Molecular Cloning and Characterization of 12-Oxophytodienoate Reductase, an Enzyme of the Octadecanoid Signaling Pathway from Arabidopsis thaliana

STRUCTURAL AND FUNCTIONAL RELATIONSHIP TO YEAST OLD YELLOW ENZYME*

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Using partial amino acid sequence information for 12-oxophytodienoate-10,11-reductase obtained from Corydalis sempervirens we have cloned the homologous enzyme from Arabidopsis thaliana. The open reading frame of the cDNA encodes a polypeptide of 372 amino acids (M, = 41,165) with significant similarity to the sequence of Old Yellow Enzyme from Saccharomyces carlsbergensis (Saito, K., Thiele, D. J., Davio, M., Lockridge, O., and Massey, V. (1991) J. Biol. Chem. 266, 20720–20724), a flavin (FMN)-protein catalyzing the NADPH-dependent reduction of the olefinic bond of αβ-unsaturated carbonyls. Specifically, all residues required for binding of FMN in Old Yellow Enzyme are conserved in the A. thaliana sequence, as are all residues associated with catalytic activity. The enzyme was functionally expressed from its cDNA in Escherichia coli and thus proven to encode OPDA reductase. Further similarities of OPDA reductase and yeast Old Yellow Enzyme include their binding to and elution by reductant from N-(4-hydroxybenzoyl)aminohexyl-Sepharose, the immunoreactivity of yeast Old Yellow Enzyme with an antiserum raised against plant OPDA reductase and the demonstration that Old Yellow Enzyme is an active OPDA reductase. It is thus conceivable that the physiological role of Old Yellow Enzymes now known from bacteria, yeasts, and higher plants, is in oxylipin metabolism.

Octadecanoids are plant signaling molecules derived from α-linolenic acid and comprise 18-carbon members as well as metabolites derived from these via β-oxidation, notably the 12-carbon compound jasmonic acid. Within the C18-group, 12-oxophytodienoic acid (OPDA) is the first cyclic metabolite in the biosynthetic pathway. OPDA originates from cyclization of 13-hydroperoxylinolenic acid by allene oxide synthase and allene oxide cyclase, the hydroperoxy precursor being derived from α-linolenic acid in a reaction catalyzed by lipoygenase (1). OPDA is then reduced to 3-oxo-2-(2′-pentenyl)-cyclopentane-1-octanoic acid by OPDA reductase (Fig. 1), and the enzyme in pure form (17) (EC 1.3.1.42) to make it accessible for reverse genetics. Here, we report the molecular cloning of OPDA reductase, an enzyme of the Octadecanoid Signaling Pathway from Arabidopsis thaliana, and its functional expression in a bacterial host. Sequence analysis has revealed a surprising similarity of OPDA reductase to Warburg’s Old Yellow Enzyme (OYE) (18, 19) (EC 1.6.99.1). This prompted a more detailed comparison of the two enzymes. We report that yeast OYE also has OPDA reductase activity. It is thus probable that the, as
**EXPERIMENTAL PROCEDURES**

**Purification and Protein Sequencing of OPDA Reductase**—OPDA reductase was purified in enzymatically active form from cell suspension cultures of *Corydalis sempervirens* as described previously (17). The protein (25 mg) was finally precipitated with methanol at −20 °C and subjected to SDS-PAGE (20) on a preparative 12% gel from which the 41-kDa band was electroeluted using a Biotrap BT1000 (Schleicher & Schuell), precipitated with methanol, and redissolved in 1 ml of double distilled water. Peptides were generated by cyanogen bromide cleavage at Met-X bonds according to Smith (21) and separated by SDS-PAGE according to Schägger and von Jagow (22). They were then electrotransferred to polyvinylidene difluoride membranes (Immobilon). Primer A (5′-GAG GGC TGC ACC CGG-3′) encompassed the translational start ATG and nucleotides 75–88 of the sequence of EST clone 179A13T7 flanked by a restriction site (underlined). Primer B (5′-GAG GCC TGC ACC CGG GGG AAA CAC-3′) encompassed nucleotides 1217–1225 of the EST sequence flanked by a Smal site (underlined). Using these primers, a cDNA fragment of the predicted size (1.18 kilobase pairs) was obtained. The amplified fragment was cloned into the vector pBS SK (+) (Stratagene, La Jolla, CA) and sequenced on both strands to provide independent verification of the nucleotide sequence obtained for the EST clone 179A13T7. Furthermore, the polymerase chain reaction-generated full-length *A. thaliana* cDNA fragment was cloned into the protein expression vector pQE-30 (Qiagen) using the BamHI/Smal restriction sites and transfected into *Escherichia coli* strain XL-1-blue (25) by electroporation (26) for protein expression studies. Nucleotide sequences were aligned using AssemblyLIGN™ (Eastman Kodak Co.), amino acid sequences were aligned using DNAsis (Hitachi) and MacVector (Kodak) software.

