Phytochemistry 98 (2014) 101–109

Contents lists available at ScienceDirect

Phytochemistry

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

Gibberellin oxidase activities in *Bradyrhizobium japonicum* bacteroids

Constanza Méndez [a], Cecilia Baginsky [b], Peter Hedden [c], Fan Gong [c], Margarita Carú [d], María Cecilia Rojas [a,*]

[a] Laboratorio de Bioorgánica, Departamento de Química, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile
[b] Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Chile, Casilla 1004, Santiago, Chile
[c] Rothamsted Research, Harpenden, Herts AL5 2JQ, United Kingdom
[d] Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

ARTICLE INFO

Article history:
Received 30 August 2013
Received in revised form 29 October 2013
Available online 27 December 2013

Keywords:
Soybean
Glycine max
Fabaceae
Bradyrhizobium japonicum
Bradyrhizobiaceae
Bacteroids
Gibberellins
Oxidases
Soybean nodules

ABSTRACT

Bradyrhizobium japonicum bacteroids isolated from root nodules of soybean (*Glycine max.*) plants converted the gibberellin (GA) precursor [$^{14}C_1$]GA_{12} into several products identified by combined gas chromatography–mass spectrometry as [$^{14}C_1$]GA_{24}, [$^{14}C_1$]GA_9, [$^{14}C_1$]GA_{15}, GA_9 17-nor-16-one and unidentified products. The oxidation of GA_{12}, catalyzed by the GA 20-oxidase, was present in symbiotic bacteroids from plants around flowering, but not in bacteroids from plants at either an early vegetative stage or at late growth stages. Expression of *cps* and *ks* genes, involved in *ent*-kaurene biosynthesis, was also demonstrated in bacteroids from soybean plants around flowering. Earlier precursors of the GA pathway, *ent*-[$^{14}C_1$]kaurenoic acid or [$^{14}C_4$]GA_{12}-aldehyde, were efficiently utilized by *B. japonicum* bacteroids to give labelled GA_9 plus intermediates partially oxidized at C-20, as well as GA_9 17-nor-16-one and an unidentified product. No 3β or 13-hydroxylated [^{14}C]GAs were detected in any of the incubations. Moreover the C_{19}-GAs [$^{14}C_1$]GA_4 or [$^{14}C_1$]GA_{20} were recovered unconverted upon incubation with the bacteroids which supports the absence of GA 3β-hydroxylase activity in *B. japonicum*. The bacterial 20-oxidase utilized the 13-hydroxylated substrates [$^{14}C_1$]GA_{53}, [$^{14}C_1$]GA_{44} or [$^{14}C_1$]GA_{19}, although with less efficiency than [$^{14}C_1$]GA_{12} to give [$^{14}C_1$]GA_{20} as final product, while the 3β-hydroxylated substrate [$^{14}C_1$]GA_{14} was converted to [$^{14}C_1$]GA_4 to a very small extent. Endogenous GA_9 and GA_{24} were identified by GC–MS in methanolic nodule extracts. These results suggest that *B. japonicum* bacteroids would synthesize GA_9 under the symbiotic conditions present in soybean root nodules.

Introduction

Gibberellins (GAs) are tetracyclic diterpenoid carboxylic acids derived from *ent*-kaurene (**3**) (Fig. 1) widely distributed in higher plants and present in some fungi and soil bacteria (MacMillan, 2002). A few GAs, (GA_1 **17**, GA_4 **13**, GA_3 **16**, GA_7 **15**) function as phytohormones regulating different aspects of plant growth and development, such as seed germination, stem elongation, and flower induction (Hedden and Thomas, 2012; Yamaguchi, 2008). Although their role in fungi and in rhizobacteria is less well understood, it has been proposed that GAs would influence growth and development of their host plants (Bottini et al., 2004; Rademacher, 1992). GAs, mainly GA_3 (**16**), are produced as secondary metabolites in large quantities by the rice pathogen *Fusarium fujikuroi*, interfering with plant defense mechanisms by suppression of jasmonate signaling and thus favouring fungal infection (Hou et al., 2010; Navarro et al., 2008). In root-colonizing soil bacteria including symbiotic *Rhizobium*, phytohormone biosynthesis, particularly of GAs, could be involved in the bacterial plant growth promotion effect, although little information is available about the bacterial GA biosynthesis pathway and its regulation (Bottini et al., 2004; Morrone et al., 2009).

