Planta (2000) 210: 979–984

Planta
© Springer-Verlag 2000

12-Oxophytodienoate reductase 3 (OPR3) is the isoenzyme involved in jasmonate biosynthesis

Florian Schaller[1], Christian Biesgen[1], Carsten Müssig[2], Thomas Altmann[2], Elmar W. Weiler[1]

[1]Lehrstuhl für Pflanzenphysiologie, Ruhr-Universität, 44780 Bochum, Germany
[2]Max-Planck-Institut für Molekulare Pflanzenphysiologie, Karl-Liebknecht-Straße 25, 14476 Golm, Germany

Received: 21 October 1999 / Accepted: 10 December 1999

Abstract. In addition to OPR1 and OPR2, two isoenzymes of 12-oxophytodienoate reductase, a third isoform (OPR3) has recently been identified in *Arabidopsis thaliana* (L.) Heynh. The expression of the *OPR3* gene is induced not only by a variety of stimuli, such as touch, wind, wounding, UV-light and application of detergent, but also by brassinosteroids. The three enzymes were expressed in a functional form in *Escherichia coli*, and OPR2 was additionally expressed in insect cell cultures and overexpressed in *A. thaliana*. Substrate conversion was analyzed using a stereospecific assay. The results show that OPR3 effectively converts the natural (9S,13S)-12-oxophytodienoic acid [K_m = 35 μM, V_{max} 53.7 nkat (mg protein)$^{-1}$] to the corresponding 3-2(2′(Z)-pentenyl) cyclopentane-1-octanoic acid (OPC-8:0) stereoisomer while OPR1 and OPR2 convert (9S,13S)-12-oxophytodienoic acid with greatly reduced efficiency compared to OPR3. Thus, OPR3 is the isoenzyme relevant for jasmonate biosynthesis.

Key words: *Arabidopsis* – Insect cell cultures – Jasmonate biosynthesis – 12-Oxophytodienoate reductase (overexpression) – 12-Oxophytodienoate reductase (stereospecificity)

Introduction

The oxylipin 12-oxophytodienoic acid (OPDA) is synthesized in plastids from α-linolenic acid and serves as a signal transducer in plant mechanotransduction (Weiler et al. 1993, Blechert et al. 1999) and as substrate for the biosynthesis of jasmonic acid (JA; Vick and Zimmerman 1984), a signalling compound which influences multiple cellular functions (reviewed by Weiler 1997). Conversion of OPDA to JA involves the reduction of the 10,11-double bond of OPDA by a flavoprotein reductase, 12-oxophytodienoic acid-10,11-reductase (OPR). The enzyme was first purified from cell cultures of *Corydalis sempervirens* (Schaller and Weiler 1997a) and the homologous cDNA was later cloned from *Arabidopsis thaliana* (Schaller and Weiler 1997b). It was then shown that two highly related genes, *OPR1* and *OPR2*, that were differentially expressed, occurred in the genome of *A. thaliana* (Biesgen and Weiler 1999).

At the same time, enzymatic analyses of *C. sempervirens* protein fractions revealed that the OPR activity could be separated into fractions differing in their preferences for OPDA stereoisomers (Fig. 1): (i) the enzyme activity originally described (Schaller and Weiler 1997a), which was shown to convert (9R,13R)-OPDA but not the natural (9S,13S)-OPDA and which is homologous to the OPR1 isozyme of *A. thaliana* (Schaller and Weiler 1997b) encoded by *OPR*1 (Biesgen and Weiler 1999), and (ii) a novel activity which reduced both enantiomers of *cis*-OPDA (Schaller et al. 1998). The enzymatic activity and substrate preference of the isozyme encoded by the *OPR*2 gene of *A. thaliana* have not been reported.

Recently, a third isoenzyme of OPR (OPR3) has been identified as a brassinosteroid-upregulated gene responsive to a variety of stimuli (Müssig et al. 2000). Thus, OPR3 steady-state mRNA levels, like those of OPR1 and OPR2 (Biesgen and Weiler 1999), increase after wounding and UV illumination. Furthermore, elevation of OPR3 mRNA levels occurs after stimulation of plants by wind and touch and after application of detergent. To elucidate the functions of the three isoforms, a range of experiments was carried out to compare the enzymatic properties of OPR1, OPR2 and OPR3, particularly their stereoisomer preference. The results clearly show that OPR3 is the isoenzyme relevant for JA biosynthesis.