**Protein Expression in *E. coli***—Expression of protein was induced by adding isopropyl-1-thio-β-D-galactopyranoside (IPTG) to log-phase bacteria up to 2 mM concentration for 5 h. In parallel, control cultures transfected with pQE-30 containing no insert, were processed. Native, hexahistidine-tagged protein was purified from bacterial lysates on Ni-nitrilotriacetate (Ni-NTA) resin according to the protocol (no. 5) provided by the supplier (Qiagen) and was obtained as a 1 mg/ml solution in 50 mM Na-phosphate, 300 mM NaCl, 10% (v/v) glycerol, 500 mM imidazole, pH 6.0. The eluate was further concentrated using Amicon Centriprep-10 ultrafiltration units and taken up in 0.1 M Tris-HCl, 0.1 M (NH₄)₂SO₄, pH 8.0 (1.2 mg/ml protein). This fraction was used for affinity chromatography.

**Affinity Chromatography on *N*-(4-Hydroxybenzoyl)aminohexyl-Sepharose—**OYE was purified from baker’s yeast (*Saccharomices cerevisiae*, 500 g of packed cells) as described elsewhere (27). The synthesis of *N*-(4-hydroxybenzoyl)aminohexyl (HBA)-Sepharose followed the protocol of Abramovits and Massey (27) and Stott et al. (28). The affinity matrix (10 ml) was equilibrated with 0.1 M Tris-HCl, 0.1 M (NH₄)₂SO₄, 10 μM phenylmethylenesulfon fluoride, pH 8.0, and used at 4 °C. Total soluble yeast protein representing 500 g of cells was applied at a flow rate of 1.5 ml/min. OYE was eluted with the equilibration buffer containing 3 mM sodium dithionite (flow rate, 1.5 ml/min) and concentrated, using an Amicon Centriprep-10 ultrafiltration unit, to a protein concentration of 1.5 mg/ml.

Prepurified protein fractions from *C. sempervirens* cell cultures (17) (2 mg) or Ni-NTA affinity purified recombinant protein from cell lysates of *E. coli* (1–2 mg) (see above) was subjected to affinity chromatography on HBA-Sepharose under the same conditions.

**Assays for OPDA Reductase Activity**—The substrate, cis-OPDA (25 mg) was synthesized enzymatically from 13-hydroperoxylinolenic acid using recombinant *A. thaliana* allene oxide synthase (29). The trans-isomer was produced from the cis-isomer by base-catalyzed enolization (30). Assays contained, in a total volume of 0.5 ml or 1.0 ml of buffer (50 mM potassium phosphate, pH 7.5) 0.1 mM substrate, 1.0 mM NADPH, and protein (10 μg of partially purified, recombinant OPDA reductase or control protein (data in Fig. 5C); 1 μg of affinity-purified OPDA reductase (data in Fig. 5F); 2 μg of partially purified OYE (data in Fig. 7)) and were incubated for 30–60 min at 25 °C (reaction linear under these conditions for 60 min). Because of potential NADPH-oxidase side reactions, all enzyme activities were based on quantitations of product formed versus substrate consumed made by gas chromatography-mass spectrometry as described in Schaller and Weiler (17). Product verification was further done by using 17H[1,18]H-JOPDA as substrate and gas chromatography-mass spectrometry analysis of the reaction products (17).

**Immunological Methods**—Antisera for OPDA reductase were raised in rabbits. Headache antisera were affinity purified using purified recombinant OPDA reductase from *C. sempervirens* (17) and diluted 1:10,000 in TBS-T buffer. Membranes were incubated in this solution for 1 h at room temperature and were then washed three times, 10 min each, in TBS-T buffer, followed by incubation with goat anti-rabbit IgG coupled to alkaline phosphatase (Promega) and enzymatic analysis as described previously (32).

