Effect of excess of cupric sulfate on sanguinarine formation and activities of amine oxidase and polyphenol oxidase in cell suspension cultures of *Papaver somniferum*

Andrea Bilková*, František Bilka, Víťazoslava Blanáriková & Lýdia Bezáková

Department of Cell and Molecular Biology of Drugs, Faculty of Pharmacy, Comenius University, Kalinčiakova 8, SK–83232 Bratislava, Slovakia; phone: ++ 421 2 50117 315, e-mail: bilkova@fpharm.uniba.sk

Abstract: Cell suspension cultures of *Papaver somniferum* produce relatively large amount of benzophenanthridine alkaloid, sanguinarine, upon elicitation. Cupric sulfate was used as an abiotic elicitor to induce sanguinarine biosynthesis in cultures of *P. somniferum*. The treated cells exhibited an increase in intracellular sanguinarine content and an increase of specific activities of amine oxidase (AO) and polyphenol oxidase (PPO), i.e. the enzymes involved in sanguinarine and morphinan biosynthetic pathway. The elicitation led to a seven-fold increase in the content of sanguinarine. Specific activities of AO and PPO were induced in both dose- and time-dependent manners up to 2.5- and 3-fold, respectively. Two isoforms of PPO (Mr 65 kDa and 40 kDa) were separated from opium poppy cell cultures by gel filtration on Sephadex G-100 column and their relative participation in the whole PPO activity was estimated.

Key words: *Papaver somniferum*, elicitation, cupric sulfate, amine oxidase, polyphenol oxidase, sanguinarine.

Abbreviations: AO, amine oxidase; BICH, benzylisoquinoline; DRR, 1,2-dehydroreticuline reductase; PPO, polyphenol oxidase; TDC, tyrosine decarboxylase.

Introduction

Plants respond actively to infection and other environmental stress factors. Defense gene products function in diverse biochemical responses that include the biosynthesis of antimicrobial phytoalexins, the deployment of antimicrobial hydrolytic enzymes and the putative reinforcement of the plant cell wall with structural glycoproteins or lignin (Facchini et al., 1996). Members of the Papaveraceae synthesize benzophenanthridine alkaloids (Kutchan & Zenk, 1993), and selected species respond to pathogen challenge or elicitor treatment with the accumulation of the antimicrobial alkaloid sanguinarine (Cline & Coscia, 1988) as a putative phytoalexin.

Sanguinarine and other benzophenanthridine alkaloids together with morphinans belong to a large group of benzylisoquinoline (BICH) alkaloids. The first steps of their biosynthetic pathway are common. They are derived from (*S*)-norcoclaurine, which is formed *via* the condensation of dopamine and 4-hydroxyphenylacetaldehyde (Stadler et al., 1989). Dopamine may arise from L-tyrosine by decarboxylation by action of tyrosine decarboxylase (TDC) followed by hydroxylation of tyramine by polyphenol oxidase (PPO); or L-tyrosine is first hydroxylated to 3,4-dihydroxyphenylalanine by PPO and then decarboxylated (Rueffer & Zenk, 1987; Stano et al., 1995). Synthesis of 4-hydroxyphenylacetaldehyde from another molecule of L-tyrosine involves its decarboxylation and deamination. Deamination may be catalyzed by transaminase (Rueffer & Zenk, 1987) or by amine oxidase (AO) (Bilková et al., 2000).

(*S*)-norcoclaurine is converted to (*S*)-reticuline by four enzymatic reactions in so-called pre-reticuline pathway. (*S*)-reticuline is a branch-point intermediate involved in the biosynthesis of many structurally diverse types of BICH alkaloids.

The first step in sanguinarine and other benzophenanthridine alkaloid biosynthesis is the conversion of the *N*-methyl group of (*S*)-reticuline into the methylene bridge moiety of (*S*)-scoulerine by the berberine bridge enzyme. Conversion of (*S*)-reticuline to its (*R*)-epimer represents the first step in the biosynthesis of morphinane alkaloids. This conversion is catalyzed by 1,2-dehydroreticuline reductase (DRR). The absence of DRR activity in opium poppy cell cultures is likely a primary reason for the lack of morphinane alkaloid

* Corresponding author