Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 68 (2007) 229–236
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Preparative enzymatic solid phase synthesis of *cis*(+)-12-oxo-phytodienoic acid – physical interaction of AOS and AOC is not necessary

Philipp Zerbe, Elmar W. Weiler, Florian Schaller *

Lehrstuhl für Pflanzenphysiologie, Ruhr-Universität Bochum, Universitätsstr. 150, D-44780 Bochum, Germany

Received 28 March 2006; received in revised form 5 October 2006
Available online 20 November 2006

Abstract

The pathway of jasmonic acid (JA) biosynthesis was established in the 1980s by Vick and Zimmerman but, until now, the preparative biosynthesis of the jasmonic acid precursors 12-oxo-phytodienoic acid (OPDA) and 3-oxo-2-[2′-pentenyl]-cyclopentan-1-octanoic acid (OPC-8:0) in their endogenous and biologically relevant *cis*(+)-configuration was only possible in small amounts and had to put up with high costs. This was mainly due to the lack of high amounts of pure and enzymatically active allene oxide cyclase (AOC), which is a key enzyme in the biosynthesis of jasmonates in that it releases, in a coupled reaction with allene oxide synthase (AOS), the first cyclic and biological active metabolite – OPDA. We describe here the expression and purification of AOS and AOC and their subsequent coupling to solid matrices to produce an enantioselective, reusable bioreactor for octadecanoid production. With the method described here it is possible to produce optically pure enantiomers of octadecanoids in high amounts in a cost- and time-efficient manner. Furthermore, it could be demonstrated that a physical interaction of AOS and AOC, hitherto postulated to be required for substrate channeling from AOS to AOC, is not necessary for the *in vitro* cyclization of the unstable epoxide generated by the AOS reaction.

Keywords: Allene oxide cyclase; Allene oxide synthase; Jasmonates; Jasmonic acid; 12-Oxo-phytodienoic acid; Octadecanoid pathway; Oxylipins; Plant hormones

1. Introduction

Since the initial discovery of methyl jasmonate (MeJA) as a secondary metabolite in essential oils of jasmin in 1962 (Demole et al., 1962), jasmonates have become known as a new class of plant hormones. In the early 1980s, their widespread occurrence throughout the plant kingdom (Meyer et al., 1984) and their growth-inhibitory (Dathe et al., 1981) and senescence-promoting activities (Ueda and Kato, 1980) have been established.

A role in plant defense was first shown by Farmer and Ryan who demonstrated the induction of proteinase inhibitors by MeJA and JA as part of the defense response against herbivorous insects (Farmer and Ryan, 1990; Farmer et al., 1991). The function in plant defense reactions was unequivocally confirmed by the analysis of mutants compromised in either the synthesis or the perception of jasmonate signals (for a review, see Schaller et al., 2005). It became increasingly clear, however, that biological activity is not limited to JA but extends to, and even differs between its many metabolites and conjugates as well as its cyclopentenone precursors (Blechert et al., 1995; Kramell et al., 1997; Stintzi et al., 2001; Taki et al., 2005).

The pathway of JA biosynthesis is shown in Fig. 1. Biosynthesis is believed to start with the release of α-linolenic acid (LA) trough the action of (a) lipase(s) possibly triggered by various local and systemic signals (Narváez-Vásquez et al., 1999; Farmer and Ryan, 1992; Mueller et al., 1993; Conconi et al., 1996). The free fatty acid is then

* Corresponding author. Tel.: +49 234 32 25771; fax: +49 234 32 14187.
E-mail address: florian.schaller@rub.de (F. Schaller).

0031-9422/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2006.10.010

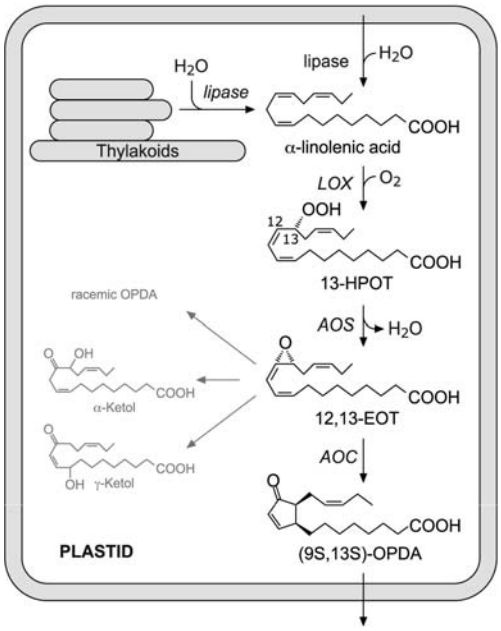

Fig. 1. The first part of the jasmonic acid biosynthetic pathway takes place in the chloroplasts leading to the first intermediate with biological activity, OPDA. LOX: 13-lipoxygenase, AOS: allene oxide synthase, AOC: allene oxide cyclase, 13-HPOT: 13-hydroperoxy-octadecatrienoic acid, 12,13-EOT: 12,13-epoxy-octadecatrienoic acid, OPDA: *cis*(+)-12-oxophytodienoic acid [(9*S*,13*S*)-12-oxo-phytodienoic acid].

oxygenated to 13-hydroperoxy-octadecatrienoic acid (HPOT) in a reaction catalyzed by a 13-lipoygenase (LOX).