Abbreviations: JA = jasmonic acid; NTA = nitrilotriacetate; OPC-8:0 = 3-oxo-2(2′(Z)-pentenyl)-cyclopentane-1-octanoic acid; OPDA = 12-oxophytodienoic acid; OPR = OPDA reductase

Correspondence to: E. W. Weiler;
E-mail: elmar.weiler@ruhr-uni-bochum.de; Fax: 49-234-7094187

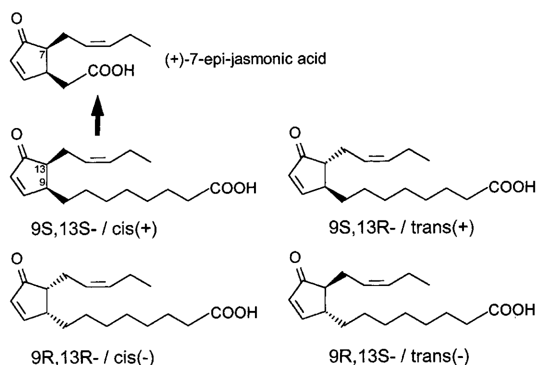

Fig. 1. Structures of the four stereoisomers of 12-oxophytodienoic acid. The naturally occurring form is 9S,13S-OPDA (*cis*(+)-OPDA). Enolization yields (9S,13R)-OPDA from (9S,13S)-OPDA and (9R,13S)-OPDA from (9R,13R)-OPDA. The terminology and enolization are the same for the OPC-8:0 isomers obtained from OPDA after reduction of the 10,11-double bond

Materials and methods

Plant material and growth conditions. Arabidopsis thaliana ecotype Columbia (Col-0) was grown on soil in a greenhouse with an 8-h photoperiod. After 3–4 weeks, at least 3 d before harvesting, plants were transferred to a growth chamber with controlled conditions (16-h photoperiod at 20 °C, 8 h darkness at 18 °C; 70% relative humidity).

Cloning of OPR2 cDNA by reverse transcriptase-polymerase chain reaction (RT-PCR). Total RNA (20 µg) was isolated from *A. thaliana* plants (line A47-19), which had been transformed with the OPR2 gene including introns under the control of the cauliflower mosaic virus (CaMV) 35S-promoter. First-strand cDNA was synthesised with 9 U avian myeloblastis virus (AMV) reverse transcriptase (Promega) 0.3 mM dNTPs, 1 pmol primer OPR2-BACK (5′-AGG TAT AAT GAC TTA GA), 1 µl RNasin (Promega) at 42 °C for 45 min. The RNA was degraded by addition of 1 µg RNase for 10 min at 37 °C. The reaction mixture was extracted with phenol/chloroform and chloroform, the DNA was precipitated with ethanol and dissolved in 20 µl water. Of this solution, 2 µl was used to amplify the *OPR2* cDNA by PCR [with 50 pmol primer, each OPR2-BACK and OPR2-FOR (5′-AAG TGT TTT TGA GAG AA), 0.2 mM dNTPs and 2.5 U *Pwo*-polymerase (Boehringer); 39 cycles: 1 min at 94 °C, 2 min at 35 °C, 2 min at 72 °C]. The reaction product was analysed by agarose gel-electrophoresis, the 1150-bp DNA product was recovered from the gel, cloned into the *Eco*RV site of the pBS-KS vector (Stratagene) and transformed into *Escherichia coli* (XL1-Blue) following standard procedures (Sambrook et al. 1989). The identity of the *OPR2* cDNA was confirmed by sequencing and comparison to the known genomic sequence (Biesgen and Weiler 1999).

Expression of OPR2 in insect cells. The *OPR2* cDNA, subcloned in pBS (see above), was isolated as a 1.2-kbp *Eco*RI fragment and cloned under the control of the polyhedrin promoter into the *Bam*HI/*Nco*I sites of the pBlueBacIII vector (Invitrogen). Transgenic baculoviruses (AcMNPV, *Autographa californica* nuclear polyhedrosis virus) were obtained by cotransfection of virus wildtype DNA and the described pBlueBacIII derivatives. The transfection mixture (100 µl Grace's insect medium, Invitrogen, Groningen, The Netherlands, 100 ng AcMNPV-DNA, 300 ng pBlueBacIII derivatives as well as 2 µl Lipofectin) was pipetted onto a layer of *Spodoptera frugiperda* (Sf9) insect cells and incubated for 4 h at room temperature. In a subsequent incubation at 27.5 °C for 5 d, a primary virus stock was prepared as described by the manufacturer. A pure, transgenic virus stock was recovered by a plaque assay of different dilutions of the primary virus stock essentially as described (Invitrogen). Any contamination with wildtype virus was excluded by PCR (as described by the manufacturer) and by visual analyses for occ^- virus plaques. The resulting virus stock (Bv-OPR2) was stored at 4 °C and used for the production of recombinant OPR2 by the method of Schmidt (1997).

