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Identification and stress-induced expression of three 3β-hydroxysteroid dehydrogenases from *Erysimum crepidifolium* Rchb. and their putative role in cardenolide biosynthesis

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ABSTRACT

3β-Hydroxysteroid dehydrogenases (3βHSD) are supposed to be involved in cardenolide biosynthesis in plants. *Erysimum crepidifolium* Rchb., a member of the Brassicaceae accumulating cardenolides, is a close relative to *Arabidopsis thaliana*. Full length cDNAs encoding for three individual 3βHSDs (*EcHSD1*, *EcHSD2*, *EcHSD3*) were isolated from *E. crepidifolium* leaves. *EcHSD1* and *EcHSD2* encode proteins assembled from 257 amino acids whereas *EcHSD3* encodes a protein assembled from 260 amino acids. All three proteins qualify as members of the short-chain dehydrogenases/reductases family of proteins (SDRs). *Ec*HSD1 and *Ec*HSD2 shared a high amino acid sequence identity of about 86% and 91% with putative 3βHSDs of *A. thaliana* (AT2G47140 and AT2G47130). *Ec*HSD3 showed high homology to the *A. thaliana* SDRs AT2G47150 (74%) and AT2G47120 (81%). All three *EcHSD* genes were expressed in *Escherichia coli* and the recombinant enzymes were characterized biochemically. All three recombinant *Ec*HSDs catalyzed the dehydrogenation of pregnenolone and the 3-reduction of 5α/β-pregnane-3,20-dione when NAD and NADH were used as cosubstrates, respectively. After exposure to different stress conditions, no increased transcription was seen for *EcHSD1* whereas *EcHSD2* was expressed four times higher under osmotic stress than under control conditions. *EcHSD3* expression was 10 times and 6 times higher after osmotic stress and MeJA treatment, respectively, than in controls.

Introduction

Cardenolides are a group of small plant natural products possessing potent biological activities. They bind to and inhibit Na^+/K^+-ATPase and have been in clinical use for many years for the treatment of heart insufficiency in humans. Besides the well-known positive effects on cardiac activity new therapeutic roles for these compounds in various diseases are discussed. The increased susceptibility of cancer cells to cardenolides in tumor therapy is of special interest (Prassas and Diamandis, 2008). Other targets are viral diseases such as HSV infections (Bertol et al., 2011). Cardenolides are still isolated from plants since their structural complexity impede their chemical synthesis. Improving and manipulating cardenolide production requires a detailed knowledge of their biosynthesis. For a long time cardenolide biosynthesis was only studied in *Digitalis* (Kreis and Müller-Uri, 2010, 2013). However molecular biology tools and protocols, as well as information on gene expression pattern are very limited in this genus. Because of its close relationship to the model plant *Arabidopsis thaliana* we recently proposed that *Erysimum* will turn out to be a more appropriate plant genus than *Digitalis* to study cardenolide biosynthesis (Munkert et al., 2011).

The genus *Erysimum* comprises about 200 species spread all over the world (Bailey et al., 2006; Polatschek, 2013a,b). For example, 19 species were described for Northern America (Rollins, 1993) and five on the Macaronesian Islands (Polatschek, 1976). Preparations from *Erysimum* species have been used in the therapy of heart insufficiency in humans due to the occurrence of cardiac glycosides (Zhu, 1989). More than 50 cardenolides have been isolated from *Erysimum* species so far (Gmelin and Bredenberg, 1966; Nagata et al., 1975, Makarevich et al., 1976). Cardenolide biosynthesis in *Erysimum* has not yet been studied in much detail (Munkert et al., 2011). It is supposed that the biosynthetic pathway follows the putative pathway described for *Digitalis* (Kreis and Müller-Uri, 2010, 2013; Sales Clemente et al., 2011). So far, only few steps

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in cardenolide biosynthesis have been studied on the molecular level. The main focus has been on the early steps of β-cardenolide genin formation, namely the conversion of pregnenolone to isoprogesterone and the reduction of progesterone to 5β-pregnane-3,20-dione (Kreis and Müller-Uri, 2013) (Fig. 1). Only recently, a full length cDNA clone encoding a progesterone 5β-reductase was isolated from *Erysimum crepidifolium* leaves and the recombinant protein over-expressed in *Escherichia coli* (Munkert et al., 2011). This enzyme is able to convert progesterone to 5β-pregnane-3,20-dione *in vitro*. Two putative biosynthetic steps upstream and downstream of progesterone, namely the conversion of pregnenolone to isoprogesterone and the conversion of 5β-pregnane-3,20-dione to 5β-pregnane-3βol,20-one, are supposed to be catalyzed by 3β-hydroxysteroid dehydrogenases (3βHSD).

