Available online at www.sciencedirect.com
SCIENCE DIRECT
Gene 362 (2005) 57–69
GENE
SECTION
EVOLUTIONARY GENOMICS
www.elsevier.com/locate/gene

Molecular cloning and tissue-specific transcriptional regulation of the first peroxidase family member, Udp1, in stinging nettle (*Urtica dioica*)

Triantafyllia G. Douroupi, Issidora S. Papassideri *, Dimitrios J. Stravopodis, Lukas H. Margaritis

Department of Cell Biology and Biophysics, Faculty of Biology, University of Athens, Panepistimiopolis, Zografou, 15784, Athens, Greece

Received 3 March 2005; received in revised form 2 June 2005; accepted 16 June 2005
Available online 10 October 2005
Received by G. Theissen

Abstract

A full-length cDNA clone, designated Udp1, was isolated from *Urtica dioica* (stinging nettle), using a polymerase chain reaction based strategy. The putative Udp1 protein is characterized by a cleavable N-terminal signal sequence, likely responsible for the rough endoplasmic reticulum entry and a 310 amino acids mature protein, containing all the important residues, which are evolutionary conserved among different members of the plant peroxidase family. A unique structural feature of the Udp1 peroxidase is defined into the short carboxyl-terminal extension, which could be associated with the vacuolar targeting process. Udp1 peroxidase is differentially regulated at the transcriptional level and is specifically expressed in the roots. Interestingly, wounding and ultraviolet radiation stress cause an ectopic induction of the Udp1 gene expression in the aerial parts of the plant. A genomic DNA fragment encoding the Udp1 peroxidase was also cloned and fully sequenced, revealing a structural organization of three exons and two introns. The phylogenetic relationships of the Udp1 protein to the *Arabidopsis thaliana* peroxidase family members were also examined and, in combination with the homology modelling approach, dictated the presence of distinct structural elements, which could be specifically involved in the determination of substrate recognition and subcellular localization of the Udp1 peroxidase.

Keywords: Cloning; Nettle peroxidase; PCR; Root expression; Transcriptional induction

1. Introduction

Peroxidases are capable of utilizing hydrogen peroxide (H_2O_2) to oxidize a wide variety of hydrogen donors, such as phenolic substances, nitrite, leuco-dyes, ascorbic acid, indole, amines and certain inorganic ions and only minor differences in substrate specificity are observed among isoenzymes for peroxidation. Like catalases, peroxidases are heme-containing proteins. They consist of an apoenzyme, which contains both carbohydrate and protein, bound to an iron porphyrin. The enzymatic reaction mechanism for peroxidation has been described in detail and shown to involve three consecutive redox stages of the enzyme, resulting in the consumption of one equivalent of H_2O_2 and the dehydrogenation (oxidation) of two equivalents of reducing substrate.

Peroxidases are widely distributed in higher plants. The number and relative concentration of isoenzymes usually vary among different tissues and developmental stages of a plant organ. Peroxidase activity can be detected in the whole lifespan of various plants: from germination to senescence. In plants, peroxidases are mainly involved in germination, cell wall formation, lignification, suberization, polymer cross-linking, auxin metabolism, cell elongation, stress and pathogen defence reactions, ethylene biosynthesis, plant growth regulation, phenolics and H_2O_2 catabolism (Scialabba et al., 2002; Roberts and Kolattukudy, 1989; Moerschbacher, 1992; Allison and Schultz, 2004). Peroxidases can create a physical barrier by catalysing cross-linking of cell wall compounds in response to different stimuli such as wounding, pathogen interactions or as a normal cell wall evolution during the growth and senescence

Abbreviations: ER, endoplasmic reticulum; N-terminal, amino-terminal; PE, positioning element; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; RT, reverse transcription; SDS, sodium dodecyl sulphate; SSC, 3 M NaCl, 0.3 M trisodium citrate solution; Udp1, *Urtica dioica* peroxidase 1; UTR, untranslated region; UE, upstream efficiency element.

* Corresponding author. Tel.: +30 210 7274546; fax: +30 210 7274742.
E-mail address: ipapasid@biol.uoa.gr (I.S. Papassideri).

0378-1119/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2005.06.039

(Passardi et al., 2005). Cross-linking of phenolic monomers during the formation of suberin and the oxidative coupling of lignin subunits are associated with reduction of cell extensibility and growth. Peroxidases are candidates for lignin unit assembly by oxidative polymerization (Lewis and Yamamoto, 1990). Plants exposed to stress upregulate their peroxidase activity. This reaction happens with various abiotic and biotic stresses such as chemical, biological (pathogens) or physical (wounding) assaults (Lavid et al., 2001; Martinez et al., 1998; Hiraga et al., 2000).