Expression of OPR1 and OPR2 in transgenic Arabidopsis plants. The *OPR1* cDNA and the *OPR2* genomic DNA were cloned into the plant transformation vector pGPTV-BAR (Becker et al. 1992) in order to achieve overexpression of either OPR isoform in transgenic *A. thaliana*. To this end, the CaMV 35S-promoter was subcloned as *Xba*I/*Hin*dIII fragment from pBI121 (Clontech, Heidelberg, Germany) into pGPTV-BAR. The resulting vector was pGPTV-BAR-p35S. The *OPR1* cDNA was recovered as a 1.2-kbp *Sma*I/*Xba*I fragment from the plasmid 179A13T7 (Schaller and Weiler 1997b) and subsequently inserted into the *Sac*I/*Hin*dIII sites of pGPTV-BAR-p35S. A 2.2-kbp *Bcl*I/*Eco*RV fragment, covering the entire *OPR2* genomic sequence, was inserted into the *Xho*I/*Eco*RI sites of pGPTV-BAR-p35S. The resulting plasmid pCBi47 for OPR2 expression was introduced into the *Agrobacterium tumefaciens* strain GV3101 (pMP90; Koncz and Schell 1986) by electroporation. Transformation of *A. thaliana* was achieved by the infiltration method (Bechthold et al. 1993). Seeds of the infiltrated T_0 plants were harvested in bulk, sown on soil and selected by spraying with 0.025% (v/v) BASTA (200 g/l glufosinate-ammonium; Hoechst), equivalent to 50 mg/l phosphinotricine, every third day. The T_1-seeds were harvested individually, sown on soil and selected as described before. Leaves of resistant plants were harvested for analyses.

Protein extraction and immunoblot analyses. Plant material was crushed in a mortar under liquid nitrogen and suspended in 50 mM potassium phosphate buffer (pH 7.5). Cell debris was removed by centrifugation at 10 000*g* and soluble proteins were separated from microsomes at 100 000*g* (25 min, 4 °C). The Sf9 insect cells and bacteria were harvested by centrifugation (5 min, 4000*g*, 4 °C), frozen in liquid nitrogen, resuspended in 300 mM NaCl, 50 mM sodium phosphate buffer (pH 7.5) and crushed by sonication (50 W, 20 s). Cell debris and inclusion bodies were separated from the soluble protein fraction by centrifugation (14 000*g*, 10 min, 4 °C).The SDS-PAGE and immunoblot analyses of crude protein followed standard procedures (Sambrook et al. 1989). A rabbit antiserum raised against native OPR1, expressed in baculovirus-infected insect cells (Biesgen and Weiler 1999), was used as first antibody in immunoblot analyses at 1:10 000 final dilution and detected by the ECL kit (Amersham) following the instructions of the manufacturer.

Bacterial expression of OPR-isoenzymes. The cDNA containing the complete coding sequence for OPR1 was cloned into the protein expression vector pQE-30 (Qiagen) as described by Schaller and Weiler (1997b). The OPR2 cDNA was cloned by a similar strategy using the *Bam*HI site of the vector pQE-31. The isoform OPR3 was also cloned into pQE-30 (Müssig et al. 2000). From this vector, all OPR isoforms were thus expressed as his_6-tagged fusion proteins. This allowed their purification on Ni-nitrilotriacetate (NTA) agarose affinity columns.

To induce protein expression, bacterial cultures (200 ml) that had been inoculated with a fresh overnight culture (1:100) and grown for 1 h at 37 °C were induced by the addition of IPTG (*iso*propyl-1-thio-*β*-D-galactopyranoside; final concentration: 2 mM) and incubated for 5 h at 37 °C. Bacteria were harvested and total soluble protein purified by Ni-NTA-chromatography following general protocols (Qiagen). The purity of the proteins was checked by SDS-PAGE and concentrations were determined against BSA as a standard (Bradford 1976).

