MALONYL COENZYME A: FLAVONE GLYCOSIDE MALONYL-TRANSFERASE FROM ILLUMINATED CELL SUSPENSION CULTURES OF PARSLEY

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1. Introduction

The formation of flavone glycosides in cell suspension cultures of parsley (*Petroselinum hortense*) and concomitant increases in the activities of a number of enzymes related to this biosynthetic pathway have been shown to be induced by treatment of the cells with light [1, 2]. As a result of these studies, it has been postulated that all of the enzymes directly involved in the synthesis of flavone glycosides are regulated interdependently in light-induced parsley cell cultures [2].

In a recent investigation on the chemical structures of the flavonoid glycosides formed after illumination of parsley cell cultures, a number of malonylated compounds have been isolated and identified [3]. One of the major components was the flavone glycoside apiin (7-O-[β-D-apiofuranosyl-(1→2)-β-D-glucosyl]-5,7,4′-trihydroxyflavone) bearing a malonyl residue in the sugar moiety of the molecule.

This communication reports the isolation of an enzyme from cell-free extracts of illuminated parsley cell suspension cultures which catalyzes the transfer of malonate from malonyl CoA to flavone glycosides. Changes in the activity of this enzyme with time after the onset of illumination lend further support to the hypothesis mentioned above.

2. Experimental

Cell suspension cultures of *Petroselinum hortense* were grown for 10 days in the dark, subsequently illuminated for 24 hr, and then harvested by vacuum filtration as described previously [2]. Two g (fresh weight) of cells and 4 ml of 20 mM potassium phosphate buffer (pH 7.5, containing 5 μl of mercaptoethanol) were homogenized 3 times for 30 sec at intervals of 30 sec in an "Ultra-Turrax" (Janke and Kunkel, Staufen). The mixture was centrifuged at 20,000 *g* for 10 min, and the supernatant was stirred for 15 min with 0.2 g of Dowex I × 2 (phosphate form, equilibrated with 0.2 M potassium phosphate, pH 7.5). The resin was removed by vacuum filtration through glass wool (solution A). One ml of the filtrate was diluted with 9 ml of a solution of serum albumin (2 mg/ml) in 20 mM potassium phosphate, pH 7.5 (solution B).

2.1. *Enzyme assay*

Fifty μl of solution B were incubated with 1 nmole of apiin dissolved in 2 μl of ethylene glycol monomethyl ether and 0.5 nmoles of [1,3-^{14}C]malonyl CoA (20,000 dpm, New England Nuclear) for 10 min at 30°. The reaction was stopped by adding 10 μl of acetic acid, and the mixture was chromatographed on Whatman No. 3 MM paper in 10% acetic acid. The labelled product was detected with a UV lamp at 350 nm or with a paper chromatogram scanner LB 280 (Berthold, Wildbad).

2.2. *Synthesis and purification of [1,3-^{14}C]malonyl apiin*

22 Nanomoles of [1,3-^{14}C]malonyl CoA (890,000 dpm, New England Nuclear), 8.65 μmoles of unlabelled malonyl CoA (Serva, Heidelberg), and 7.6 μmoles of apiin dissolved in 430 μl ethylene glycol monomethyl ether were incubated with 10 ml of solution A at 30°

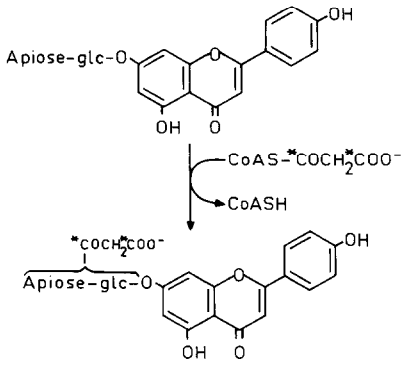

Fig. 1. Formation of malonyl apiin from apiin and malonyl CoA.

for 60 min. The reaction was stopped by adding 1 ml of acetic acid. This mixture was chromatographed on 12 sheets of Whatman 3 MM paper in 10% acetic acid. Radioactive zones absorbing UV light at 350 nm (R_f 0.30) were eluted with methanol–H_2O (1:1), and the solvent was then evaporated. The residue was dissolved in 0.5 ml of ethylene glycol monomethyl ether and passed through a Sephadex LH-20 column (1.7 × 33.5 cm) with methanol. Fractions 31–34 (see fig. 2) were combined and used for studies on the properties of malonyl apiin.

