Enzymes of General Phenylpropanoid Metabolism and of Flavonoid Glycoside Biosynthesis in Parsley

Differential inducibility by light during the growth of cell suspension cultures

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ABSTRACT

Several enzymes of phenylpropanoid metabolism showed large changes in their inducibility by light during the growth cycle of cell suspension cultures from parsley (Petroselinum hortense Hoffm.). Two of the three enzymes of general phenylpropanoid metabolism (group I) and six of the approximately 13 enzymes of the flavone and flavonol glycoside pathways (group II) were investigated. Both enzymes of group I (phenylalanine ammonia-lyase and 4-coumarate:coenzyme A ligase) were most efficiently induced at two different stages: first, soon after starting a new culture, and second, near the beginning of the stationary phase. In contrast, the enzymes of group II (acetyl-coenzyme A carboxylase, flavanone synthase, chalcone isomerase, UDP-apiose synthase, and at least one of two malonyltransferases) were maximally induced during exponential growth of the culture. This result supports the conclusions drawn from previous data that the two groups are regulated differentially and that the enzymes within each group are regulated in a coordinated manner.

The enzymes of general phenylpropanoid metabolism (group I) and of the flavone and flavonol glycoside pathways (group II) can be clearly distinguished in cultured parsley cells because of characteristic differences in their regulation. The main criteria used for the classification of the two groups are: (a) enzyme induction (only group I) in freshly inoculated cultures in the absence of light; and (b) different time courses of changes in the enzyme activities after simultaneous induction of both groups by UV irradiation of cell cultures (9).

After induction by continuous irradiation, the enzymes of group I reach maximal activities several hours earlier than the enzymes of group II. The peak positions of the individual enzymes may vary considerably with the growth stage of the cell culture. However, under the conditions tested maximal activities of all enzymes were reached within the range from 15 to 40 h after the onset of induction. At least about 80% of the maximal enzyme activity was obtained in all cases after 20 h of continuous irradiation (3, 8, 11, 18, 21). This period of induction was therefore used throughout the present experiments, where changes in the inducibility of the enzymes of both groups were investigated in a comparative study.

MATERIALS AND METHODS

Cell Cultures. Cell suspension cultures of parsley (Petroselinum hortense Hoffm.) were propagated in a fermentor (17) containing 20 liters of medium I (7). Samples of 80 to 200 ml depending on the density of cells, were taken at various times and irradiated as described previously (11). The conductivity of the medium was measured (7) both before and after irradiation. Cell fresh weight was determined after suction of an air stream through the cells for 2 min on a porous glass filter. The cells were then frozen by immersion in liquid N₂ and stored at −70 C for up to several months without loss of enzyme activities.

Enzyme Assays. Standard procedures were used for preparing crude cell extracts and measuring flavonoid concentration (11) and the activities of phenylalanine ammonia-lyase (PAL) (22), 4-coumarate:CoA ligase (4CL) (17), acetyl-CoA carboxylase (ACC) (3, 16), flavanone synthase (FLS) (20), chalcone isomerase (CHI) (15), UDP-apiose synthase (UAS) (4, 11), and malonyl-CoA:flavone/flavonol 7-O-glycoside malonyltransferase (5) (malonyltransferase 1 = MAT 1). Malonyl-CoA:flavonol 3-O-glucoside malonyltransferase (malonyltransferase 2 = MAT 2) activity was measured with the same assay as used for MAT 1, except that isorhamnetin 3-O-glucoside was used as substrate instead of apigenin. The data are expressed in μkat/kg of protein. One unit of enzyme activity (1 kat) is the amount of enzyme required for the formation of 1 mol of product in 1 s under the assay conditions.

Protein Determination. Protein was determined by a modified Lowry method (2), measuring A at 750 nm. The changes in extractable cellular protein during growth of a culture are shown in Figure 1.

RESULTS

The increase in cell fresh weight was used as a measure of growth of the culture. The portion of the resulting growth curve which is of interest for the present experiments is shown in Figure 1. For easy comparison of the following data with earlier results (8), the changes in the conductivity of the culture medium are also shown. Furthermore, Figure 1 shows the amounts of flavonoid glycosides accumulated in the cells within 20 h of irradiation at the various growth stages of the culture. It should be noted, however, that the concentration of these compounds can be taken only as a very rough estimate of the rate of flavonoid biosynthesis. The rate of flavonoid degradation changes considerably with the age of parsley cell cultures and is particularly high in young cultures (1).