Production of substrates for enzymatic assays. Racemic *cis*-OPDA was produced enzymatically with linolenic acid as substrate as described by Laudert et al. (1997). Optically pure (9S,13S)-OPDA (*cis*(+)-OPDA) was isolated from the racemic mixture by HPLC

(column: Chiralcel OJ, Diacel; Mallinckrodt, Griesheim, Germany); HPLC-solvent: *n*-hexane:*iso*propanol:acetic acid 1000:32:5, by vol). *Trans*-12-oxophytodienoic acids were produced by alkaline enolisation of the *cis*-isoforms. Racemic *cis*-3-oxo-2(2′(*Z*)-pentenyl)-cyclopentaneoctanoid acid (*cis*-OPC-8:0) was synthesised by enzymatic reduction of racemic *cis*-OPDA using Old Yellow Enzyme (OYE) from yeast. To this end, OYE was purified by affinity chromatography as described by Abramovitz and Massey (1976). Racemic *cis*-OPDA (100 μg) was incubated with 10 μg purified OYE in the presence of 10 mM NADPH in 0.5 ml of 50 mM potassium phosphate buffer (pH 7.5) for 2 h at 30 °C. The reaction was stopped by acidification, the reaction products were extracted with ethyl acetate and purified by HPLC, (column: Nucleosil 100, Knauer, Bad Homburg, Germany; HPLC-solvent: *n*-hexane:*iso*propanol:acetic acid 100:1.6:0.11, by vol.).

Assay of OPR activity. The activity of OPR was determined as described by Schaller et al. (1998). The amounts of protein and the incubation conditions are specified in the results section. Unless stated otherwise, incubation mixtures contained, in a total volume of 0.5 ml, 1 mM NADPH, 0.1 mM substrate and the appropriate amount of protein in 50 mM potassium phosphate buffer, (pH 7.5), and the reaction was allowed to proceed for 30 min at 25 °C, which is within the linear range. Chiral GC-MS was performed as described in Schaller et al. (1998), standard GC-MS as described in Schaller and Weiler (1997a).

Isolation and blotting of RNA. Total RNA was isolated, size-fractionated, transferred to nylon membranes and hybridized as described by Biesgen and Weiler (1999).

Results and discussion

OPR2 preferentially catalyzes the reduction of (9R,13R)-OPDA. It has recently been shown that purified and recombinant OPR1 is specific for *cis*-OPDA, but prefers the (9R,13R)-enantiomer (Schaller et al. 1998) which is not found in plants (Laudert et al. 1997; Stelmach et al. 1998). The identification of two *OPR* genes in *A. thaliana* (Biesgen and Weiler 1999) and the demonstration of two enzymatic OPR activities in fractionated extracts from *Corydalis sempervirens* (Schaller et al. 1998) suggested that the reductase specific for the natural *cis*-OPDA enantiomer [*cis*(+)-OPDA = (9S,13S)-OPDA] might be encoded by *OPR2*.

Therefore, OPR2 was expressed in two heterologous expression systems using *Spodoptera fugiperda* Sf9 cells or *E. coli* as host cells. In both systems, the enzyme could be expressed in functional form. The OPR2 in the total cellular protein from Sf9-cells as well as his$_6$-tagged OPR2 purified from *E. coli* lysates, like OPR1, preferentially reduced *cis*-OPDA over *trans*-OPDA using NADPH (data not shown) as the reductant (Fig. 2) but strongly preferred the unnatural (9R, 13R)-enantiomer (Fig. 2). Transgenic *A. thaliana* lines overexpressing OPR2 under the control of the 35S-promoter to various extents exhibited an increased ratio of (9R,13R)-OPC-8:0/(9S,13S)-OPC-8:0 as the reaction products formed from racemic-*cis*-OPDA by total soluble leaf protein isolated from the plants (Fig. 3). Comparable results were obtained with plants expressing OPR1 (data not shown). The results rule out the possibility that the substrate specificity of recombinant OPR1 or OPR2 differs from those of the enzymes synthesized *in planta*. It can be concluded that neither OPR1 nor OPR2 represent

