UV DOSE-DEPENDENT INDUCTION OF ENZYMES RELATED TO FLAVONOID BIOSYNTHESIS IN CELL SUSPENSION CULTURES OF PARSLEY

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1. Introduction

Flavonoid biosynthesis in parsley cell suspension cultures can be induced by white light [1], the UV part of which plays an essential role in this response [2,3]. The enzymes involved in the flavonoid pathway are coordinately regulated in two groups. Group 1 consists of the enzymes of the general phenylpropanoid metabolism; group II consists of the enzymes which are exclusively related to the formation of flavonoid compounds [4]. In response to continuous illumination with white light, time courses of induced activities of both enzyme groups differ markedly [4]. Phytochrome is involved in the effect of light on flavonoid biosynthesis. This was shown by the physiological effectiveness of phytochrome after UV irradiation [3,5]. Enzymes of both groups are stimulated by phytochrome to the same extent. It is feasible that for each enzyme of the flavonoid pathway the same quantitative relationship to the inducing agent (UV receptor, phytochrome) exists [3].

The present paper describes the UV dose-dependent synthesis of flavonoids which is correlated to a concomitant induction of phenylalanine ammonia-lyase, PAL, a representative of enzyme group I and chalcone-flavanone isomerase, CFI, a representative of enzyme group II.

2. Experimental

Cell suspension cultures from parsley (*Petroselinum hortense* Hoffm.) were obtained and grown as described previously [1,2], subculturing every 7 days. Seven-day-old cultures (about 8 g fresh weight per 40 ml suspension) were used for experiments. Growth of the cultures in the dark and irradiations were done under sterile conditions with continuous shaking at 25°C. Samples (1.5 g of packed cells resuspended in 15 ml of medium) were irradiated in 5 × 5 cm glass Petri dishes covered with plastic lids which had a good transmission in the near ultraviolet [2]. UV was obtained from Osram 40 W/73 lamps (λ max = 350 nm).

Extracts were prepared by homogenizing 1 g of cells (fresh weight) in 5 ml 0.1 M borate buffer, pH 8.8 in a Sorvall Omni-Mixer. The homogenate was centrifuged at 35 000 *g* for 15 min and the supernatant fluid was used for the assays of flavonoids and enzymes which were determined in the same extract. The flavonoid content was determined by measuring the absorbance at 370 nm. The assays of PAL [6] and CFI [7] have been described elsewhere. Proportionality between extract volume and enzyme activities was always observed. The PAL reaction was linear with time for more than 2 hr and the CFI reaction for at least 2 min.

3. Results and discussion

In previous investigations UV<350 nm wad demonstrated to trigger flavonoid synthesis in parsley cell suspension cultures [2,3]. The visible range of the spectrum proved to be without detectable effect. Action spectra are consistent with maximum quantum efficiency below 300 nm. The quantitative relationship between UV doses and amounts of induced flavonoids is shown in fig.1. The existence of reciprocity and of linear UV dose dependency points to a sequence of linearly related metabolic reactions, translating the stimulus induced by a certain amount

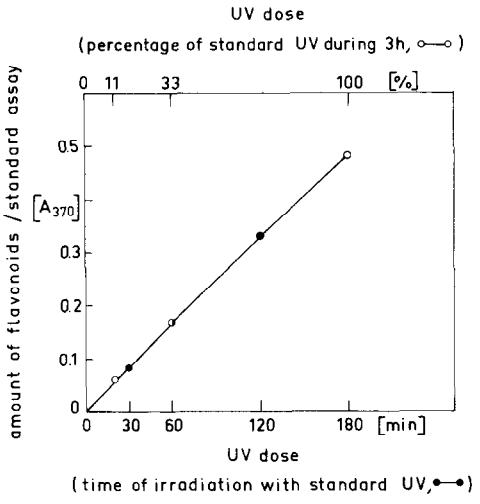

Fig. 1. UV dose-dependent flavonoid synthesis (test of Reciprocity Law) in parsley cell cultures. The UV dose was varied by means of the time of irradiation with standard UV (0.2 $W \cdot m^{-2}$), or by means of reducing the standard intensity to 33% or 11% (using screens) with a constant irradiation time of 3 hr. Flavonoids were determined at the time of maximum accumulation. Dark values were substracted. The given values represent means of 5 independent experiments.

