Biosynthesis and accumulation of rosmarinic acid in suspension cultures of Coleus blumei

Maike Petersen, Elisabeth Szabo, Juliane Meinhard, Barbara Karwatzki, Claudia Gertlowski, Bettina Kempin & Elisabeth Fuß
Institut für Entwicklungs- und Molekularbiologie der Pflanzen, Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, D-40225 Düsseldorf, Germany

Key words: biosynthesis, caffeic acid esters, Coleus blumei (Lamiaceae), plant cell cultures, rosmarinic acid, secondary product formation

Abstract

This communication reviews data on the accumulation and biosynthesis of rosmarinic acid in cell suspension cultures of Coleus blumei. The influence of the medium, mainly the carbohydrate source on growth and rosmarinic acid production in these cell cultures is described. The biosynthetic pathway of rosmarinic acid was elucidated in Coleus blumei cell cultures: eight enzymatic activities are involved in the transformation of the precursors phenylalanine and tyrosine to the end product rosmarinic acid.

Abbreviations: CAH – cinnamic acid 4-hydroxylase; 4CL – 4-coumarate:CoA ligase; HPPR – hydroxyphenylpyruvate reductase; 3-H – hydroxycinnamoyl-hydroxyphenyllactate 3-hydroxylase; 3'-H – hydroxycinnamoyl-hydroxyphenyllactate 3'-hydroxylase; PAL – phenylalanine ammonia-lyase; RAS – rosmarinic acid synthase (hydroxycinnamoyl-CoA:hydroxyphenyllactate hydroxycinnamoyl transferase); TAT – tyrosine aminotransferase

Rosmarinic acid, an ester of caffeic acid and 3,4-dihydroxyphenyllactate (Fig. 1), is a common constituent mainly of species belonging to the families Lamiaceae and Boraginaceae (Mølgaard & Ravn 1988). The compound was, however, also detected in some species of other families of the dicotyledonous angiosperms (Harborne 1966; Hiller & Kothe 1967; Lamaison et al. 1990; De Tommasi et al. 1991) as well as in ferns (Blechnum spec.; Harborne 1966; Häusler et al. 1992) and hornworts (Takeda et al. 1990 and Petersen unpubl.). Therefore, the occurrence of rosmarinic acid is not satisfactory as a chemotaxonomic marker.

The structure of rosmarinic acid isolated from Rosmarinus spec. was elucidated by Scarpati and Oriente in 1958. The biosynthetic precursors of rosmarinic acid, phenylalanine and tyrosine, were identified by feeding radioactively labelled amino acids to plants of Mentha spec. and cell cultures of Coleus blumei (Ellis & Towers 1970; Razzaque & Ellis 1977).

Cell suspension cultures of Coleus blumei proved to be a very suitable system for the elucidation of the biosynthetic pathway of rosmarinic acid in this species. Suspension cultures in a modified B5-medium (Petersen & Alfermann 1988) with 2% sucrose accumulate about 2–3% of rosmarinic acid related to the cell dry weight. This accumulation can be raised to about 20% of the cell dry weight by higher sucrose contents in the medium (Zenk et al. 1977; Petersen & Alfermann 1988; Gertlowski & Petersen 1993). Rosmarinic acid accumulation starts at the end of the growth phase and takes place during only five days of the culture period (Fig. 2). At this time, the enzymes involved in the biosynthesis of rosmarinic acid show high activities, thus facilitating their identification and isolation.

The influence of different sugars and sugar concentrations as well as some mineral nutrients on the accumulation of rosmarinic acid was investigated (Gertlowski 1991; Gertlowski & Petersen 1993). Increasing concentrations of sucrose from 1% to 6% in the...
culture medium promoted dry weight accumulation and rosmarinic acid concentration related to the dry weight, thus resulting in an increasing productivity of the cell cultures for rosmarinic acid. Sucrose was rapidly cleaved into glucose and fructose, and glucose was consumed more rapidly than fructose. However, glucose or fructose added to the culture medium either separately or simultaneously at equimolar concentrations, did not promote the same high rosmarinic acid accumulation as did sucrose. The start of rosmarinic acid accumulation coincides with the depletion of phosphate from the culture medium at day 4–5 of the culture period. Our hypothesis is, that rosmarinic acid produc-

tion starts when a nutrient (e.g. phosphate) becomes growth-limiting, and the amount of rosmarinic acid synthesized is determined by the remaining amount of carbohydrates in the cells and the culture medium. Lower phosphate concentrations in the culture medium resulted in an increased rosmarinic acid accumulation. This is in agreement to multiple reports on the promoting effect of lower phosphate and nitrate concentrations on the production of phenolic natural compounds.

Microspectrophotometric determinations (Chaprin & Ellis 1984) as well as the isolation of protoplasts and vacuoles (Häusler et al. 1993) revealed the vacuolar localization of the high amounts of rosmarinic acid synthesized by suspension cultures of *Coleus blumei*. The distribution of rosmarinic acid between protoplasts and vacuoles is the same as for vacuolar marker enzymes, such as acid phosphatase, phosphodiesterase and N-acetylglucosaminidase (Häusler et al. 1993). The mechanisms of transport of rosmarinic acid into and its accumulation in the vacuole of *Coleus blumei* cells are still under investigation.

A pathway for the biosynthesis of rosmarinic acid (Fig. 1) was proposed, based on the enzyme activities detected in protein extracts from suspension cultures of *Coleus blumei* (Petersen et al. 1993). Phenylalanine is oxidatively desaminated by phenylalanine ammonia-lyase (PAL) and the resulting cinnamic acid hydroxylated to 4-coumarate by cytochrome P-450-dependent cinnamic acid 4-hydroxylase (CAH). 4-Coumarate is activated with help of coenzyme A by 4-coumarate:CoA ligase (4CL). This enzyme from *Coleus blumei* cells accepts caffeic acid as substrate as well. The coenzyme A-

Fig. 1. Proposed biosynthetic pathway for rosmarinic acid in cell cultures of *Coleus blumei*. PAL = phenylalanine ammonia-lyase; CAH = cinnamic acid 4-hydroxylase; 4CL = 4-coumarate:CoA ligase; TAT = tyrosine aminotransferase; HPPR = hydroxyphenylpyruvate reductase; RAS = rosmarinic acid synthase (hydroxycinnamoyle-CoA:hydroxyphenyllactate hydroxycinnamoyltransferase); 3-H = hydroxycinnamoyl-hydroxyphenyllactate 3-hydroxylase; 3’-H = hydroxycinnamoyl-hydroxyphenyllactate 3’-hydroxylase.

Fig. 2. Growth (□, ○) and rosmarinic acid accumulation (□, ○) of suspension cultures of *Coleus blumei* in medium with 2% (CB2, □, ○) and 4% sucrose (CB4, ○, □) during a cultivation period of 14 days.