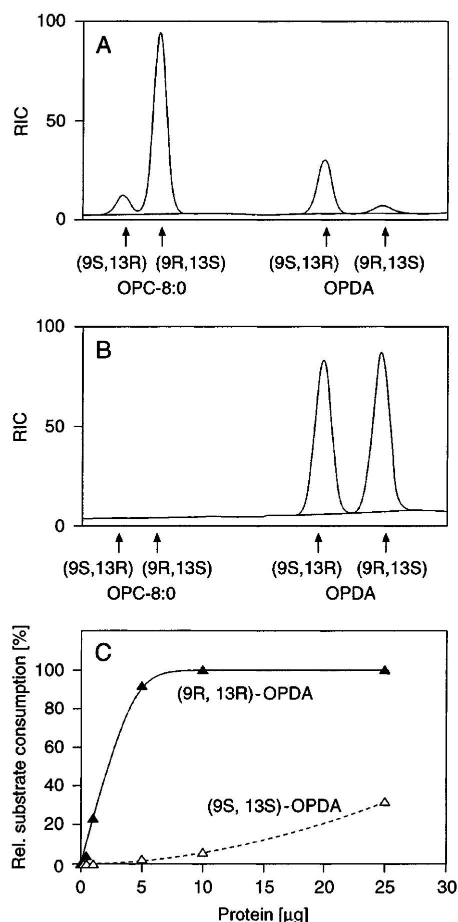

Fig. 2A–C. The OPDA reductase 2 predominantly reduces (9R,13R)-OPDA (*cis*(–)-OPDA, see Fig. 1). **A** Protein extracts from Sf9 cells expressing OPR2 produce a 9:1 mixture of (9R,13R)-OPC-8:0 and (9S,13S)-OPC-8:0 from racemic *cis*-OPDA while control cells (**B**) do not convert this substrate. Shown are reconstructed ion current (*RIC*) traces of GC-MS recordings. Note that, due to better chromatographic separation, reaction mixtures have been enolized prior to GC-MS analysis by alkaline treatment to convert the *cis*-isomers into the *trans*-isomers (see Fig. 1, for nomenclature). The substrate, a 1:1 mixture of (9R,13R)- and (9S,13S)-OPDA is thus converted to (9R,13S)- and (9S,13R)-OPDA, respectively (**B**). In **A** and **B**, (9S,13R)-OPC-8:0 corresponds to (9S,13S)-OPC-8:0 enzymatically produced from (9S,13S)-OPDA and (9R,13S)-OPC-8:0 corresponds to (9R,13R)-OPC-8:0 produced from (9R,13R)-OPDA. **C** The OPR2 expressed in *E. coli* as a his$_6$-fusion protein and purified to homogeneity by Ni-NTA affinity chromatography strongly prefers (9R,13R)-OPDA over (9S,13S)-OPDA. Reactions were carried out under standard conditions with racemic *cis*-OPDA as substrate (0.1 mM) for 30 min at 25 °C

the enzyme responsible for the conversion of (9S,13S)-OPDA to (9S,13S)-OPC-8:0 in the biosynthetic pathway leading to (+)-7-epi-JA. Both OPR1 and OPR2 may rather function in removal of (9R,13R)-OPDA resulting from spontaneous cyclization of 12,13-epoxytrienoic acid, the allene oxide which is the precursor for OPDA. It has been shown that enzymatic cyclization involving allene oxide cyclase exclusively produces (9S,13S)-OPDA (Laudert et al. 1997; Ziegler et al. 1997) whereas spontaneous cyclization in aqueous solution yields racemic *cis*-OPDA (e.g. Laudert et al. 1997).

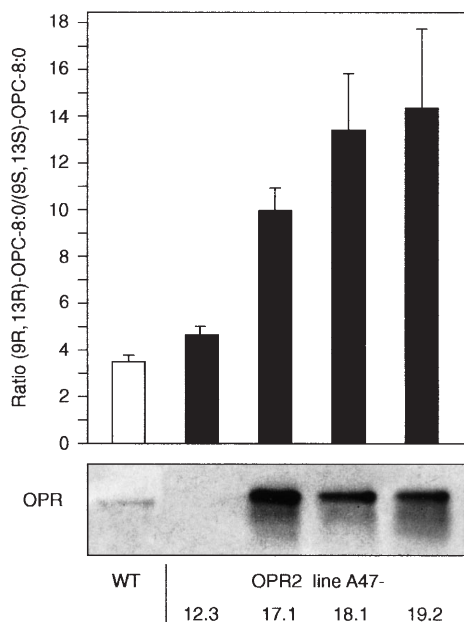

Fig. 3. Activity of OPR in crude extracts from wild-type (*WT*) and four transgenic *Arabidopsis thaliana* lines expressing OPR2 under the control of the 35S-CaMV promoter. The columns give the ratios (±SD, $n = 3$) of the two *cis*-isomers of OPC-8:0 formed from racemic *cis*-OPDA used as a substrate (0.1 mM; the actual analysis has been carried out after enolization to the *trans*-forms, cf. Fig. 2A,B). Shown below the columns are immunoblot analyses of the OPR polypeptides using a polyclonal antibody raised against OPR1 (which recognizes OPR2 to a comparable extent). With increasing overexpression of OPR2, the ratio of OPC-8:0-isomers shifts towards the (9R,13R)-form, indicating that OPR2, *in planta*, also utilizes (9R,13R)-OPDA as the preferred substrate

