in 2.5 ml buffer (see extraction buffer above) and desalted on Sephadex G 25 (PD 10, Pharmacia, Freiburg, F.R.G.). IME was measured in the fraction from 0 to 35% saturation. This fraction was free of β-glucosidase activity which would hydrolyze the product of the IME-reaction, the biochanin A 7-O-glucoside. The IGT was measured in the fraction from 35 to 80% saturation and

the CHS was assayed in a fraction between 50 to 80%.

For determination of ICDH/NAD and Cytochrome C_{min}-oxidase the following extracts were prepared: 7 g cells and 14 ml buffer (20 mM K_2HPO_4/KH_2PO_4, pH 7.6 containing 1.4 mM mercaptoethanol) were homogenized with an ultraturrax (see above) for 5 sec. After centrifugation for 5 min at $1000 \times g$ the supernatant was centrifuged for 15 min at $20{,}000 \times g$. The pellet was dissolved in 5 ml buffer (20 mM K_2HPO_4/KH_2PO_4, pH 7.6 containing 5 M glycerin). This solution was sonicated (Branson B 15-Sonifier, Titanoxid-microtip, grade 6) for 4 times 10 sec each.

Assays for enzyme activities

All enzymes were assayed according to previously published methods with only minor modifications. The following enzymes were assayed by direct photometric measurement. Glc6PDH and ICDH/NADP were measured by the increase of NADPH at 340 nm according to Bergmeyer [19]. The ICDH/NAD was assayed according to Cox [20] by monitoring the increase of NADH at 340 nm. The aldolase was assayed according to [19] by monitoring the decrease of NADH at 366 nm.

The cytochrome *c*-oxidase was assayed according to Wharton and Tzagoloff [21] by monitoring the decrease of reduced cytochrome *c* at 550 nm. Peroxidases were assayed according to [19] by monitoring the H_2O_2 dependent oxidation of guajacol at 456 nm. ANA-esterases have been measured as previously described [22] by monitoring the appearance of α-naphthol at 235 nm.

PAL was measured by monitoring the formation of *t*-cinnamic acid at 290 nm according to [23]. CHI was assayed by monitoring the disappearance of isoliquiritigenin at 395 nm according to [24]. The β-glucosidase was assayed according to Hösel and Barz [8] using a test buffer of pH 7.5 by monitoring the formation of *p*-nitrophenol at 400 nm.

The enzyme activities of IGT and IME have been determined using a HPLC procedure which was previously described [3]. IGT: The reaction mixture contained 130 µl buffer (200 mM TRIS/HCl, pH 8.5 containing 40 mM mercaptoethanol), 10 µl of a 20 mM-solution of UDP-glucose, 10 µl of a 2 mM-solution of biochanin A in methanol and 50 µl enzyme preparation. The enzyme reaction was incubated for 30 min at 30 °C and stopped by addition of 600 µl methanol [25]. Biochanin A 7-O-glucoside was separated and quantitated by HPLC [3]. The β-glucosidase (see above) was not active under the assay conditions of IGT and did not interfer with the IGT-assay.

IME: The assay was performed using BGM as substrate [7]. The amount of biochanin A 7-O-glucoside was quantitated by HPLC.

The CHS-assay was performed by using 2-[^{14}C]-malonyl-CoA and *p*-coumaroyl-CoA as substrates [26]. Naringenin was identified by TLC cochromatography (cellulose, Merck, Darmstadt, F.R.G.) in four solvent systems. 1) 25% ethanol, 2) 30% HOAc, 3) $CHCl_3$:acetic acid:H_2O 10:9:1, 4) Butanol: acetic acid:H_2O 5:2:1.

Protein determination

Protein concentrations were determined by using the method of Bradford [27] using bovine serum albumin as reference.

Extraction of cells and medium and analysis of isoflavones

Extraction of isoflavones, their conjugates and phytoalexins from cells and medium were carried out as described previously [6, 10]. Phenolics were separated and quantitated by HPLC [3].