Plant peroxidases exhibit considerable amount of sequence similarities in regions that build up the heme-binding catalytic site. On the other hand, it has been really difficult to assign specific functions to the large variety of peroxidases that have been purified, characterized and also localized to certain cell compartments. Identification of in vivo specific substrates and regulatory mechanisms of gene expression for each peroxidase remains still unclear. Extensive search of the nucleotide sequence databases for plant peroxidases belonging to Rosales resulted in only 30 sequences, whereas 2428 peroxidase sequences have already been deposited from plants belonging to Eurosids I. Moreover, only 1 of the 30 sequences corresponds to a full-length cDNA clone of a class III peroxidase (*Ficus carica* peroxidase mRNA, AF479623), while the rest of them mainly correspond to ascorbic peroxidases or EST fragments. Therefore, cloning and characterization of genes encoding peroxidases from diverse plant species are crucial and important steps towards understanding the function and regulation of individual members of this multi-gene family. *Urtica dioica* (stinging nettle) belongs to Eurosids I, Rosales and has been extensively studied for its medicinal applications, as well as for causing contact urticaria and allergic responses (Oliver et al., 1991).

In the present study we describe, for the first time, the isolation and characterization of a full-length cDNA clone, designated Udp1, encoding a stinging nettle cationic peroxidase. The cloning strategy was based on a reverse transcription PCR (RT-PCR) approach, using degenerated primers designed against plant peroxidase conserved motifs and subsequent rapid amplification reactions of cDNA ends (RACE). The nucleotide sequence analysis of the Udp1 cDNA clone revealed that the predicted open reading frame contains a 337 amino acid residues putative protein, including an N-terminal signal peptide. A genomic DNA fragment, designated *gUdp1,* corresponding to the full-length Udp1 cDNA, was also cloned and fully sequenced. Comparative analysis between the obtained sequences of the genomic fragment and the cDNA clone demonstrated a structural organization of three exons and two introns. Consequent functional studies by Northern blot analysis disclosed the root-specific Udp1 transcriptional activity and its ectopic inducible profile by certain factors, such as mechanical stress and ultraviolet radiation. Multiple sequence alignments, in combination with the molecular modelling approach, among different family members were able to dictate the presence of unique and evolutionary conserved structural elements, which could be likely associated with distinct functions of the Udp1 peroxidase.

2. Materials and methods

2.1. Plant material and exposure of plants to stress

Stinging nettle (*Urtica dioica*) plants were grown from seeds. Seeds were surface-disinfected and allowed to germinate in the dark, before planting in sterile sand pot cultures. The growth chamber was maintained at 25 °C, with a 16 h photoperiod. Plants were irrigated with Hoagland's solution.

Leaves from two months old plants were sliced into approximately 10 mm sections and floated on sterile water for 48 h. Two months old plants were exposed to ultraviolet radiation at a distance of 30 cm, for 1 h. UV radiation was generated by Philips TL12 fluorescent tubes (λmax 315 nm). Two months old *Urtica dioica* plants were chilled for 48 h, at 4 °C.

2.2. Molecular cloning techniques

Unless stated otherwise, all conventional molecular cloning techniques were performed as previously described by Sambrook et al. (1989).

2.3. RNA isolation

Total RNA was isolated according to the procedure developed by Jacobs-Lorena (1980) and modified by Bouhin et al. (1992). Poly(A)$^+$ RNA was purified directly from crude extracts with DYNAL “Dynabeads mRNA DIRECT™ Kit”, according to manufacturer's protocol.

2.4. Polymerase chain reaction (PCR) and reverse transcription (RT) PCR

Amplification reactions were carried out with deoxynucleotides, buffers and enzyme concentrations as recommended by the enzyme manufacturer (New England Biolabs Vent DNA polymerase, with proofreading exonuclease activity). Reactions were performed on an MJ Research Minicycler™ thermocycler with an initial denaturation step at 94 °C for 3 min, followed by 30 cycles at 94 °C for 1 min, 58 °C for 1 min and 72 °C for 1 min. Á final polymerization step at 72 °C for 15 min was added after the completion of 30 cycles. The amplification reactions with *Urtica dioica* genomic DNA as a template were carried out under standard PCR conditions, with the exception of a critical modification in the cycling parameters. The initial denaturation step at 94 °C for 3 min was followed by 35 cycles at 94 °C for 1 min, 53 °C for 1.5 min and 72 °C for 3 min. A final polymerization and extension step at 72 °C for 20 min was added after the end of 35 cycles. The following oligonucleotide primers were used in the present study: (a) primers corresponding to highly conserved regions of plant peroxidases, sense A (5′-CACTTCCACGACTGCTTTG-3′), sense B (5′-GTTTCTTGTGCTGACATGCTCGC-3′) and antisense C (5′-GAGGTTGGTGTAGTAGGCGTT-3′), (b) Udp1-specific primer, antisense D (5′-GTGTGTGATCCAAGGAGAAC-3′), (c) primers, sense H (5′-GCTTGGTTAGTAGTTATTAG-3′) and