Curiously, yeast old yellow enzyme (OYE), a close relative of *A. thaliana* OPR1 and OPR2, prefers (9S,13S)-OPDA over (9R,13R)-OPDA (ratio 2:1; Schaller et al. 1998), thus showing that, in the plant, another OPR isoform could be the enzyme involved in the biosynthesis of (+)-7-epi-JA.

OPR3 is the isoform involved in biosynthesis in Arabidopsis thaliana. Originally, OPR3 was isolated as a cDNA representing a brassinosteroid-inducible gene (Müssig et al. 2000). In order to compare the enzymatic properties of OPR1, OPR2 and OPR3, all isoforms were expressed from their cDNAs using the same expression vector, which results in the formation of N-terminally tagged proteins that can be purified to high extents on Ni-NTA chelating agarose.

Comparison of purified OPR1, OPR2 and OPR3 for their ability to reduce (9S,13S)-OPDA, the naturally occurring isomer, showed that OPR3 is highly effective while OPR1 and OPR2 carried out the reaction at a very much lower specific activity [OPR3 17.8 nkat (mg protein)$^{-1}$ OPR2 50 pkat (mg protein)$^{-1}$, OPR1 117 pkat (mg protein)$^{-1}$]. Thus OPR3, rather than its relatives OPR1 and OPR2, is the enzyme that is involved in JA biosynthesis in *A. thaliana* (Fig. 4). The OPR3 isoform also (even slightly better) reduces (9R,13R)-OPDA, and strongly prefers the two OPDA *cis*-isomers over the two

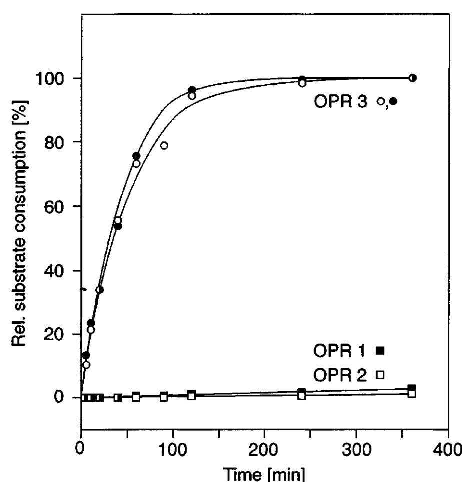

Fig. 4. Relative rates of reduction by OPR1, OPR2 and OPR3 of the natural optical isomer (9S,13S)-OPDA. The three OPR isoenzymes were expressed as his$_6$-tagged fusion proteins in *E. coli*, purified by Ni-NTA-agarose chromatography and the same amount of pure polypeptide (0.4 µg) was used under standard conditions with pure (9S,13S)-OPDA (0.1 mM) as the substrate. Typical curves are shown for OPR1 and OPR2, the *open* and *closed* circles represent two independent, representative experiments run with OPR3

trans-isomers. Again, the (9R,13S)-(*trans*(−)-)isomer is slightly preferred over the (9S,13R)-(*trans*(+)-)isomer (Fig. 5). Only (9S,13S)-OPDA is the reaction product of the allene oxide synthase/cyclase reaction. This isomer enolizes slowly in aqueous conditions to the *trans*-form, (9S,13R)-OPDA. This substrate preference, together with the much higher specific activity for (9S,13S)-OPDA compared to OPR1 and OPR2, clearly places OPR3 in the biosynthetic pathway for JA. The efficiency with which OPR3 reduces the primary product of the allene oxide cyclization reaction may be the reason why (9S,13R)-OPDA is hardly ever found in plants. The