Results

Time course of enzyme activities during the growth cycle of chickpea cell cultures ILC 3279

Chickpea cell suspension culture ILC 3279 had previously been characterized with regard to growth parameters, the accumulation of isoflavones, their glucoside and malonylglucoside conjugates and the pterocarpan phytoalexins [10] during a growth cycle. Growth-related changes of enzyme activites of primary and secondary metabolism were measured in this study during the growth period of 8 days.

The enzyme aldolase, ICDH/NADP and Glc6PDH of primary metabolism and PAL, CHS, CHI, IGT, β-glucosidase and IME of the secondary pathways were monitored. The data obtained with six of these enzymes are shown in Fig. 1. Glc6PDH, PAL, CHS, CHI and IGT expressed highest specific activities four or five days after inoculation into fresh medium. At this time the cells were in the late linear growth phase and contained maximum amounts of soluble protein (Fig. 1,A). In contrast the time course of IME activity (Fig. 1,H) was just reverse

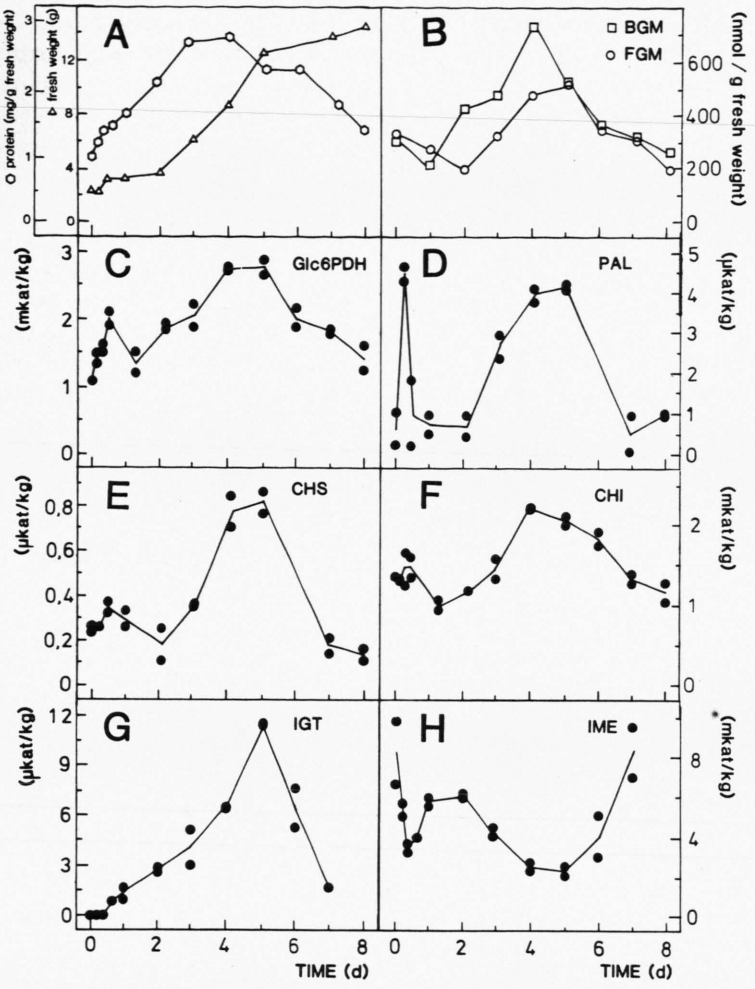

Fig. 1. Fresh weight and protein content (A), accumulation of BGM and FGM (B), and time course of enzyme activities (C–H) during a growth cycle of chickpea cell suspension culture ILC 3279. Each point (●) represents the mean value of the specific enzyme activity from two or more enzyme assays. The two data points at each time interval stem from two separate experiments which documents the reproducibility of the experimental design.

and expressed a minimum on day four and five of the culture period. β-glucosidase activity was present at a constant level over the first seven days (data not shown). Fig. 1, B shows the accumulation of the 7-O-glucoside-6″-O-malonate of biochanin A and formononetin (BGM and FGM) during the culture period. They both reached maximum level on days four and five. Aldolase activity decreased rapidly between the third and fourth day whereas the activity of ICDH/NADP was nearly constant over the whole growth cycle (data not shown).