2.3. *Hydrolysis*

Approx. 50 μg of [1,3-^{14}C]malonyl apiin (4,000 dpm) dissolved in 10 μl of ethylene glycol monomethyl ether were hydrolyzed with 40 μl of 1 N KOH at room temp. for 4 hr. The mixture was then acidified with 50 μl of 1 N HCl and chromatographed on Whatman No. 1 paper in n-butanol/ethanol/H_2O (4:1:1).

Radioactivity was determined by scintillation spectrometry in toluene containing 5 g of PPO per liter.

High voltage electrophoresis was carried out on MN 214 paper (Macherey-Nagel, Duren) in 20 mM potassium phosphate, pH 7.5, at 35 V/cm (45 mA).

3. Results and discussion

A crude enzyme preparation from illuminated cell suspension cultures of parsley catalyzed the transfer of the malonyl residue from malonyl CoA to the flavone diglycoside apiin as shown in fig. 1. An ion exchange resin was used in order to remove endogenous substrates from the protein solution.

The labelled product of the enzymatic reaction was easily separated by paper chromatography from radioactive malonyl CoA and malonate, both migrating at the front of the solvent system, and was isolated

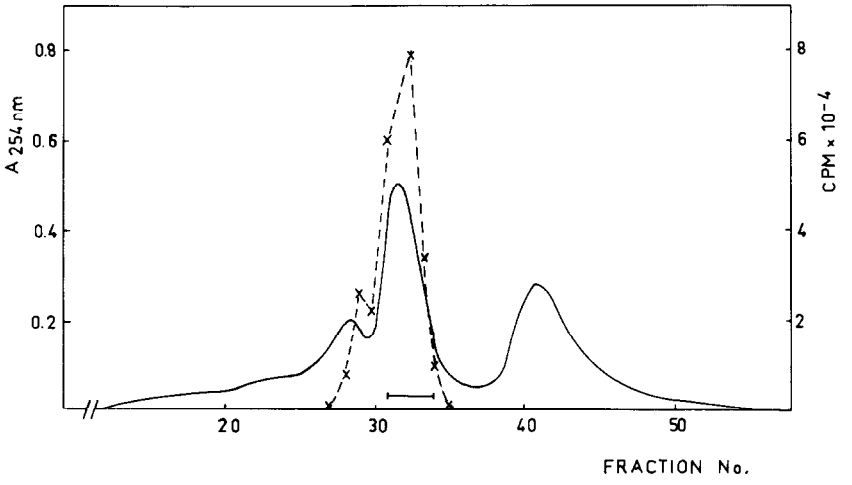

Fig. 2. Separation of [1,3-^{14}C]malonyl apiin from apiin by gel chromatography on a Sephadex LH-20 column. The bar indicates the fractions combined. (——): Absorbance at 254 nm; (– – –): radioactivity (cpm/fraction).

together with the non-labelled substrate apiin. Subsequent separation of ^{14}C-labelled malonyl apiin and apiin itself was achieved by gel chromatography on a Sephadex LH-20 column (fig. 2). Fractions containing malonyl apiin were identified by the radioactive label associated with the compound absorbing UV light at 254 nm. The intact incorporation of one mole of malonate and the formation of one glycosidic ester linkage per mole of apiin is concluded from the following results.

i) The UV spectrum of malonyl apiin was identical with that of apiin. This suggests that the aglycone moiety of the product was not altered by the transferase reaction.