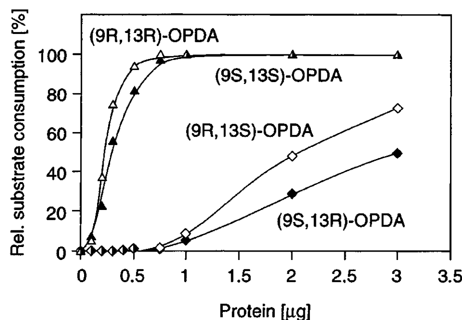

Fig. 5. Isomer preference of OPR3. The OPR3 isoenzyme was expressed in *E. coli* as a his$_6$-fusion protein, purified on Ni-NTA-agarose and used as an enzyme source with either racemic *cis*-OPDA or racemic *trans*-OPDA. The enzyme prefers the *cis*-isomers over the *trans*-forms and, within each group, has a slight preference for the 9R- as compared to the (natural) 9S-forms. The effective reduction of both *cis*-isomers of OPDA has potential physiological significance, as OPR3 would not only utilize OPDA produced by allene oxide cyclase [which is the (9S,13S)-enantiomer], but likewise the (9R,13R)-form which might occur when the 12,13-epoxytrienoic acid formed by allene oxide synthase cyclizes non-enzymatically (leading to racemic *cis*-OPDA)

isoform OPR3 shows a broad pH optimum around pH 7–8 and has a K_m for NADPH of 12 μM [substrate (9S,13S)-OPDA] and the following kinetic properties at saturating NADPH concentrations (1 mM) for the substrate (9S,13S)-OPDA: K_m = 35 μM, V_{max} = 53.7 nkat per mg protein.

It has been shown by Müssig et al. (2000) that OPR3 mRNA levels do rapidly and transiently increase after touch- and wind-stimulation, after wounding and UV-irradiation. These are all conditions that have been associated with octadecanoid signal transduction (Farmer and Ryan 1990; Falkenstein et al. 1991; Conconi et al. 1996; Stelmach et al. 1998). Allene oxide synthase (AOS), the first enzyme specific for the jasmonate pathway, is upregulated by, for example, wounding and particularly by intermediates (OPDA) and by the end-product of the pathway, JA, at the level of mRNA, protein and enzymatic activity (Laudert and Weiler 1998). Although all enzymes of JA biosynthesis are present at low levels in non-induced plants, it is becoming increasingly clear that key steps in the pathway are subject to inductive control which may serve to increase metabolite flow through, and thus output of, the signalling pathway. The ODPA reductase operates at such a decisive point in the pathway. The enzyme is cytosolic and accepts OPDA, produced in the chloroplast, and converts it to OPC-8:0, which is utilized as substrate for β-oxidation to JA in the peroxisomes. Regulation of OPR activity may thus be used by the cell to determine the distribution of OPDA for JA biosynthesis and for OPDA-signalling. The latter compound has been implied in mechanotransduction in *Bryonia dioica* (Weiler et al. 1994; Blechert et al. 1999) and *Phaseolus vulgaris* (Stelmach et al. 1998). However, at present, it is not known if *OPR*3 fulfils such a regulatory role, because it is still unknown whether or not protein and enzyme activity levels do actually change in response to stimuli that alter the steady-state mRNA levels. For OPR1 and OPR2, it has been shown that mRNA levels, but not polypeptide or activity levels, change in response to wounding, heat or cold treatment, or UV-C illumination (Biesgen and Weiler 1999).

The observation that OPR3 mRNA levels are also upregulated by 24-epi-brassinolide (Müssig et al. 2000) suggests a functional relationship between octadecanoid and brassinolide signalling. Brassinosteroids are present at very low concentrations in plant tissues but pollen contains relatively high brassinosteroid levels and JA is required for pollen fertility (Xie et al. 1998); induction of *OPR*3 gene expression by membrane damage after detergent treatment is impaired in the brassinolide-deficient mutant *cbb1* (Müssig et al. 2000). These observations point to connections between the brassinolide and jasmonate signalling systems. More work, however, is required to substantiate this hypothesis.

This work was supported by the Deutsche Forschungsgemeinschaft (Bonn) and by Fonds der Chemischen Industrie (literature provision).