In this investigation CHS was detected for the first time in chickpea cells using published assay methods [26]. Great care was taken to identify the ^{14}C-labelled reaction product as the flavanone naringenin by cochromatography and radioscanning techniques.

Repeated determinations of enzyme activities based on parallel extraction procedure demonstrated that the reproducibility of the data in Fig. 1 was within 10% for Glc6PDH and appr. 30% for PAL, CHS and IGT. The observed differences are more likely due to variations in the cell cultures than in the procedures used for protein extraction or enzyme assays.

Effect of elicitor application on enzyme activities

To investigate how elicitor application affected changes of enzyme activities the polysaccharide elicitor isolated from *A. rabiei* mycelium [18] was added to chickpea cell suspension culture ILC 3279. Previous investigations [28] had shown that application of elicitor at the early linear phase of growth

induced maximum levels of medicarpin and maackiain. Under these conditions maximum accumulation of the phytoalexins is reached 8 h after elicitor application (Fig. 2).

During the period of rapid phytoalexin accumulation and subsequently for up to 24 h after elicitor application the enzyme activities of altogether 13 enzymes (Table I) of primary and secondary metabolism have been measured.

Among the 7 enzymes of primary metabolism measured in these studies only Glc6PDH showed a significant increase in specific activity with a maximum 4 h after elicitor application (Fig. 2, A). After altogether 12 h the enzyme activity had again decreased to the control level.

The level of activities of two enzymes that are required for phenylpropane and flavonoid metabolism, PAL and CHS (Fig. 2, B and C) rapidly increased in response to elicitor application and showed maximum activities after 4 h. The data in Fig. 2 further show the rapid subsequent decline in PAL activity reaching values below those obtained for the control assays and the comparatively much slower decrease in the enzyme activity of CHS. In contrast the activity of CHI was not influenced by the elicitor treatment (Fig. 2, E). The isoflavone 7-O-glucosyltransferase (IGT) which is specific for the isoflavones biochanin A and formononetin [5, 25] was substantially induced by the elicitor. The highest activity was reached after 8 h (Fig. 2, D). On the other hand the activities of IME and β-glucosidase were not influenced by the elicitor (data not shown). Despite of significant elicitor-induced changes in the activities of several enzymes (Table I) the growth of the cell cultures was not affected as measured by fresh weight, protein content of cells or phosphate concentration in the nutrient medium (data not shown). No browning reactions of the cells after elicitor treatment could be observed.

The sensitivity of chickpea cell suspension culture ILC 3279 towards elicitor treatment varies greatly during a growth cycle. When the elicitor was applied either concomitant with the inoculation of cells into fresh medium or at the early stationary phase (day 7) the activities of both PAL and Glc6PDH were not significantly altered (Fig. 3) even though there is a

