Available online at www.sciencedirect.com
SCIENCE DIRECT
Archives of Biochemistry and Biophysics 447 (2006) 87–95
ABB
www.elsevier.com/locate/yabbi

Lanosterol biosynthesis in plants

Mariya D. Kolesnikova [a], Quanbo Xiong [b], Silvia Lodeiro [a], Ling Hua [c], Seiichi P.T. Matsuda [a,b,*]

[a] Department of Chemistry, Rice University, 6100 S. Main Street, Houston, TX 77005, USA
[b] Department of Biochemistry and Cell Biology, Rice University, 6100 S. Main Street, Houston, TX 77005, USA
[c] Department of Chemistry, Southern Methodist University, Dallas, TX 75275, USA

Received 17 November 2005, and in revised form 21 December 2005
Available online 11 January 2006

Abstract

Plants biosynthesize sterols from cycloartenol using a pathway distinct from the animal and fungal route through lanosterol. Described herein are genome-mining experiments revealing that *Arabidopsis* encodes, in addition to cycloartenol synthase, an accurate lanosterol synthase (*LSS*)—the first example of lanosterol synthases cloned from a plant. The coexistence of cycloartenol synthase and lanosterol synthase implies specific roles for both cyclopropyl and conventional sterols in plants. Phylogenetic reconstructions reveal that lanosterol synthases are broadly distributed in eudicots but evolved independently from those in animals and fungi. Novel catalytic motifs establish that plant lanosterol synthases comprise a third catalytically distinct class of lanosterol synthase.

Keywords: Sterol biosynthesis; Cycloartenol; Lanosterol

Plants use a circuitous pathway to generate the tetracyclic ring system of their membrane sterols. Instead of producing the tetracycle lanosterol [1,2] as do animals [3–5][1] and fungi [6–8], plants make the pentacycle cycloartenol [9] as the initial cyclic sterol precursor (Fig. 1) [10,11]. An additional enzyme, cycloeucalenol-obtusifoliol isomerase [12], is required to open the extraneous cyclopropyl ring in order to obtain the tetracyclic sterol ring system. The shorter sterol biosynthetic pathway through lanosterol has not been discovered in plants. Nevertheless, some observations hint that lanosterol plays roles in plants. Lanosterol is metabolized by some plants [13–16] and is biosynthesized by *Euphorbia lathyris* latex [17]. The lanostane skeletons of various 4,4-dimethyl Δ^8 sterol saponins are not readily rationalized as cycloartenol metabolites because 4,4-dimethyl cyclopropyl sterols are poor substrates for plant cycloeucalenol isomerases [18–20]. These observations would be easily explained by the presence of a lanosterol synthase in plants.

Herein, we describe an *Arabidopsis thaliana* gene *At3g45130* that encodes the first lanosterol synthase cloned from a plant. A survey of known genomic sequences of other plants suggests that lanosterol synthase is widespread among eudicots. Plant lanosterol synthases evolved independently and achieve product accuracy using catalytic motifs distinct from those in other lanosterol synthases. The lanosterol and lanostane saponins that have been found sporadically in plants may arise from an accurate lanosterol synthase rather than a nonspecific cycloartenol synthase.

Materials and methods

Materials

Restriction enzymes *Not*I, *Sal*I, *Bsr*GI, and Quick Ligation Kits were purchased from New England Biolabs (Beverly, MA). Triple Master polymerase for PCR reac-

* Corresponding author. Fax: +1 713 348 5154.
E-mail address: matsuda@rice.edu (S.P.T. Matsuda).

[1] Some animals do not biosynthesize sterols but rely on dietary sources. Sea cucumbers are exceptional in that they biosynthesize sterols from parkeol.