3β-Hydroxysteroid dehydrogenases (EC 1.1.1.51) catalyze the dehydrogenation of 3β-hydroxysteroids to their respective 3-oxo intermediate and *vice versa*. Several 3βHSDs have been isolated from animals, bacteria and higher plants. In animals the enzyme is essential for the biosynthesis of all classes of steroid hormones (Hoffmann and Maser, 2007), in bacteria 3βHSDs are involved in steroid degradation (Kramm et al., 2012) and in higher plants they are supposed to be involved in the formation of steroids including cardiac glycosides, an important group of plant natural products (Kreis and Müller-Uri, 2010). The molecular biology and phylogeny of the animal and bacterial 3β-hydroxysteroid dehydrogenases has been studied in some detail. Bifunctional 3βHSDs catalyze in addition to 3β-dehydrogenation the isomerisation of Δ^5-3-ketosteroids (Simard et al., 2005) or possess 17β-dehydrogenase activity

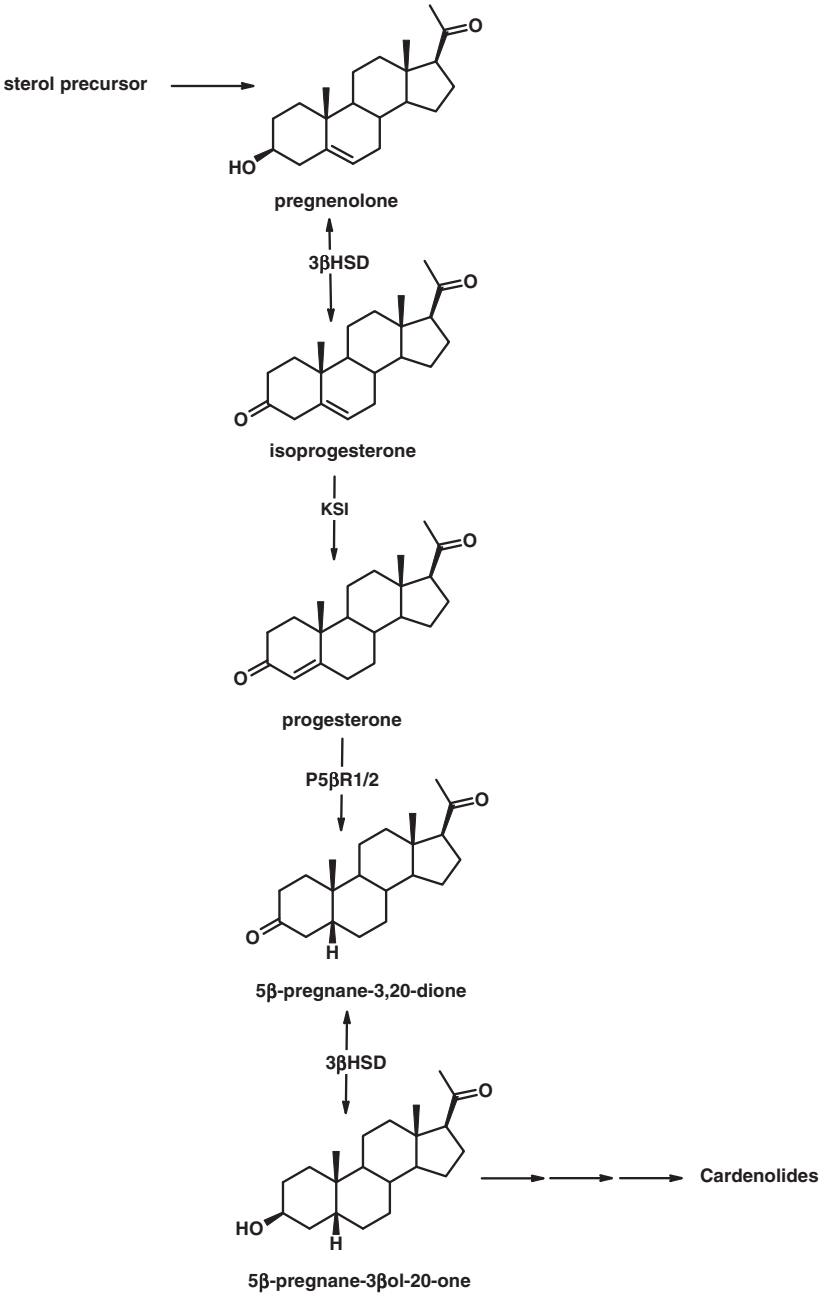

Fig. 1. Partial biosynthetic pathway for cardenolides. 3βHSD, 3β-hydroxysteroid dehydrogenase; KSI, Δ^5-3-ketosteroid isomerase; P5βR1/2, progesterone 5β-reductase 1 or 2.