References

Abramovitz AS, Massey V (1976) Purification of intact Old Yellow Enzyme using an affinity matrix for the sole chromatographic step. J Biol Chem 251: 5321–5326

Bechthold N, Ellis J, Pelletier G (1993) In planta *Agrobacterium* mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. C R Acad Sci Paris 316: 1194–1199

Becker D, Kemper E, Schell J, Masterson R (1992) New plant binary vectors with selectable markers located proximal to the left T-DNA border. Plant Mol Biol 20: 1195–1197

Biesgen C, Weiler EW (1999) Structure and regulation of *OPR1* and *OPR2*, two closely related genes encoding 12-oxophytodienoic acid-10,11-reductases from *Arabidopsis thaliana*. Planta 208: 155–165

Blechert S, Bockelmann C, Füßlein M, von Schrader T, Stelmach BA, Niesel U, Weiler EW (1999) Structure-activity relationships reveal two sub-groups of active octadecanoids in elicitation of the tendril coiling response of *Bryonia dioica*. Planta 207: 470–479

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254

Conconi A, Smerdon MJ, Howe GA, Ryan CA (1996) The octadecanoid signalling pathway in plants mediates a response to ultraviolet radiation. Nature 383: 826–829

Falkenstein E, Groth B, Mithöfer A, Weiler EW (1991) Methyljasmonate and α-linolenic acid are potent inducers of tendril coiling. Planta 185: 316–322

Farmer EE, Ryan CA (1990) Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. Proc Natl Acad Sci USA 87: 7713–7716

Koncz C, Schell J (1986) The promoter of TL-DNA gene 5 controls the tissue-specific expression of chimaeric genes carried by novel type of *Agrobacterium* binary vector. Mol Gen Genet 204: 383–396

Laudert D, Hennig P, Stelmach BA, Müller A, Andert L, Weiler EW (1997) Analysis of 12-oxo-phytodienoic acid enantiomers in biological samples by capillary gas chromatography-mass spectrometry using cyclodextrin stationary phases. Anal Biochem 246: 211–217

Laudert D, Weiler EW (1998) Allene oxide synthase: a major control point in *Arabidopsis thaliana* octadecanoid signalling. Plant J 15: 675–684

Müssig C, Biesgen C, Lisso J, Uwer U, Weiler EW, Altmann T (2000) A novel stress-inducible 12-oxophytodienoate reductase from *Arabidopsis thaliana* provides a potential link between brassinosteroid action and jasmonic acid synthesis. J Plant Physiol, in press

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor, New York

Schaller F, Weiler EW (1997a) Enzymes of octadecanoid biosynthesis in plants. 12-Oxophytodienoate-10,11-reductase. Eur J Biochem 245: 294–299

Schaller F, Weiler EW (1997b) Molecular cloning and characterization of 12-oxophytodienoate reductase, an enzyme of the octadecanoid signalling pathway from *Arabidopsis thaliana*. J Biol Chem 44: 28066–28072

Schaller F, Hennig P, Weiler EW (1998) 12-Oxophytodienoate-10,11-reductase: occurrence of two isoenzymes with different specificity against stereoisomers of 12-oxophytodienoic acid. Plant Physiol 118: 1345–1351

Schmidt RC (1997) Molekularbiologische und biochemische Untersuchungen der Nitrilase-Isoformen 1 und 2 aus *Arabidopsis thaliana* (L.) Heynh. Dissertation, Bochum

Stelmach BA, Müller A, Hennig P, Laudert D, Andert L, Weiler EW (1998) Quantitation of the octadecanoid 12-oxo-phytodienoic acid, a signalling compound in plant mechanotransduction. Phytochemistry 47: 539–546

Vick BA, Zimmerman DC (1984) Biosynthesis of jasmonic acid by serveral plant species. Plant Physiol 75: 458–461

Weiler EW (1997) Octadecanoid-mediated signal transduction in higher plants. Naturwissenschaften 84: 340–349

Weiler EW, Albrecht T, Groth B, Xia ZQ, Luxem M, Liss H, Andert L, Spengler P (1993) Evidence for the involvement of jasmonates and their octadecanoid precursors in the tendril coiling response of *Bryonia dioica*. Phytochemistry 32: 591–600

Weiler EW, Kutchan TM, Gorba T, Brodschelm W, Niesel U, Bublitz F (1994) The *Pseudomonas* phytotoxin coronatine mimics octadecanoid signalling molecules of higher plants. FEBS Lett 345: 9–13

Xie DX, Flys BF, James S, Nietro-Rostro M, Turner JG (1998) *Coi1*: an *Arabidopsis* gene required for jasmonate-regulated defense and fertility. Science 280: 1091–1094

Ziegler J, Hamberg M, Miersch O, Parthier B (1997) Purification and characterization of allene oxide cyclase from dry corn seeds. Plant Physiol 114: 565–573