AOS catalyzes the dehydration of the hydroperoxides to form an unstable epoxide (12,13-epoxy-octadecatrienoic acid, EOT) which either spontaneously dissociates to a mixture of α- and γ-ketols (80% and 10%, respectively) and racemic *cis*-OPDA (10%) or, in a concerted action with AOC, is converted into enantiomerically pure 9*S*,13*S*-OPDA (i.e. *cis*(+)-OPDA) (Hamberg and Fahlstadius, 1990; Laudert et al., 1997), the first cyclic and biologically active compound of the pathway. Reduction of the 10,11-double bond by a NADPH-dependent OPDA-reductase yields 3-oxo-2-(2′-pentenyl)-cyclopentane-1-octanoic acid (OPC-8:0) which undergoes three cycles of β-oxidation to yield the end product of the pathway, i.e. JA with an acyl-CoA oxidase (ACX1) and a thiolase (KAT2) being involved in the β-oxidation reaction (Cruz Castillo et al., 2004; Li et al., 2005).

The conversion of LA to OPDA is localized in the chloroplasts (Vick and Zimmerman, 1987; Froehlich et al., 2001; Stenzel et al., 2003), while the reduction of OPDA to OPC-8:0 (Schaller et al., 2000; Strassner et al., 2002) and the three steps of β-oxidation, i.e. conversion of OPC-8:0 to JA, occur in peroxisomes (Gerhardt, 1983; Vick and Zimmerman, 1984; Li et al., 2005).

An interesting aspect of the AOC reaction in vitro is the apparent competition between the enzyme-catalyzed formation of optically pure *cis*(+)-OPDA, and the spontaneous decomposition of its substrate, the unstable allene oxide, to form α- and γ-ketols, which can also be found in planta (Theodoulou et al., 2005), and racemic *cis*-OPDA (Fig. 1). The extremely short half-life of allene oxides ($t_{1/2}$ less than 30 s in water; Brash et al., 1988) and the optical purity of natural OPDA suggest a tight coupling of the AOS and AOC reactions possibly in a synthase-cyclase complex in vivo. Functional coupling of the two reactions is also observed in vitro: AOC from potato or recombinant *Arabidopsis* AOC2 in combination with recombinant *Arabidopsis* AOS produced highly asymmetrical *cis*-OPDA consisting almost exclusively of the (9*S*,13*S*)-enantiomer (Laudert et al., 1997).

Although research in recent years generally confirmed the Vick and Zimmerman pathway of JA biosynthesis (the octadecanoid pathway) and brought considerable progress with respect to the biochemistry of the enzymes involved as well as the molecular organization and regulation of the pathway, the preparative biosynthesis of large amounts of optically pure octadecanoid metabolites was possible in only a few laboratories and was expensive, labor-intensive, and time consuming. Here we describe an efficient expression system for AOC2 and the coupling of AOS and AOC to agarose, allowing for the large-scale preparation of optically pure OPDA from unpurified linolenic acid as starting material.

2. Results and discussion

Biological activity is not limited to jasmonic acid but extends to its many conjugates and its biosynthetic precursor, 12-oxo-phytodienoic acid (OPDA). OPDA can either substitute for jasmonic acid (JA) in JA-deficient plants as a signal for the induction of defense responses (Stintzi et al., 2001), or else, may itself be the primary signal as it has been shown for e.g. the mechanotransduction in *Bryonia dioica* (Falkenstein et al., 1991; Weiler et al., 1993). The synthesis of optically pure *cis*(+)-OPDA as the biologically relevant isomer in preparative amounts becomes more and more important since it allows researchers to address long-standing open questions like (i) how OPDA is transported from plastids into the microbodies (ii) if biological activity in different assays is really limited to *cis*(+)-OPDA, and (iii) if OPDA, like JA, can be further derivatized for modulation of its activity (for review, see Schaller et al., 2005).

Aiming to establish a preparative method for "on-column"-synthesis of optically pure *cis*(+)-OPDA, constructs of *Arabidopsis thaliana* allene oxide synthase (AOS) and allene oxide cyclase 2 (AOC2) for heterologous expression in *E.coli* were prepared according to Laudert et al. (1996) and Stenzel et al. (2003) (for details see Section 4). Recombinant AOC2 was purified to homogeneity yielding up to 10 mg/l culture (data not shown). AOS expressed in *E. coli* was solubilized from the bacterial membrane fraction with 0.1% (v/v) Triton X-100, and further purified to homogeneity via Ni-NTA affinity chromatography with a maximum yield of 15 mg/l of culture (data not shown). Enzymatic activity was demonstrated for both purified