GA biosynthesis has been described in detail at the level of chemical reactions, genes and enzymes in higher plants (Hedden and Phillips, 2000; MacMillan, 1997) and in the rice pathogen *Fusarium fujikuroi* (Hedden et al., 2002; Tudzynski, 2005). Basically the reaction sequence to the main final products GA_3 (**16**) and/or GA_1 (**17**) from geranylgeranyl diphosphate (GGDP **1**) consists of two cyclization reactions followed by multiple oxidative steps (Fig. 1). *ent*-Kaurene (**3**), the first committed intermediate, is synthesized from GGDP (**1**) through *ent*-copalyl diphosphate (CDP **2**) by two distinct cyclases in plants (Sun and Kamiya, 1994; Yamaguchi et al., 1996) or by a bifunctional cyclase in *F. fujikuroi* (Toyomasu et al., 2000). Bifunctional diterpene cyclases have also been found in two other GA-producing fungi, *Phaeosphaeria* sp. (Kawaide et al., 1997) and *Sphaceloma manihoticola* (Bömke et al., 2008). Further oxidation of *ent*-kaurene (**3**) at positions 19, 7 and 6, is catalyzed by P450 monooxygenases in both systems to give GA_{12}-aldehyde (**6**) and GA_{12} (**9**). 3β-Hydroxylation of

* Corresponding author. Tel.: +56 2 978 7317; fax: +56 2 271 3888.
E-mail addresses: cons.mendez@gmail.com (C. Méndez), cbaginsk@uchile.cl (C. Baginsky), peter.hedden@rothamsted.ac.uk (P. Hedden), fan.gong@rothamsted.ac.uk (F. Gong), mcaru@uchile.cl (M. Carú), crojas@uchile.cl (M.C. Rojas).

0031-9422/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.phytochem.2013.11.013

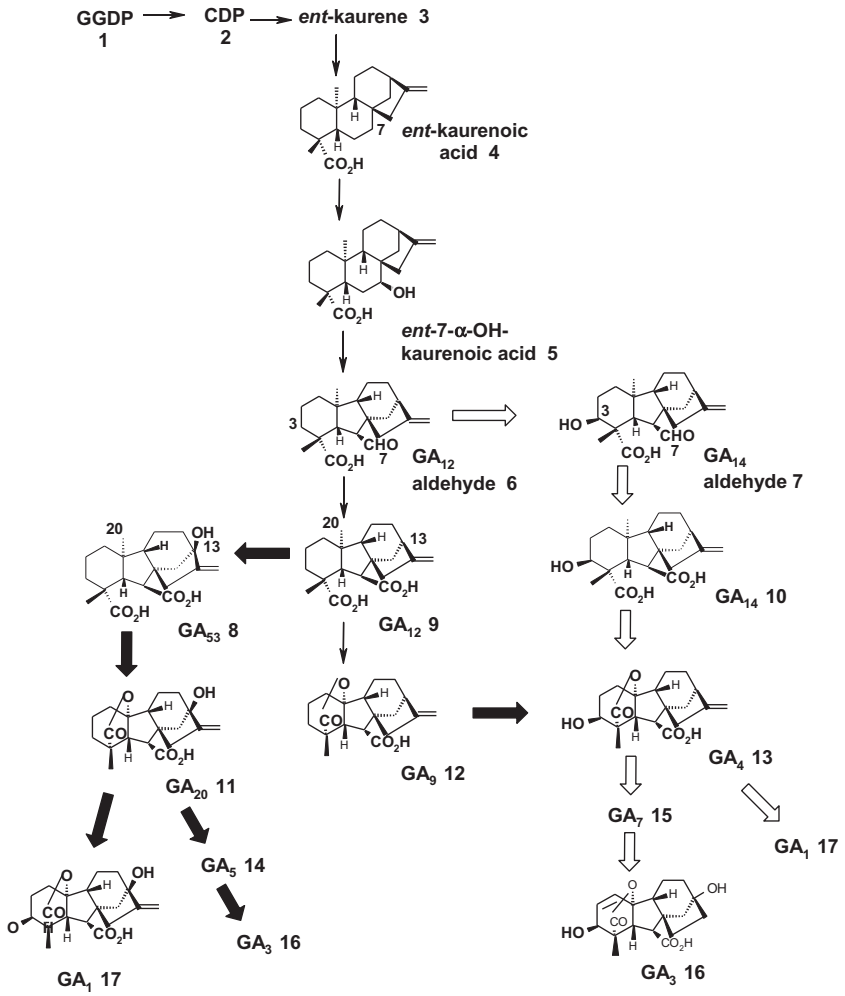

Fig. 1. Main intermediates of the GA biosynthesis pathway in plants (solid arrows) or in the fungus *F. fujkuroi* (empty arrows). Reactions common to both systems are indicated with thin arrows.

