Phytochemistry 70 (2009) 1485–1503

Contents lists available at ScienceDirect

Phytochemistry

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

Review

Methods for the analysis of oxylipins in plants

Cornelia Göbel, Ivo Feussner *

Georg-August-University, Albrecht-von-Haller-Institute for Plant Science, Department of Plant Biochemistry, D-37077 Göttingen, Germany

ARTICLE INFO

Article history:
Received 14 March 2009
Received in revised form 14 July 2009
Available online 6 September 2009

Keywords:
Gas chromatography
Lipid peroxidation
Liquid chromatography
Mass spectrometry

ABSTRACT

Plant oxylipins comprise a highly diverse and complex class of molecules that are derived from lipid oxidation. The initial oxidation of unsaturated fatty acids may either occur by enzymatic or chemical reactions. A large variety of oxylipin classes are generated by an array of alternative reactions further converting hydroperoxy fatty acids. The structural diversity of oxylipins is further increased by their occurrence either as free fatty acid derivatives or as esters in complex lipids. Lipid peroxidation is common to all biological systems, appearing in developmentally regulated processes and as a response to environmental changes. The oxylipins formed may perform various biological roles; some of them have signaling functions. In order to elucidate the roles of oxylipins in a given biological context, comprehensive analytical assays are available for determining the oxylipin profiles of plant tissues. This review summarizes indirect methods to estimate the general peroxidation state of a sample and more sophisticated techniques for the identification, structure determination and quantification of oxylipins.

Contents

Abbreviations: ALA, α-linolenic acid; AOC, allene oxide cyclase; AOS, allene oxide synthase; CI, chemical ionization; CID, collisionally induced dissociation; DAD, diode array detection; DES, divinyl ether synthase; DGDG, digalactosyl diacylglycerol; DGMG, digalactosyl monoacylglycerol; dn-oPDA, (7*S*,11*S*)-10-oxo dinor-phytodienoic acid; α-DOX, α-dioxygenase; EAS, epoxy alcohol synthase; EI, electron ionization; ESI, electrospray ionization; GC, gas chromatography; HPL, hydroperoxide lyase; HPLC, high-performance liquid chromatography; JA, jasmonic acid; LA, linoleic acid; LOX, lipoxygenase; MDA, malondialdehyde; MGDG, monogalactosyl diacylglycerol; MGMG, monogalactosyl monoacylglycerol; MS, mass spectrometry; NAE, *N*-acylethanolamine; NMR, nuclear magnetic resonance; $^1O_2$, singlet oxygen; oPDA, (9*S*,13*S*)-12-oxo phytodienoic acid; PG, phosphatidylglycerol; PI, phosphatidylinositol; PUFA, polyunsaturated fatty acid; PXG, peroxygenase; ROS, reactive oxygen species; SPE, spontaneous photon emission; TBA, thiobarbituric acid; TOF, time-of-flight; UPLC, ultra-performance liquid chromatography; VOC, volatile organic compound; VPE, vapor-phase extraction.

* Corresponding author. Tel.: +49 551 39 5743; fax: +49 551 39 5749.
E-mail address: ifeussn@uni-goettingen.de (I. Feussner).

0031-9422/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2009.07.040

1. Introduction

The term oxylipin covers a class of compounds of several hundred distinct oxidized lipophilic molecules or so-called molecular species (Andreou et al., 2009). This review will focus on analytical methods and techniques for the global analysis and quantification of oxylipins. A first section will give an overview about the complex structural diversity of oxylipins, which is due to the existence of a multitude of reactions leading to their generation. For more interested readers this journal issue provides several more articles giving more detailed information about the different biosynthetic pathways and their biological roles.

2. Diversity of oxylipins

Plant oxylipins are derived from the oxidation of the most abundant polyunsaturated fatty acids (PUFAs) in plants (Mosblech et al., 2009). These are linoleic acid (LA, 18:2($n-6$): where x:y(z) is a fatty acid containing x carbons and y double bonds in position z counting from the methyl end), α-linolenic acid (ALA, 18:3($n-3$)) and in addition in plants, in which glycolipid biosynthesis occurs mainly in the plastids, roughanic acid (16:3($n-3$)). Oxylipins formed in plants include hydroperoxy-, hydroxy-, oxo- and epoxy-fatty acids, divinyl ethers, volatile aldehydes and the plant hormone, jasmonic acid (JA) (Grechkin, 1998). The structural diversity of oxylipins is further increased by their esterification in complex glycerolipids (glycolipids, phospholipids, and neutral lipids) and their conjugation to amino acids and other metabolites, such as sulfate, glutathione, ethanolamine, and carbohydrates (Mosblech et al., 2009). Due to their great structural variety, oxylipins may have various biological roles as second messengers and antimicrobial, anti-insecticidial as well as antifungal compounds (Blée, 2002; Howe and Jander, 2008). These products of lipid peroxidation may either originate from chemical oxidation or are synthesized by the action of various enzymes, such as lipoxygenases (LOXs) or CYP74 enzymes (Schneider et al., 2007a; Stumpe and Feussner, 2006).

