Phytochemistry 156 (2018) 9–19

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

PRISEs (progesterone 5β-reductase and/or iridoid synthase-like 1,4-enone reductases): Catalytic and substrate promiscuity allows for realization of multiple pathways in plant metabolism

Karin Schmidt[a], Jan Petersen[b], Jennifer Munkert[b], Claudia Egerer-Sieber[a], Michael Hornig[b], Yves A. Muller[a], Wolfgang Kreis[b,*]

[a] Division of Biotechnology, Department of Biology, Friedrich-Alexander-University Erlangen-Nürnberg, D-91054 Erlangen, Germany
[b] Division of Pharmaceutical Biology, Department of Biology, Friedrich-Alexander-University Erlangen-Nürnberg, D-91058 Erlangen, Germany

ARTICLE INFO

Keywords:
Cardenolide biosynthesis
Iridoid biosynthesis
1,4-Enone reductase
Pathway evolution
Site-directed mutagenesis
Short-chain dehydrogenase
X-ray crystallography

ABSTRACT

PRISEs (progesterone 5β-reductase and/or iridoid synthase-like 1,4-enone reductases) are involved in cardenolide and iridoid biosynthesis. We here investigated a PRISE (r*At*St5βR) from *Arabidopsis thaliana*, a plant producing neither cardenolides nor iridoids. The structure of r*At*St5βR was elucidated with X-ray crystallography and compared to the known structures of PRISEs from *Catharanthus roseus* (r*Cr*ISY) and *Digitalis lanata* (r*Dl*P5βR). The three enzymes show a high degree of sequence and structure conservation in the active site. Amino acids previously considered to allow discrimination between progesterone 5β-reductase and iridoid synthase were interchanged among r*At*St5βR, r*Cr*ISY and r*Dl*P5βR applying site-directed mutagenesis. Structural homologous substitutions had different effects, and changes in progesterone 5β-reductase and iridoid synthase activity were not correlated in all cases. Our results help to explain fortuitous emergence of metabolic pathways and product accumulation. The fact that PRISEs are found ubiquitously in spermatophytes insinuates that PRISEs might have a more general function in plant metabolism such as, for example, the detoxification of reactive carbonyl species.

1. Introduction

A key challenge in plant specialized metabolism ('secondary metabolism') is understanding how new pathways have emerged during evolution. Pathways can evolve and existing metabolic grids can be extended using pre-existing enzymes which can recruit intermediates from 'underground metabolism' (D'Ari & Casedesús, 1998; Notebaart et al., 2014) to form new metabolites that may prove to be beneficial for the producer. In this context, enzymes with relaxed substrate and reaction specificities, sometimes called 'promiscuous', can play important roles in pathway evolution (Khersonsky and Tawfik, 2010). According to Hult and Berglund (2007) enzyme promiscuity can be classified into three different types, a) condition promiscuity, b) substrate promiscuity and c) catalytic promiscuity. The latter can be either accidental when the side reaction is catalyzed by the wild-type enzyme, or induced when a new reaction is established after mutations. Induced catalytic promiscuity can be explained by a gene duplication event followed by mutational changes. However, the progenitor enzyme may also acquire a second function accidentally, while maintaining its original activity. PRISEs are an ideal group of enzymes to study this issue with.

Genes encoding PRISEs belong to the VEP1 gene family which originated from α-proteobacteria (Tarrío et al., 2011). VEP1-encoded PRISEs have been detected in many seed plants (*e.g.* Bauer et al., 2010; Munkert et al., 2015a, b; Rudolph et al., 2016). These enzymes have also been termed progesterone 5β-reductases (*e.g.*, Gärtner et al., 1994; Herl et al., 2006a,b), 3-oxo-$\Delta^{4,5}$-steroid 5β-reductase (*e.g.*, Herl et al., 2009), or iridoid synthases (*e.g.*, Geu-Flores et al., 2012). PRISEs can reduce the C=C double-bond of progesterone in 5β-cardenolide (CAR) formation and/or catalyze the reduction and cyclization of 8-oxogeranial to generate an iridoid scaffold, serving as a building block for monoterpene indole alkaloid (MIA) biosynthesis (Fig. 1). So far, no convincing evidence has been provided allowing a structural discrimination of iridoid synthases from progesterone 5β-reductases. Therefore, Petersen et al. (2016) suggested to bracket these enzymes and call them PRISEs (progesterone-5β-reductase and/or iridoid synthase activity displaying 1,4-enone reductases), a term that is also used here. The role of PRISEs in cardenolide and iridoid (especially MIA) formation has been studied in some detail (*e.g.*, Munkert et al., 2015a,b;