GA_{12}-aldehyde (**6**) in *F. fujikuroi* or 13-hydroxylation of GA_{12} (**9**) in most plants give alternative pathways to the 3β, 13-hydroxylated final products, GA_1 (**17**) and/or GA_3 (**16**) (Hedden et al., 2002). Subsequent oxidation of C-20 to CO_2 to give the C_{19}-GAs is catalyzed by 2-oxoglutarate-dependent dioxygenases in plants (Hedden et al., 2002; MacMillan, 1997) or by P450 moooxygenases in fungi (Tudzynski et al., 2002; Bömke et al., 2008). The final reactions of the pathway in *F. fujikuroi* are introduction of a 1,2 double bond followed by 13-hydroxylation (Tudzynski et al., 2003), in contrast to most plants in which 3β-hydroxylation occurs at the end of the pathway (MacMillan, 1997) (Fig. 1). In plants, an additional hydroxylation at C-2 deactivates the GA (Hedden and Thomas, 2012; Yamaguchi, 2008). In reference to the enzymes involved, four of the five oxidases required for GA_3 (**16**) biosynthesis are P450 monooxygenases in *F. fujikuroi* (Hedden et al., 2002). The fifth oxidase that introduces a 1,2-carbon double bond into GA_4 (**13**) to give GA_7 (**15**) has recently been demonstrated to be a 2-oxoglutarate-dependent dioxygenase (Bhattacharya et al., 2012). In plants, besides two P450 monooxygenases that catalyze the steps from *ent*-kaurene (**3**) to GA_{12} (**9**), three 2-oxoglutarate-dependent dioxygenases, 20-oxidase, 3β-hydroxylase and 2β-hydroxylase, participate in the biosynthesis and deactivation of bioactive GAs (Hedden and Thomas, 2012; MacMillan, 1997). The significant differences between GA biosynthesis in plants and fungi demonstrated that both organisms have evolved the GA biosynthetic pathway independently (Bömke and Tudzynski, 2009; Hedden et al., 2002).

In contrast to plants and fungi, scarce information is available about GA biosynthesis in bacterial systems. Very low levels of the C_{19}-GAs GA_1 (**17**) and GA_3 (**16**), about 20–400 pg mL^{-1}, have been found in liquid cultures of some rhizobacteria, including *Rhizobium phaseoli* (Atzhorn et al., 1988), *Azospirillum lipoferum* (Bottini et al., 1989; Piccoli et al., 1999), *Azospirillum brasilense* (Janzen et al., 1992) and *Bacillus sp.* (Gutierrez-Mañero et al., 2001). Also, a few conversions of exogenous deuterium-labelled GAs by *A. lipoferum* have been described (Bottini et al., 2004; Piccoli and Bottini, 1994; Piccoli et al., 1996) indicating that GA_1 (**17**) and GA_3 (**16**) would be synthesized by different pathways. Specifically $[^2H_2]GA_{20}$ (**11**) was partially converted to $[^2H_2]GA_1$ (**17**), while added $[^2H_2]GA_9$ (**12**) was transformed exclusively into $[^2H_2]GA_3$ (**16**). Conversion of $[^2H_2]GA_{20}$ (**11**) to $[^2H_2]GA_1$ (**17**) (3β-hydroxylation) was also demonstrated *in vivo*, in seedlings of rice dwarf mutants that lack 3β-hydroxylase inoculated with *A. lipoferum* (Cassán et al., 2001a, 2001b). These results suggest that GA biosynthesis by *rhizobacteria* would differ from that in fungi and plants, although the molecular organization of the GA pathway is still unclear and many steps have not been directly demonstrated or the intermediates identified.

The only bacterial system in which genes for GA biosynthesis have been described is the soybean symbiont *Bradyrhizobium japonicum* (Morrone et al., 2009; Tully et al., 1998). An operon of eight genes was found in the genome of *B. japonicum* USDA110 that encodes an *ent*-copalyl diphosphate synthase (CPS), an *ent*-