2.1. Non-enzymatic lipid peroxidation

Non-enzymatic peroxidation of membrane lipids is catalyzed during oxidative stress and formation of reactive oxygen species (ROS), such as hydrogen peroxide and singlet oxygen (Müller, 2004). The toxic effect of ROS may be mainly caused by their conversion to the highly reactive hydroxyl radical. This radical attacks membrane lipids and leads to the oxidation of the most abundant PUFAs of plant membranes, LA and ALA. The resulting racemic mixtures of peroxy fatty acid radicals may start a radical chain reaction, yielding the formation and accumulation of racemic hydroperoxy fatty acids, which are predominately esterified to complex lipids in membranes, but also occur in the cytosol as free acids (Fig. 1, right hand side). In the case of hydroperoxy fatty acids and peroxy radicals with more than two double bonds, this oxidation process can also include intramolecular radical chain reactions leading up to the generation of unstable bicyclic endoperoxy hydroperoxides with a prostaglandin G-ring system (G_1-type phytoprostanes). Spontaneous reduction and rearrangement reactions will finally lead to the formation of A_1-, B_1-, D_1-, E_1-, F_1-, and deoxy-J_1-type phytoprostanes which are structurally analogous to the arachidonate-derived isoprostanes in animals (Imbusch and Müller, 2000a,b). A side-reaction of the phytoprostane pathway may be the breakdown of G_1-type phytoprostanes to produce malondialdehyde (MDA) (Müller, 2004). Other well-characterized products of peroxy radical chemistry are di- and trihydroxy fatty acids, epoxy alcohols, ketodienes, ketotrienes and alkenals (Gardner, 1989).

2.2. Enzymatic oxylipin biosynthesis

Oxylipin biosynthesis is initiated by the enzymatic formation of hydroperoxy fatty acids catalyzed by LOX (EC 1.13.11.12) (Feussner and Wasternack, 2002) or α-dioxygenase (α-DOX) (Hamberg et al., 2005) (Fig. 1, left hand side). LOX catalyzes the regio- and stereo-specific dioxygenation of PUFAs containing a (1*Z*,4*Z*)-pentadiene system as this is the case for the most abundant PUFAs in plants, LA, ALA and roughanic acid (Andreou and Feussner, 2009; Feussner and Wasternack, 2002). Plant LOXs are classified based on their positional specificity in introducing molecular oxygen into LA. The oxygenation can occur either at carbon atom 9 (9-LOX) or at carbon atom 13 (13-LOX) of the hydrocarbon backbone (Schneider et al., 2007a). These different specificities lead to the formation of the corresponding hydroperoxides, the 9-hydroperoxy and the 13-hydroperoxy derivatives of the substrate (Liavonchanka and Feussner, 2006). Plant LOXs have been considered to oxygenate mainly free PUFAs for many years. But more and more studies provide evidence that the enzymes are also capable of oxygenation of PUFAs bound to complex lipids, e.g., phospholipids or triacylglycerols (Brash et al., 1987; Feussner et al., 1997a; Holtman et al., 1997). Genes encoding LOXs are present as multigene families in all plant species analyzed so far, for instance, with six members in *Arabidopsis thaliana* or at least 14 in potato (Feussner and Wasternack, 2002). This diversity of LOX isoforms may be associated with different physiological functions of LOXs that might provide distinct pools of hydroperoxy PUFAs as substrates for alternative pathways of further conversion (Feussner and Wasternack, 2002; Liavonchanka and Feussner, 2006). The α-oxygenation catalyzed by α-DOX leads to the formation of unstable (2*R*)-hydroperoxy derivatives of PUFA, which can be further converted into the corresponding α-hydroxy fatty acids, chain-shortened aldehydes as well as chain-shortened fatty acids (Hamberg et al., 1999).

The metabolism of the LOX-derived hydroperoxy fatty acids can occur by up to six alternative pathways. All of these metabolic routes are named after their first committed enzymatic reaction step. Four of the alternative reactions are catalyzed by an atypical family of cytochrome P450 monooxygenases, the CYP74 enzymes. These are allene oxide synthase (AOS, EC 4.2.1.92), hydroperoxide lyase (HPL), divinyl ether synthase (DES) and epoxy alcohol synthase (EAS) (Blée, 2002; Howe and Schilmiller, 2002; Lee et al., 2008; Matsui, 2006; Stumpe and Feussner, 2006). These reactions have been well-characterized in different plant species.

The occurrence of oxylipins as metabolites of free fatty acids has been intensively reported from various plants. In their free form oxylipins can be associated with soluble compartments, such as the cytosol, the stroma of plastids or the peroxisomal matrix (Browse, 2005; Mosblech et al., 2009). Recent studies indicate that oxylipins can occur in different forms in plant cells because they have also been found to be esterified with complex lipids in the cell. The most prominent group of metabolites containing esterified oxylipins are the so-called Arabidopsides which are derivatives of the plastidial glycolipids, mono- and digalactosyl diacylglycerol (MGDG and DGDG). They contain (9*S*,13*S*)-12-oxo phytodienoic acid (oPDA) and/or (7*S*,11*S*)-10-oxo dinor-phytodienoic acid (dn-oPDA) instead of fatty acid moieties at sn_1 and sn_2 positions (Hisamatsu et al., 2003, 2005; Nakajyo et al., 2006) and some Arabidopsides additionally contain oPDA/dn-oPDA acylated to the galactose group (Andersson et al., 2006; Kourtchenko et al., 2007) (Table 3). Further oxidized glycolipids having one or two oxylipin chains are still being discovered and can contain, for example, ketols at sn_1 and/or sn_2 positions beside oPDA/dn-oPDA (Buseman et al., 2006; Glauser et al., 2008b; Stelmach