* Corresponding author.
E-mail address: wolfgang.kreis@fau.de (W. Kreis).

https://doi.org/10.1016/j.phytochem.2018.08.012
Received 29 March 2018; Received in revised form 3 August 2018; Accepted 17 August 2018

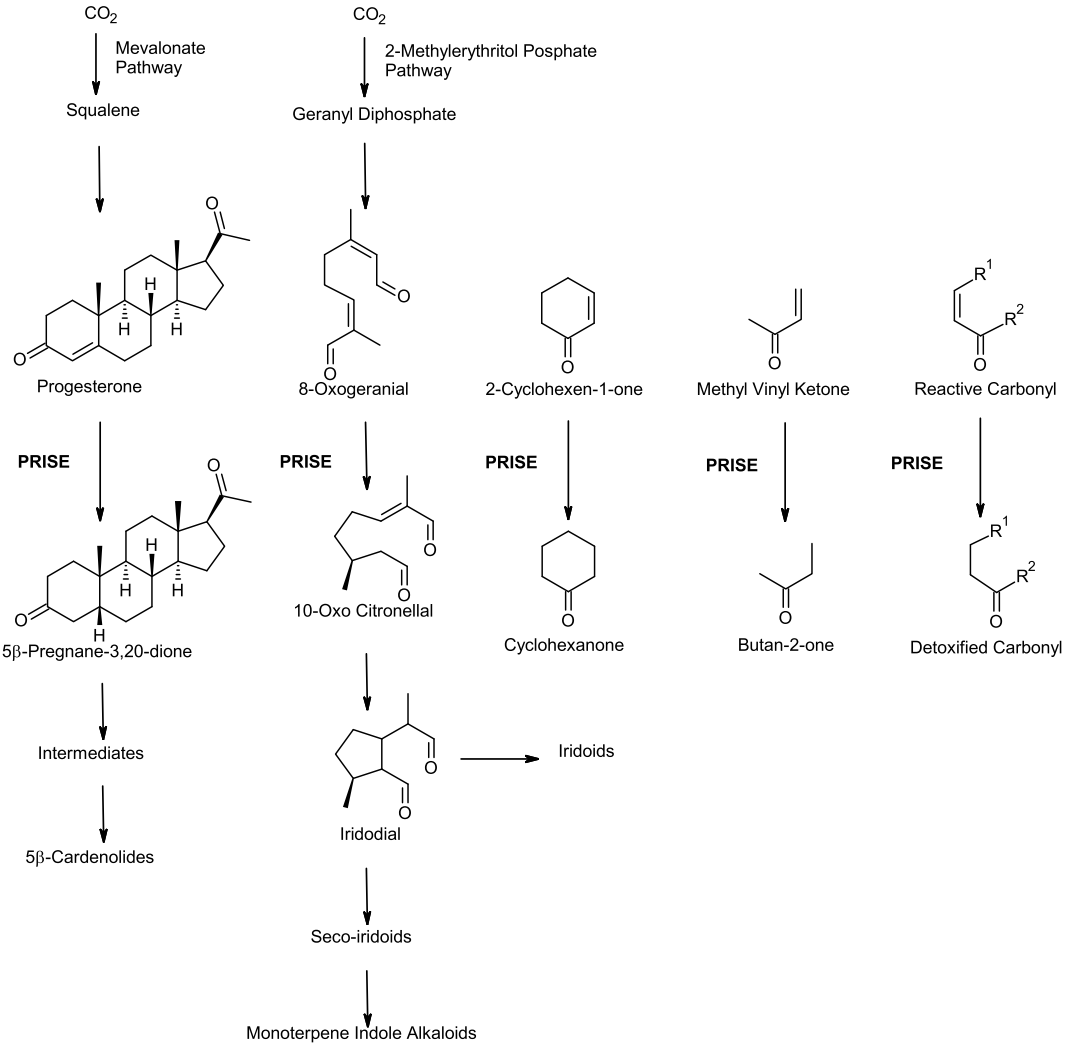

Fig. 1. Substrates of PRISEs and the products formed. A wide range of reactive carbonyl compounds is accepted by the enzymes including intermediates of specialized metabolism, such as progesterone in 5β-cardenolide formation and 8-oxogeranial in iridoid/monoterpene indole alkaloid formation.

Geu-Flores et al., 2012; Kries et al., 2016; Xiang et al., 2017, Kries et al., 2017). In contrast to *Cr*ISY (from *Catharanthus roseus*) *in planta* loss of function studies are still missing for the *Digitalis lanata Dl*P5βRs. *Arabidopsis thaliana* plants in which *At*St5βR is knocked-down show a phenotype with an altered vein patterning (Jun et al., 2002).

VEP1-encoded PRISEs, which display a high sequence identity, belong to the SDR (short-chain dehydrogenase) superfamily (Kallberg et al., 2002) whose genome-wide inventory and diversification patterns in plants have been investigated recently by Moummou et al. (2012). PRISEs of various origin have been demonstrated to convert progesterone and/or 8-oxogeranial independent of the occurrence of 5β-cardenolides or iridoids (Fig. 2) hinting at a more general function of PRISEs in plant metabolism or development. Progesterone and 8-oxogeranial are not the only PRISE substrates, and CAR and MIA not the only natural products in whose formation PRISEs are involved. Several 1,4-enones, such as 2-cyclohexen-1-one, methyl vinyl ketone or citral are also accepted (*e.g.*, Burda et al., 2009; Burda, 2010; Durchschein et al., 2012) (Figs. 1 and 2).