**RESULTS AND DISCUSSION**

**Partial Amino Acid Sequence of OPDA Reductase**—To obtain partial amino acid sequence information, OPDA reductase (25 mg) was purified from cell suspension cultures of *C. sempervirens* and obtained as a soluble, yellow preparation containing predominantly a single, 41-kDa, polypeptide (17). Treatment with CNBr yielded, on SDS-PAGE using 16.5% separating gels, seven fragments ranging from 4 to 12 kDa in apparent molecular masses. N-terminal Edman gas-phase sequencing of the two predominant peptides yielded single sequences in each
Fig. 2. Nucleotide and deduced amino acid sequence of 12-oxophytodienoate-10,11-reductase from A. thaliana. The nucleotide sequence has been obtained independently from EST clone 178A13T7 and from a full-length PCR fragment amplified from cDNA derived from leaf mRNA. The sequencing of both DNAs gave identical results. Aligned to the deduced amino acid sequence are the two partial amino acid sequences obtained from peptide sequencing. Asterisks denote stop codons.

Fig. 3. Amino acid sequence similarity matrix for members of the Old Yellow Enzyme family. OYE 1, S. carlsbergensis (accession no. Q02899) (19); OYE 2 and OYE 3, S. cerevisiae (accession nos. Q05558 and P41816) (28, 34); OYE A, K. lactis (accession no. P40952) (35); EBPI, C. albicans estrogen-binding protein (accession no. L25759) (37); OYE A and OYE B, S. pombe (accession nos. Q09670 and Q9671); Morph-RED, morphine reductase from Pseudomonas putida (accession no. U37350) (36); OPDA-RED, OPDA reductase from A. thaliana, this study (accession no. Y19617). Calculations were performed using the software MacVector.

More detailed analysis of the sequences strengthens the evidence that the protein encoded by EST clone 178A13T7 belongs to the OYE family of FMN-containing reductases (Fig. 4). From the crystal structure of OYE1 from S. cerevisiae, it has been deduced that the following amino acids (numbering as in Fox and Karplus (38)) contribute to the FMN binding site: Pro35, Thr 37, Gly 72, Gln 114, Arg 243, Gly 324, Asn 325, Phe 326, His 375 (numbering as in Fig. 2) and two more are substituted similar amino acids (Gly 72 → Ala 64 and Gly 345 → Ala 324). There is only a single amino acid (Asn 325) conserved between OYES and with the Candida albicans estrogen-binding protein (EBI in Fig. 3), which is an o xo reductase (37).

OYE 1    OYE 2    OYE 3    KYE 1    EBPI    OPDA  RED    Morph-RED    OYE A    OYE B
OYE 1    100       92.0       100       71.6       72.3       67.9       100
OYE 2    92.0       100       80.5       51.5       100
OYE 3    100       100       100       100       100
KYE 1    71.6       72.3       67.9       100       100
EBPI     44.7       46.2       45.0       45.0       100
OPDA-RED 37.5       39.0       39.1       39.1       39.1       32.4       100
Morph-RED 36.7       36.6       38.7       37.8       36.9       44.5       100
OYE A    36.2       37.3       37.8       38.6       31.8       36.3       33.9       100
OYE B    36.1       36.6       36.5       36.5       31.4       35.4       30.9       57.1       100

of which are clearly very similar and preceded by a methionine in the A. thaliana-deduced sequence, in agreement with the CNBr cleavage sites.

A comparison of the complete amino acid sequence deduced from the cDNA with sequence information already available in data bases (Fig. 3) revealed significant similarities with all yeast OYES, namely OYE1 from S. carlsbergensis (19), OYE2 and OYE3 from S. cerevisiae (28, 34) (OYE1 to OYE3 in Fig. 3), Schizosaccharomyces pombe (OYE A and B) and Klyveromyces lactis (35) (KY1) as well as with a recently cloned, bacterial morphine reductase (36), which is more similar to the putative OPDA reductase than to OYES and with the Candida albicans estrogen-binding protein (EBBI in Fig. 3), which is an o xo reductase (37).
is also the preferred electron donor of OPDA reductase (17). Furthermore, the turn between the β4-α4 domains of OYE contains a characteristic structural signature of unknown function conserved among OYE subfamily members C-terminally adjacent the active site (amino acids 207–222 of OYE1 (39)). This stretch is also conserved between OYE and the *A. thaliana* protein (11 amino acids out of 16 are identical). OYE1 is a member of the α,β-ββ-barrel class of proteins (38). Using the software Swiss-Model/RasMol v2.5 (40, 41), the predictions of the most likely three dimensional structure of the *A. thaliana* protein yields an almost perfect fit to the three-dimensional structure of yeast OYE1 and clearly that of an α,β-ββ-barrel protein. Thus, information from the cDNA sequence and from structure predictions (not shown) leaves little doubt that the protein encoded by EST clone 179A13T7 represents a plant homolog of OYE. No indication of putative targeting sequences or transit-peptide sequences can be derived from the deduced amino acid sequence, in agreement with biochemical data showing that OPDA reductase is, in all probability, a cytosolic enzyme (17).