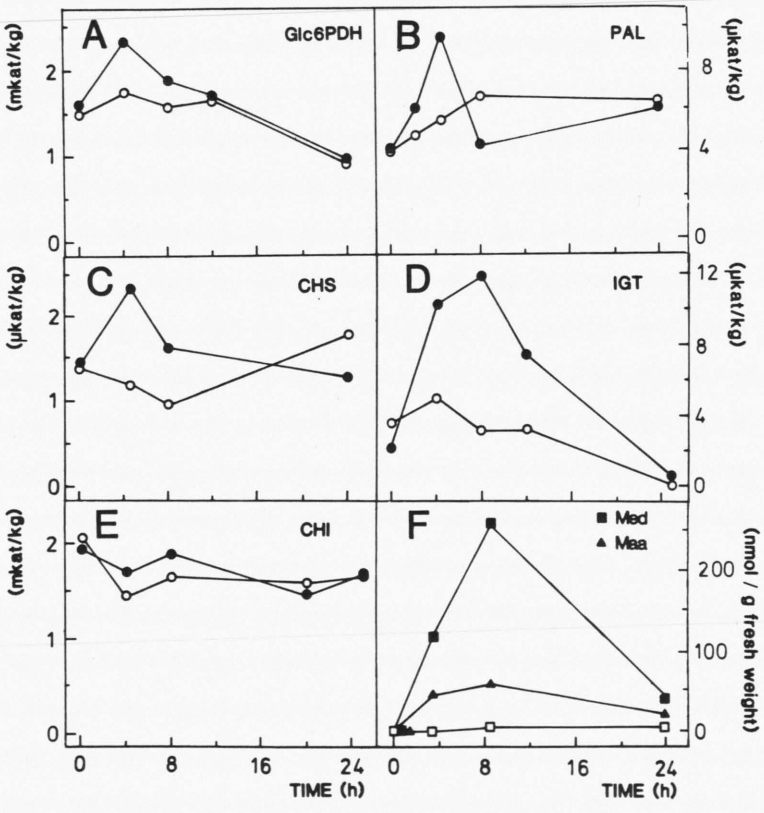

Fig. 2. Accumulation of the phytoalexins medicarpin (■) and maackiain (▲) (F) and changes of enzyme activities (●) (A–E) after application of *Ascochyta rabiei* elicitor to chickpea cell suspension culture ILC 3279. Elicitor (5 mg/flask) was applied on day three after inoculation of cells into new medium. Controls (○: enzyme data; □: constitutive level of medicarpin) were given sterile water.

Table I. Effect of glucan elicitor (5 mg/40 ml medium) from *Ascochyta rabiei* on enzymes of primary and secondary metabolism in ILC 3279 chickpea cell suspension culture. The elicitor was applied three days after inoculation of cells into fresh medium; controls were given equivalent amounts of water.

Enzyme	Enzyme reactions/pathways	Effect of elicitor on enzyme activity
	Primary metabolism	
Aldolase	glycolysis	no effect
Isocitrate dehydrogenase NADP-dependent	proton/electrontransfer in intermediary metabolism	no effect
Isocitrate dehydrogenase NAD-dependent	tricarboxylic acid cycle	no effect
Cytochrome-*c* oxidase	respiratory chain	no effect
Glucose-6-phosphate dehydrogenase	pentosephosphate pathway	47% increase in 4 h
Peroxidase	oxidation of phenolics	no effect
α-Naphthylacetate esterase	general acylesterase	no effect
	Secondary metabolism	
Phenylalanine-ammonia lyase	deamination of L-Phe; starter enzyme of phenylpropanoid metabolism	94% increase in 4 h
Chalcone synthase	formation of naringenin; C_{15}-intermediate of isoflavonoid biosynthesis	65% increase in 4.5 h
Chalcone isomerase	formation of 5-deoxyflavanone	no effect
β-Glucosidase	hydrolysis of isoflavone 7-O-glucosides	no effect
Isoflavone malonylesterase	malonatehemiester hydrolysis of isoflavone 7-O-glucoside-6″-malonate	no effect
Isoflavone 7-O-glucosyl-transferase	7-O-glucosylation of isoflavones by UDP-glucose	263% increase in 8 h