0003-9861/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.abb.2005.12.010

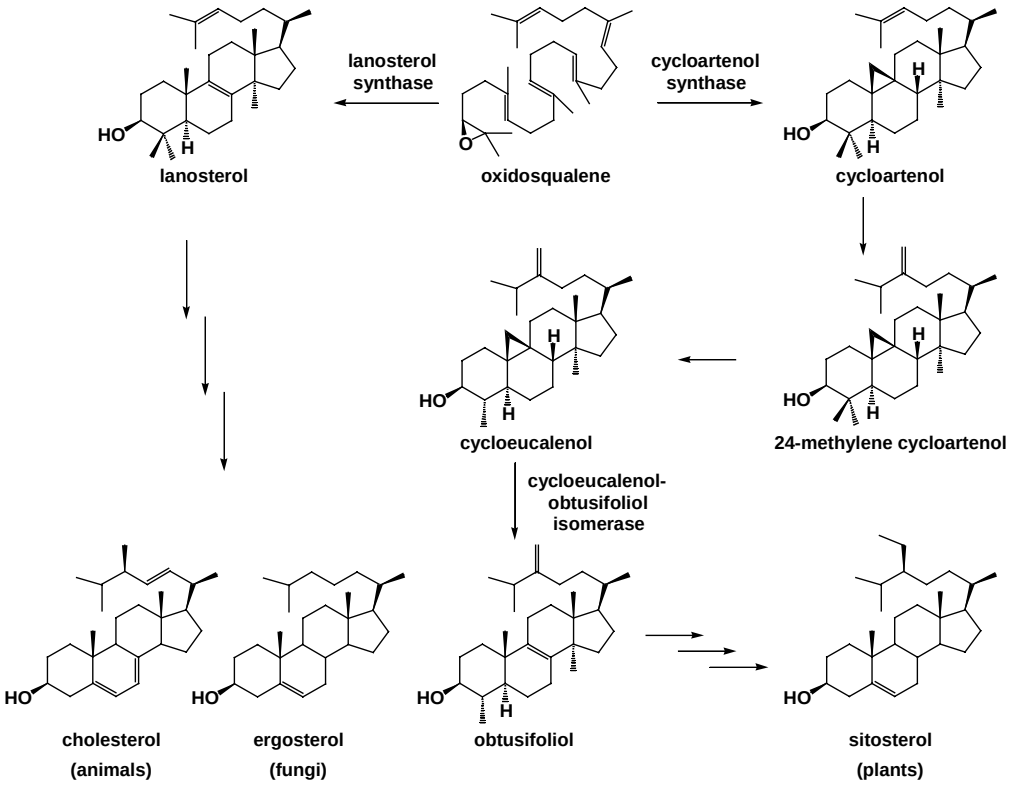

Fig. 1. Reactions catalyzed by cycloartenol synthase and lanosterol synthase. Plants and some protists use cycloartenol synthase to cyclize oxidosqualene to the pentacyclic sterol precursor cycloartenol. In contrast, dinoflagellates, trypanosomes, animals, and fungi use lanosterol synthase to generate lanosterol.

tions was obtained from Eppendorf (Westbury, NY). Gel purifications were performed using the Qiagen Gel Extraction Kit (Qiagen, Valencia, CA). *Escherichia coli* strain DH5α (ATCC, Manassas, VA) was used for plasmid manipulations. Media components were purchased from United States Biological (Swampscott, MA). Heme (in the form of hemin chloride), ergosterol, and bis(trimethylsilyl)trifluoroacetamide (BSTFA)[2] were from Sigma–Aldrich (St. Louis, MO). Pyridine, TLC plates (Merck brand), solvents for extraction and saponification were purchased from Fisher Scientific (Fair Lawn, NJ).

Sequence identification, cloning, and subcloning

Sequences similar to the *Arabidopsis* cycloartenol synthase were obtained by a tblastn [21] search against the *A. thaliana* genome [22]. A cDNA corresponding to the *A. thaliana* coding sequence *At3g45130* was cloned by hybridizing a radiolabeled probe obtained by PCR-amplification of cDNA. After screening 3×10^5 colonies from an *A. thaliana* young seedling cDNA library [23], a clone with a 2.4-kbp insert was obtained and was named pLH1.1. The *Not*I fragment of pLH1.1 was subcloned into pBluescript II KS (+) to give pLH1.2. The plasmid pLH1.2 was sequenced and revealed that the second exon of LH1.2 was absent. The *Not*I–*Sal*I fragment was cloned from the plasmid pLH1.2 into the galactose-inducible yeast expression vector pRS316GAL [24] to give pLH1.19.

The primer pair 5′-TAATGTCGACTAATATGTGGA GGTTAAAGTTA-3′ and 5′-TATGAGAGCACTGTAC AAAACATGGTGCTATT-3′ was designed to amplify the 538 bp at the 5′ end of the expected coding sequence and thereby obtain a properly spliced fragment. These primers were used to PCR-amplify an *A. thaliana* cDNA library [25] (95 °C, 1 min; 65 °C, 30 s; 72 °C, 3 min with final 5 min extension at 72 °C). The PCR reaction mixtures (50 μl) contained 0.2 μg of cDNA library, 20 μl of 2.5 × Triple Master Mix, 20 pmol of each primer, 3 U of the Triple Master polymerase. The amplicon was gel-purified and cloned into pLH1.19 using *Sal*I and *Bsr*GI restriction enzymes. The resultant expression plasmid was named pLH1.25 and was confirmed to contain the whole length of the ORF (2271 bp), including the exon that was missing in plasmids pLH1.2 and pLH1.19.

Yeast transformation and selection conditions

Saccharomyces cerevisiae strains SMY8 [7] and RXY6 [26] were transformed with pLH1.25 using the lithium acetate method [27]. Transformants were selected for uracil prototrophy at 30 °C on synthetic complete medium lacking uracil [28] solidified with 1.5% agar and supplemented

[2] *Abbreviations used:* BSTFA, bis(trimethylsilyl)trifluoroacetamide; NSL, nonsaponifiable lipids.