Functional Expression of OPDA Reductase from E. coli—The polymerase chain reaction-generated cDNA encompassing the complete coding sequence of the putative OPDA reductase from *A. thaliana* was inserted, as a BamHI/SmaI restriction fragment, into the multiple cloning site of the protein expression vector FIG. 4.

**FIG. 4. Comparison of amino acid sequences of members of the Old Yellow Enzyme family.** All abbreviations of enzymes as in Fig. 3. **CR-OYE,** deduced amino acid sequence of a short partial cDNA of unknown function isolated from *Chenopodium rubrum* (46). Aligned using MacDNAs. Consensus sequence: boldface capitals, residues conserved in all sequences; capital letters, conserved in all but one sequences; small letters, conserved in all but two sequences; *, amino acid difference specific to OPDA reductase; conserved substitutions: n, nonpolar; p, polar; b, basic; a, acidic.
from the HBA-Sepharose affinity column. For enzyme assays, fraction A (designated as "E") and fraction E (designated as "E") in part 28070 with the empty vector pQE-30 was always processed in parallel. Ni-NTA-agarose. For controls, protein from cells transfected with pQE-30-AT1 was affinity-purified on the polypeptide patterns from cells transfected with either pQE-30-AT1 or pQE-30 alone, even after Ni-NTA-agarose affinity chromatography (Fig. 5A, lanes e versus E), since the expression level was too low to allow for effective competition of the tagged fusion protein with endogenous bacterial proteins for the metal binding sites on the column. The relevant area in the electropherograms was masked by a prominent ~43-kDa polypeptide that co-eluted on Ni-NTA-agarose. The successful expression of the desired 41-kDa protein, and its binding to the affinity matrix, was, however, unequivocally demonstrated by immunoblotting using an antiserum against the C. sempervirens enzyme (17) (Fig. 5B). This protein was absent in control bacteria. Although some reduction of the substrate, OPDA, was observed in crude soluble protein fractions from control (pQE-30 transfected) bacteria, the determination of OPDA reductase activity in the fraction eluting from Ni-NTA-agarose revealed a clear increase in OPDA reductase activity in the immunoreactive protein fraction obtained from pQE-30-AT1-transfected cells (Fig. 5C; protein as in Fig. 5B, lane E versus lane e). For structural identification of the reaction product, see below (Fig. 7).

A characteristic property of yeast OYE is its specific retention on HBA-Sepharose and elution with the reductant, sodium dithionite (27). It was found that the recombinant OPDA reductase (affinity-purified on Ni-NTA-agarose) was also retained by this column and eluted by sodium dithionite (Fig. 5, D and E). The experiment revealed some heterogeneity of the recombinant enzyme, a fast eluting fraction, which was an active OPDA reductase, a slower eluting, likewise enzymatically active fraction, and a strongly retained, immunoreactive polypeptide migrating slightly slower (43 kDa) in SDS-PAGE and being devoid of enzymatic activity. Clearly, OPDA reductase activity was associated with the recombinant 41-kDa polypeptide. The 43-kDa band probably represents reductase inactivated by some post-translational modification by the bacterial host cell, while the fast eluting, active reductase (E1 fraction, Fig. 5, D-F) is front-end eluted upon contact with reductant under our fast flow conditions.