PRISEs can be induced by wounding (Yang et al., 1997) as well as other biotic or abiotic stress situations (Pérez-Bermúdez et al., 2010). It is assumed that reactive electrophile species, such as methyl vinyl ketone, can act as mediators in defence signal transduction (Almeras et al., 2003). Hence, the reported stress-induced protein upregulation may directly be linked to a biochemical reaction that detoxifies reactive electrophile species bearing a 1,4-enone structure (Fig. 1).

We here determined the X-ray structure of a PRISE (r*At*St5βR; locus At4g24220) from *Arabidopsis thaliana* (Brassicaceae) and hence the first structure of a PRISE family member from a plant containing neither cardenolides nor iridoids (IRI-/CAR-). Therefore this enzyme appears to be a good starting point to study additional and yet overlooked physiological roles of PRISEs. The structure of r*At*St5βR is compared to the structure of PRISEs from *Digitalis lanata* (IRI-/CAR+) and *Catharanthus roseus* (IRI+/CAR-) and is used to discuss the structural basis for the observed changes in the activity of the r*At*St5βR, r*Dl*P5βR and r*Cr*ISY mutant enzymes generated here. We propose that accidental rather than induced catalytic promiscuity allowed for the use of PRISEs in both the 5β-cardenolide and the monoterpene indole alkaloid pathway. Our combined observations provide reasons to surmise that the main physiological role of PRISEs could be of a more general nature such as for example the deactivation of stress-related α,β-unsaturated ketones.

2. Results and discussion

2.1. Taxonomic, biochemical and genetic evidence questions the discrimination between progesterone 5β-reductases and iridoid synthases

Our recent database search (November 2017) using NCBI's BLASTP 2.5.0 + algorithms and the *D. lanata* progesterone 5β-reductase (*Dl*P5βR; GenBank AAS93804.1) sequence as the query template yielded 876 hits in 198 species of seed plants when the query cover