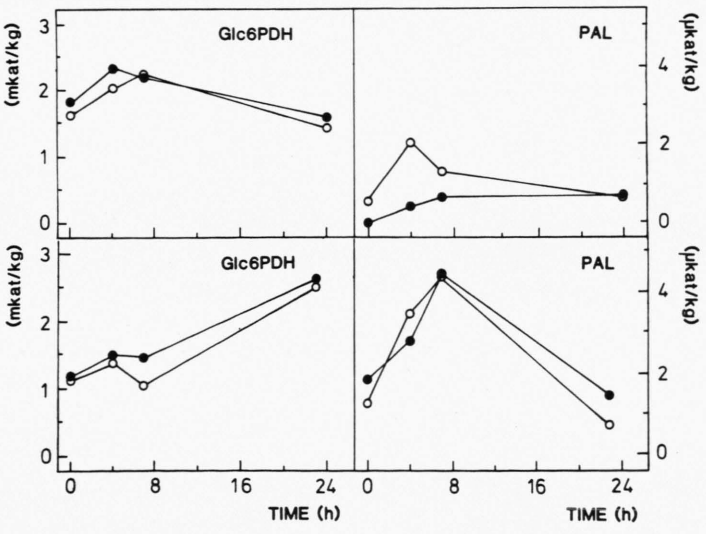

Fig. 3. Specific activities of Glc6PDH and PAL in chickpea cell suspension culture ILC 3279 after application of elicitor (●) at day seven of the growth cycle (upper graphs) and at time of inoculation of cells into fresh medium (lower graphs). Control flasks (○) received equivalent amounts of sterile water.

large change elicited in 3 day old cultures (Fig. 2, A and B).

The activity of CHI at these two stages of the growth cycle did not change in response to elicitation (data not shown) as was also observed at 3 days (Fig. 2,E).

Discussion

Phytoalexins play a pivotal role in the defense reactions of higher plants against fungal pathogens [14, 29]. Homogenous populations of rapidly growing plant cell suspension cultures challenged with fungal elicitors represent suitable model systems for the biochemical elucidation of such plant defense reactions and induction processes of enzymes [13, 29, 30].

Upon elicitation the cell suspension culture established from the chickpea cultivar ILC 3279 rapidly accumulates the pterocarpan phytoalexins medicarpin and maackiain (Fig. 2,F). This response has been observed as a long time stable property of the cell culture [10]. Furthermore, this culture has turned out to be a good source for the isolation of new enzymes involved in late stages of pterocarpan biosynthesis [31, 32]. The data obtained in this study with the various enzymes of primary and secondary metabolism are summarized in Table I. Elicitor application at day three of the growth curve thus leads to specific induction of those enzymes which are directly or indirectly involved in isoflavonoid/pterocarpan biosynthesis (Fig. 2). Other areas of metabolism (glycolysis, citric acid cycle and the respiratory chain, respectively) are not influenced by this treatment (Table I).

The cell culture ILC 3279 constitutively accumulates the isoflavone conjugates BGM and FGM (Fig. 1,B). Maximum levels are reached in the linear growth phase. This period of the growth cycle is also characterized by maximum specific activities of PAL, CHS, CHI and IGT (Fig. 1, D–G). This observation points to a correlation between the accumulation of the main phenolic constitutents and the activities of enzymes involved in the biosynthesis. On the other hand, malonylesterase (IME), which is involved in the demalonylation of the conjugates and thus in the turnover of FGM [4, 7] possesses minimum specific activities (Fig. 1,H) at this time. β-glucosidase activity showed no significant fluctuations during a growth cycle.

Among the enzymes of primary metabolism only Glc6PDH expressed maximum activity at day four and five of the growth curve (Fig. 1,C). Furthermore, the chickpea cell culture responded to elicitor application with a 50% increase in Glc6PDH activity (Fig. 2,A). This enzyme catalyses the starter reaction of the pentose phosphate pathway which yields erythrose-4-phosphate for the synthesis of aromatic amino acids. It is assumed that the enzyme is thus correlated with secondary metabolism (Fig. 1 and 2). In contrast, Glc6PDH activity is not increased in *Phaseolus vulgaris* cell cultures upon elicitation [13]. Glc6PDH, PAL, CHS and CHI all possess short periods of high activities shortly after inoculation of cells into new medium (Fig. 1). This increase in enzyme activity, most typically shown by PAL, is the result of the inducing action of the yeast extract in the PRL-4c chickpea culture medium [10] as shown by appropriate controls without yeast extract (Daniel and Barz, unpublished).