Next, it was determined whether the enzyme purified from C. sempervirens cell suspension cultures would also be retained on HBA-Sepharose. This was the case (Fig. 6, lanes 3–7); electrophoretically pure polypeptide was obtained by sodium dithionite elution (Fig. 6, lane 5). As a control, reference (27) yeast OYE was also purified (Fig. 6, lane 1) for further characterization. The yeast fraction was checked for its immunoreactivity with the OPDA reductase antiserum from the higher plant (Fig. 6, lane 2); there was a clear and specific decoration

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**Fig. 5.** A, Coomassie-stained polypeptides separated by SDS-PAGE (12% gel) and representing lanes 1 and L, whole cell lysates; w and W, column effluents; and e and E, eluates from Ni-NTA affinity columns of bacterial protein obtained from E. coli XL1 blue cells transfected with pQE-30-AT1 (harboring the OPDA reductase cDNA; L, W, E) or transfected with pQE-30 (control plasmid without insert; I, w, e). B, immunoblot of the same fractions developed with antisera raised against OPDA reductase from C. sempervirens (17). C, OPDA reductase activity in affinity (Ni-NTA)-purified fractions containing recombinant OPDA reductase (E, corresponding to protein shown in lanes E in part A and B of this figure) or control protein (e, corresponding to protein shown in lanes e in part A and B of this figure). In both cases, 10 µg of protein were used under otherwise standard conditions. D, elution from HBA-Sepharose of protein from the Ni-NTA-purified fraction containing recombinant OPDA reductase (designated E in all other parts of this figure). The arrow denotes the start of the elution process. Fractions collected separately are numbered E-1 to E-6. E, immunoblot analysis and F, relative OPDA reductase activity in fractions E-1 to E-6 collected from the HBA-Sepharose affinity column. For enzyme assays, 1 µg of protein from each fraction was used.

pQE-30. The construct (pQE-30-AT1) was electroporated into the E. coli host XL1-blue. IPTG-induced expression from this vector results in the formation of an N-terminally hexa-histidine (His6)-tagged protein. Although different concentrations of IPTG (0.1–2 mM), temperatures ranging from 28 to 37 °C, and induction periods between 1 and 16 h were tested, under all conditions, most of the expressed protein was associated with inclusion bodies, and only a minor portion remained soluble (not shown). Thus, total soluble protein from lysates of cells transformed with pQE-30-AT1 was affinity-purified on Ni-NTA-agarose. For controls, protein from cells transfected with the empty vector pQE-30 was always processed in parallel. As can be seen from Fig. 5A, there was little difference in the polypeptide patterns from cells transfected with either pQE-30-AT1 or pQE-30 alone, even after Ni-NTA-agarose affinity chromatography (Fig. 5A, lanes e versus E), since the expression level was too low to allow for effective competition of the tagged fusion protein with endogenous bacterial proteins for the metal binding sites on the column. The relevant area in the electropherograms was masked by a prominent ~43-kDa polypeptide that co-eluted on Ni-NTA-agarose. The successful expression of the desired 41-kDa protein, and its binding to the affinity matrix, was, however, unequivocally demonstrated by immunoblotting using an antiserum against the C. sempervirens enzyme (17) (Fig. 5B). This protein was absent in control bacteria. Although some reduction of the substrate, OPDA, was observed in crude soluble protein fractions from control (pQE-30 transfected) bacteria, the determination of OPDA reductase activity in the fraction eluting from Ni-NTA-agarose revealed a clear increase in OPDA reductase activity in the immunoreactive protein fraction obtained from pQE-30-AT1-transfected cells (Fig. 5C; protein as in Fig. 5B, lane E versus lane e). For structural identification of the reaction product, see below (Fig. 7).

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**Fig. 6.** Purification of Old Yellow Enzyme from S. cerevisiae and of OPDA reductase from C. sempervirens on an affinity column of HBA-Sepharose and immunological similarity of the two enzymes. Lane 1, affinity-purified OYE preparation obtained from S. cerevisiae according to the reference procedure (27); lane 2, immunoblot of the protein in lane 1; lane 3, OPDA reductase partially purified from C. sempervirens according to the reference procedure (17); lane 4: HBA Sepharose column effluent of C. sempervirens protein representing the stage of purity shown in lane 3, lane 5, eluate from the HBA Sepharose column representing C. sempervirens protein bound to the affinity matrix; lane 6, immunoblot corresponding to lane 4; lane 7, immunoblot corresponding to lane 5. Protein (lanes 1 and 3–5) stained with Coomassie; for immunoblots (lanes 2, 6, and 7), an antiserum raised against purified OPDA reductase from C. sempervirens (17) was used.
of the 43-kDa polypeptide representing yeast OYE. Altogether, these results strengthen the conclusions drawn from the sequence information and prove that yeast OYE and higher plant OPDA reductase are similar in their size, overall biochemical properties, structure, and immunoreactivity and thus must be quite closely related. Similarities in enzymatic reactivity of OPDA reductase and OYE were suggested earlier by the observation that OPDA reductase would convert 2-cyclohexenone, a standard OYE substrate (17).