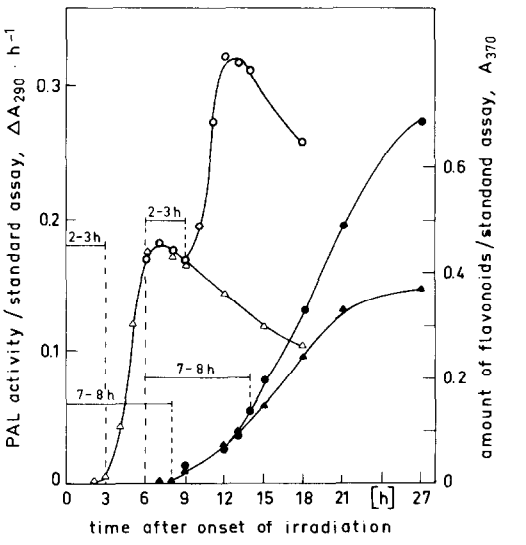

Fig. 2. Secondary induction of phenylalanine ammonia-lyase (PAL, open symbols) and flavonoids (closed symbols) in parsley cell cultures. Cells were irradiated twice, at 0 hr and after 6 hr with 15 min of standard UV (1.2 $W \cdot m^{-2}$). For the whole experiment samples of the same original culture were used. Dark values were subtracted. Corresponding results were reproduced in three independent experiments.

of UV quanta into a proportional number of flavonoid molecules. This biochemical process can also be followed at the level of the related enzymes. PAL, the enzyme initiating the flavonoid (phenylpropanoid) pathway, was studied in detail (fig. 2). In this experiment flavonoid synthesis was induced by a UV dose which was about 10% of the saturating dose. Six hours later the same UV dose was applied for a second time. Both irradiations resulted in additive (identical) accumulation curves for PAL activity as well as for flavonoid formation. Lag phases were unchanged. Obviously the processes of synthesis and degradation of PAL and flavonoids were not influenced by the first induction. This also supports previous results that there was no unspecific or damaging effect on the cells due to such low dose UV irradiations [2].

Induction of PAL activity in the parsley cultures is achieved by the novo synthesis. This was concluded from the incorporation of [^{35}S] methionine into PAL subunits in response to light [8]. Furthermore, labeling experiments with ^{15}N under inductive and noninductive conditions, using isopycnic CsCl density gradient centrifugation indicate an increased steady state rate of PAL synthesis in response to irradiation [9].

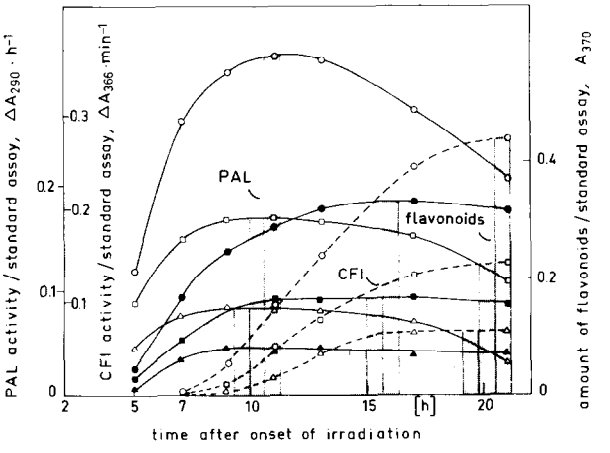

Fig. 3. UV dose-dependent induction of phenylalanine ammonia-lyase (PAL), chalcone-flavanone isomerase (CFI) and flavonoids in parsley cell cultures. Cells were irradiated for 5 min (triangles), 10 min (squares) and 20 min (circles) with standard UV at 0.5 $W \cdot m^{-2}$. Dark values remain unchanged during the course of the experiment, and were subtracted. Such linear UV dose dependency of the two induced enzyme activities and of flavonoid synthesis can be reproduced independent of the developmental state of the cell culture. Accumulation curves from parallel experiments, however, differ in time courses and in absolute values (see fig. 2).

Simultaneously with PAL the other enzymes of the general phenylpropanoid pathway as well as those of the specific flavonoid pathway are induced upon irradiation. A different regulation of these two groups of enzymes was observed [4]. Obviously there is no difference, however, in the relative response to phytochrome [5] or to UV (fig. 3) of PAL (group I enzyme) and CFI (group II enzyme) activity. Linear UV dose-dependency has been demonstrated at the time of maximum accumulation for both these enzymes just as for flavonoid synthesis (fig. 3). These results indicate that induced enzyme activities measured in vitro at the same time represent relative changes in the in vivo activity. Flavonoid biosynthesis in the parsley cell culture might be a promising model system for further studies of a metabolic pathway which is regulated by de novo enzyme synthesis.

Flavonoid synthesis may generally be regulated by a mechanism similar to that observed in the parsley culture. In other systems concomitant induction by light [10] or other factors [11,12] has also been demonstrated for 2 or all the 3 enzymes of the general phenylpropanoid metabolism. Quantitative relations between the inducing agent and the activities of PAL and CFI have been shown in the case of phytochrome-induced anthocyanin and flavonol synthesis in mustard cotyledons [13].

The UV effect described here is not limited to the cell culture system. UV is morphogenetically active in the intact parsley plant too [3]. The quantum efficiency is high enough to allow a physiological effectiveness under conditions of natural daylight. The linear relationship between UV dose and flavonoid synthesis can be assumed to represent an efficient protective mechanism against a surplus of damaging solar UV radiation which then could be absorbed by the flavonoid pigments accumulated in the outer cell layers of the plant.

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