ii) The electrophoretic mobility (1 cm/6 hr under the experimental conditions) indicates that the isolated compound contained a free carboxyl group.

iii) After alkaline hydrolysis of [1,3-^{14}C]malonyl apiin and paper chromatography of the products formed, all of the radioactivity was associated with a spot corresponding to an authentic sample of malonic acid (R_f 0.71), while a spot absorbing UV light at 350 nm corresponded to apiin (R_f 0.36).

iv) The specific radioactivity of the [1,3-^{14}C]malonyl apiin isolated from the Sephadex LH-20 column was, within the limits of the experimental error, identical with that of the [1,3-^{14}C]malonyl CoA used for the enzymatic reaction (10^5 dpm/μmole). The extinction coefficient determined for apiin at 336 nm was used for these calculations.

Attempts are underway to determine the position of the malonyl residue in the glycoside by mass spectrometry [4]. To the author's knowledge, the only detailed data on malonylated flavonoid compounds reported thus far in the literature are those on isoflavone 7-O-glucosides bearing a malonyl residue in the 6-position of the glucose [5].

When the cell cultures were illuminated for different periods of time (from 2 to 32 hr) before enzyme extraction, highest specific activities were observed about 24 hr after the onset of illumination. Fig. 3 shows that increases in the activity of the malonyl transferase occurred after a lag period of about 4 hr. The changes in enzyme activity from hours 4 to 24 were identical with those reported for the enzymes of "Group II" as defined in a previous communication [2].

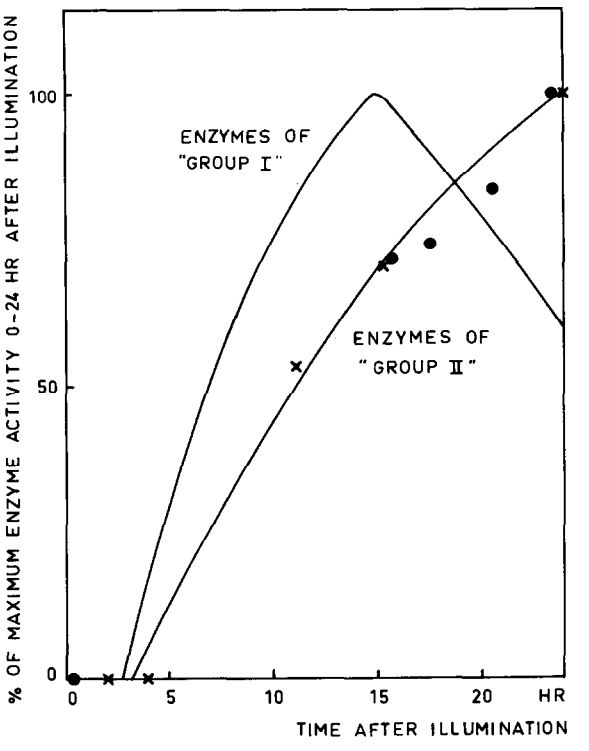

Fig. 3. Relative changes in malonyl transferase activity after the onset of illumination. The two symbols indicate results obtained from two separate experiments. Solid lines represent data reported previously [2]. All values given for enzyme activities were corrected for activities measured in dark-grown cells (approx. 30% of maximum enzyme activity in the case of malonyl transferase).

This observation strongly supports the hypothesis that all of the enzymes directly related to the formation of flavonoid glycosides in illuminated cell suspension cultures from parsley are regulated interdependently and differently from those acting on phenylpropanoid compounds ("Group I") [2].

Labelled products were also obtained when the enzyme was incubated with [1,3-^{14}C]malonyl CoA and apigenin-7-O-glucoside or 3′-methoxyapiin (graveobiosid B), two other flavone glycosides occurring in illuminated parsley cell suspension cultures in the non-acylated and in the malonylated form [3]. This suggests that the malonyl transferase plays a general role in the conversion of flavonoid glycosides to malonyl esters in these cultures.

Acknowledgements

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