**Yeast Old Yellow Enzyme Has OPDA Reductase Activity**—Although being the first discovered flavoprotein (18), the biochemical function of Warburg’s Old Yellow Enzyme is still uncertain (42). The enzyme catalyzes the oxidation of a range of cyclic α,β-unsaturated carbonyl compounds to phenols with concurrent reduction of the olefinic bond of a second substrate molecule, while also catalyzing the NADPH-dependent reduction of the olefinic bond of a second substrate molecule, catalyzing the oxidation of a range of cyclic α,β-unsaturated carbonyl compounds, such as in morphine (36), 4,4-dimethyl-2-cyclohexenone (42), or 2-cyclohexenone (34, 42), OPDA reductase is the only member within this family whose biological function is currently known. The proof that yeast OYE also has OPDA reductase activity suggests a physiological function of OYE in oxypin metabolism.

**Conclusions**—OPDA reductase catalyzes a decisive step in biosynthesis of plant octadeconoids, signal transducers in mechanotransduction, pathogen, and herbivore defense. Molecular cloning has revealed that the enzyme is the first plant member of the OYE family of reductases that now comprises members in bacteria, fungi, and higher plants. While all OYE catalyze the NAPDH-dependent reduction of olefinic bonds of α,β-unsaturated carbonyls, such as in morphine (36), 4,4-dimethyl-2-cyclohexenone (42), or 2-cyclohexenone (34, 42), OPDA reductase is the only member within this family whose biological function is currently known. The proof that yeast OYE also has OPDA reductase activity suggests a physiological function of OYE in oxypin metabolism.

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**REFERENCES**

1. Vick, B. A., and Zimmerman, D. C. (1984) *Plant Physiol.* 75, 458–461
2. Sembdner, G., and Parthier, B. (1993) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 44, 569–589
3. Turner, E. E., and Ryan, C. A. (1990) *Proc. Natl. Acad. Sci. U. S. A.* 87, 7713–7716
4. Howe, G. A., Lightner, J., Browse, J., and Ryan, C. A. (1996) *Plant Cell* 8, 2007–2017
5. Gundlach, H., Müller, M. J., Kutschat, T., and Zenk, M. H. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 2389–2393
6. Falkenstein, E., Groth, B., Mithöfer, A., and Weiler, E. W. (1991) *Plant Biol.* 185, 136–322
7. Weiler, E. W., Kutschat, T. M., Gorba, T., Brodschelm, W., Niesel, U., and Bublitz, F. (1994) *FEBS Lett.* 345, 9–13
8. Ueda, J., and Kato, J. (1980) *Plant Physiol.* 66, 246–249
9. Boland, W., Hupke, J., Donath, J., Nuske, J., and Bublitz, F. (1995) *Angew. Chem.* 107, 1715–1717
10. Bruin, J., Dicke, M., and Sabelis, M. W. (1992) *Expierientia* 48, 525–529
11. Weiler, E. W., Albrecht, T., Groth, E., Xia, Z.-Q., Luxem, M., Lies, H., Andert, L., and Spengler, P. (1995) *Phytochemistry* 32, 591–600
12. Krumm, T., Bandemer, K., and Boland, W. (1995) *FEBS Lett.* 377, 523–529
13. Blee, E., and Joyard, J. (1996) *Plant Biol.* 110, 445–454
14. Lauß, D., Pfannschmidt, U., Leitsspech, F., Holländer-Czytko, H., and Weiler, E. W. (1996) *Plant Mol. Biol.* 31, 323–335
15. Bell, E., Cremeen, R. A., and Mullet, J. E. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 8675–8679
16. Song, W.-C., Funk, C. D., and Brash, A. R. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 90, 8519–8523
17. Schaller, F., and Weiler, E. W. (1997) *Eur. J. Biochem.* 245, 294–299
18. Warburg, O., and Christian, W. (1933) *Biochim. Biophys. Acta* 6097–6106
19. Sembdner, G., and Parthier, B. (1993) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 44, 1715–1717
20. Schaller, F., and Weiler, E. W. (1997) *Eur. J. Biochem.* 245, 294–299
21. Warburg, O., and Christian, W. (1933) *Biochem. Z.* 266, 377–411
22. Saito, K., Thiele, D. J., Davis, M., Lockridge, O., and Massey, V. (1991) *J. Biol. Chem.* 266, 20720–20724
23. Lauß, D., Hennig, P., Stelmach, B. A., Müller, A., Andert, L., and Weiler, E. W. (1996) *Plant Mol. Biol.* 31, 323–335
24. Bell, E., Cremeen, R. A., and Mullet, J. E. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 8675–8679
25. Song, W.-C., Funk, C. D., and Brash, A. R. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 90, 8519–8523
26. Sembdner, G., and Parthier, B. (1993) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 44, 1715–1717
27. Schaller, F., and Weiler, E. W. (1997) *Eur. J. Biochem.* 245, 294–299
28. Warburg, O., and Christian, W. (1933) *Biochem. Z.* 266, 377–411
29. Saito, K., Thiele, D. J., Davis, M., Lockridge, O., and Massey, V. (1991) *J. Biol. Chem.* 266, 20720–20724
30. Lauß, D., Hennig, P., Stelmach, B. A., Müller, A., Andert, L., and Weiler, E. W. (1996) *Plant Mol. Biol.* 31, 323–335
31. Bell, E., Cremeen, R. A., and Mullet, J. E. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 8675–8679
32. Song, W.-C., Funk, C. D., and Brash, A. R. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 90, 8519–8523
33. Kozak, M. (1987) *Nucleic Acids Res.* 15, 8125–8130
34. Niino, Y. S., Chakraborty, S., Brown, B. J., and Massey, V. (1995) *J. Biol. Chem.* 270, 6719–6726
35. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U. S. A.* 74, 5463–5467
36. French, C. E., and Bruce, N. (1995) *Biochem. J.* 312, 671–678
37. Madani, N. D., Malloy, P. J., Rodriguez-Pombo, P., Krishnan, A. V., and Feldman, D. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 922–926
38. Fox, K. M., and Karplus, P. A. (1994) *Structure* 2, 1089–1105
39. Karplus, P. A., Fox, K. M., and Massey, V. (1995) *FASEB J.* 9, 1518–1526