In agreement with several other studies in plant cell cultures [review 14] the enzymatic activities of PAL and CHS were readily increased by the elicitor application (Fig. 1, B and C, Table I). Maximum values for both enzymes were obtained after 4 h with maximum values for the phytoalexins being reached 8 h after elicitor application (Fig. 2,F). These data seem to support the well known time course relationship between biosynthetic enzymes and their products [13, 30, 33]. In connection with chalcone synthase it should be mentioned that this enzyme catalyzes the formation of phloroglucinol chalcone and not of the deoxychalcone isoliquiritigenin which is involved in medicarpin and maackiain formation [11]. Other reports on elicitor induction of CHS [13, 33] have also presented evidence that a chalcone synthase is rapidly stimulated though it is obviously not directly involved in the biosynthetic pathway of the phytoalexins investigated.

Glc6PDH and PAL show significant induction in the linear growth phase, but not at the time of inoculation or in the stationary phase (Fig. 3). Other investigations have also shown that the inducibility of certain enzymes greatly varies during a complete growth cycle [33, 34]. This seemingly limited period of elicitor-caused enzyme induction in the chickpea cell culture is confronted with the observations (Daniel and Barz, unpublished) that phytoalexin accumulation can be elicited at every day of the growth curve shown in Fig. 1.

The CHI activity which was detected by using isoliquiritigenin as substrate was not effected by the elicitor. The very high constitutive activity of CHI in comparison with PAL or CHS might be sufficient for isoflavonoid formation. In the cell culture of *Phaseolus vulgaris* this enzyme was induced after elicitor treatment [13].

In addition to the phytoalexins medicarpin and maackiain the level of the formononetin-7-O-glucoside-6″-malonate FGM is enhanced [28, 35] by elicitor treatment of chickpea cell suspension culture ILC 3279. This observation readily explains the pronounced increase in IGT activity (Fig. 2, D, Table I) because this enzyme is required for the formation of the intermediate formononetin 7-O-glucoside. Additional activation of the specific malonyltransferase [6] could also be expected. Formononetin functions as an intermediate in both phytoalexin biosynthesis [11] and isoflavone conjugate formation [5]. Therefore, the substantial increase in glucosyltransferase activity might be a detoxification reaction for excess formononetin which is not required for phytoalexin production. A similar situation has been observed in herbicide-challenged soybean leaves [36] where a substantial increase in pterocarpans is accompanied by increased levels of isoflavone glucosides and a substantial induction of the isoflavone-specific glucosyltransferase. Furthermore, the two investigations [36, this report] also agree that maximum IGT activity is reached some hours later than observed for Glc6PDH, PAL and CHS and that the time period of high glucosyltransferase activity lasts much longer than for the three enzymes mentioned above (Fig. 2).

The strong elicitor-induced accumulation of phytoalexins at day 7 of the growth curve (Daniel and Barz, unpublished) without the additional induction of Glc6PDH and PAL activities (Fig. 3) might be explained with our recent observation (Jaques and Barz, unpublished) that the cells from this growth phase synthesize the phytoalexins from isoflavone material obtained by the hydrolysis of FGM. Elicitor-treatment of chickpea cells at day 3 of the growth curve apparently leads to the induction of enzymes of phenylpropanoid biosynthesis and the accumulation of both pterocarpan phytoalexins and FGM. However, elicitor application to cells during stationary phase of growth seems to lead to phytoalexin formation at the expense of isoflavone conjugates. The regulatory pattern of this switch in cellular metabolism is represently under investigation.

In general, the data presented in this report show that the chickpea cell suspension culture ILC 3279 represents a suitable experimental system for investigations on elicitor-induced changes in levels of secondary compounds and biosynthetic enzymes. The data especially indicate that enzymes located at branching points of related pathways readily respond to elicitor application.

Acknowledgements

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