Figure 2 illustrates the observed changes in the inducibility of PAL and 4CL within 20 h of continuous irradiation. The curves are similar for the two enzymes. Both were maximally inducible at a very early stage and also later, shortly before the stationary phase of the culture. An intermediate period of lower inducibility around the exponential growth phase was found but was less
Changes in cell fresh weight (A), conductivity of the medium (O, •), extractable cellular protein (x), and light-induced flavonoid glycoside production (□) during growth of a parsley cell suspension culture in a 20-liter fermentor. Samples were taken from the culture at the times indicated. The conductivity was measured both before (●) and after (○) UV irradiation of the cells for 20 h. Cell fresh weight and flavonoids were measured after irradiation. Broken curves in lower panels indicate changes in cell fresh weight as shown in upper panel.

FIG. 1. Changes in inducibility of two enzymes of group I. The same samples of cells as shown in Figure 1 were used after irradiation, and the enzyme activities were measured in extracts from frozen cells. For comparison, the dotted line indicates the changes in the inducibility of one typical representative of group II, flavanone synthase, under the same conditions (see Fig. 3). The data were not corrected for levels of enzyme activity in the dark. These are very low or undetectable for most enzymes of both groups but can be up to one-third of the induced level in a few cases (3, 11). For abbreviations, see under “Materials and Methods.”

FIG. 2. Changes in inducibility of six enzymes of group II. Dotted line indicates changes in inducibility of a representative of group I, PAL, under the same conditions. For further details, see legend of Figure 2. For abbreviations, see under “Materials and Methods.”

DISCUSSION

The present results provide further evidence for a differential regulation of the enzymes of general phenylpropanoid metabolism and of the flavonoid glycoside pathways, despite their close metabolic interrelation. The three enzymes of group I supply the precursors for several phenylpropanoid pathways, including the synthesis of flavonoids and esters of substituted cinnamic acids. In addition to flavonoid glycosides, cinnamate esters have been pronounced in this than in previous experiments (8, 22).

The curves obtained for changes in the inducibility of the enzymes of group II differed markedly from the curves for the group I enzymes (Fig. 3). The inducibility of all group II enzymes with the exception of malonyltransferase had a maximum during the period of relatively low inducibility of PAL and 4CL. Very poor induction of almost all group II enzymes was found at a late period when the enzymes of group I exhibited a second maximum of inducibility.
was identified as caffeate (3,4-dihydroxycinnamate) (19). It is likely that the induction of the group I enzymes is related to the formation not only of flavonoids but also of cinnamate esters and perhaps even of other phenylpropanoid derivatives.

The occurrence in parsley cell cultures of a late peak for the inducibility of the group I enzymes corresponds to a similar phenomenon observed with other cell cultures (6). The reason for this similarity is not known. However, the points of high activity for enzymes of group I and of depletion of nitrate from the culture medium coincided in the three cases investigated (6).

The early peak for the inducibility of the group I enzymes at the initial stage of the parsley cell culture is probably a result of two superimposed induction mechanisms. Part of the induction is brought about by irradiation, another part by dilution of the cultures into fresh medium. The efficiency of the dilution effect increases exponentially with decreasing cell density (12, 14), and the combined effects of irradiation and dilution are larger than additive (12). No explanation can be given for the minimum preceding the second peak not being more pronounced in the present case (cf. 8, 22). Previous results (8) suggested that the use of a longer irradiation period (20 h as compared with 15 h) has only a small effect on the shape of the inducibility curve for PAL.

The peak for the inducibility of the group II enzymes coincides with the period of highest mitotic activity of the culture (J. Vieregge, unpublished results). It is not known whether direct relation exists between these two phenomena. It should be noted that highest activities of the enzymes involved in flavonoid biosynthesis in intact parsley plants were found in very young leaf tissue where cells could still undergo division (13).

In contrast to the differential regulation of the two groups, the enzymes within each group seem to be regulated in a coordinated manner, perhaps with the exception of MAT 2. This conclusion is in agreement with all previous results (3, 11, 14). The molecular mechanisms causing the coordination are not known. We are presently involved in comparative studies of the mRNA activities for both groups under various conditions of induction (10, 21). Recently, we have shown that the coordinated regulation of enzyme activities through the existence of polycistronic mRNAs is very unlikely for both group I and group II (10).

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