**FIG. 7.** Analysis of reaction products obtained from incubations of purified yeast OYE and of recombinant *A. thaliana* OPDA reductase with pentadeuterated cis- (±)-OPDA. Reaction products were extracted from the enzyme assay mixtures after 60 min of incubation at 30 °C, converted into the trans-isomers and methyl esters and then analyzed by capillary GC-MS (17). Shown are A, total ion current (TIC) trace of the reaction products obtained with purified OYE (2 μg of protein, otherwise standard conditions) and B, TIC trace of the reaction products obtained with purified recombinant *A. thaliana* OPDA reductase expressed in *E. coli* (10 μg of protein, otherwise identical conditions). The trans-OPDA methyl ester eluted at t = 831 s, trans-OPC-8:0 methyl ester eluted at t = 800 s. C, electron impact (70 eV) mass spectrum of the reaction product (t = 800 s) obtained with OYE; D, electron impact (70 eV) mass spectrum of the reaction product (t = 800 s) obtained with OPDA reductase and fragmentation pattern of pentadeuterated OPC-8:0 methyl ester.
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40. Peitsch, M. C. (1996) Biochem. Soc. Trans. 24, 274–279
41. Sayle, R. A., and Milner-White, E. J. (1995) Trends Biochem. Sci. 20, 374–376
42. Vaz, A. D. N., Chakraborty, S., and Massey, V. (1995) Biochemistry 34, 4246–4256
43. Coetzee, D. J., Kock, J. L. F., Botha, A., Van Dyk, M. S., Smit, E. J., Botes, P. J., and Augustyn, O. P. H. (1992) Syst. Appl. Microbiol. 15, 311–318
44. Kock, J. L. F., Coetzee, D. J., Van Dyk, M. S., Cloete, F. C., Van Wyk, V., and Augustyn, O. P. H. (1991) S. Afr. J. Sci. 87, 73–76
45. Botha, A., Kock, J. L. F., Van Vuuren, D. J., and Van Dyk, M. S. (1994) S. Afr. J. Sci. 90, 694–696
46. Peters, W., Van der Knaap, E., and Kende, H. (1996) J. Plant Physiol. 149, 233–236