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Biochemistry

Cloning and functional expression of a cDNA encoding a pheromone gland-specific acyl-CoA Δ^{11}-desaturase of the cabbage looper moth, *Trichoplusia ni*

(acyl-CoA desaturase/Lepidoptera/pheromone biosynthesis)

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ABSTRACT **Desaturation of coenzyme-A esters of saturated fatty acids is a common feature of sex pheromone biosynthetic pathways in the Lepidoptera. The enzymes that catalyze this step share several biochemical properties with the ubiquitous acyl-CoA Δ^9-desaturases of animals and fungi, suggesting a common ancestral origin. Unlike metabolic acyl-CoA Δ^9-desaturases, pheromone desaturases have evolved unusual regio- and stereoselective activities that contribute to the remarkable diversity of chemical structures used as pheromones in this large taxonomic group. In this report, we describe the isolation of a cDNA encoding a pheromone gland desaturase from the cabbage looper moth, *Trichoplusia ni*, a species in which all unsaturated pheromone products are produced via a Δ^{11}Z-desaturation mechanism. The largest ORF of the ≈1,250-bp cDNA encodes a 349-aa apoprotein (PDesat-Tn Δ^{11}Z) with a predicted molecular mass of 40,240 Da. Its hydrophobicity profile is similar overall to those of rat and yeast Δ^9-desaturases, suggesting conserved transmembrane topology. A 182-aa core domain delimited by conserved histidine-rich motifs implicated in iron-binding and catalysis has 72 and 58% similarity (including conservative substitutions) to acyl-CoA Δ^9Z-desaturases of rat and yeast, respectively. Northern blot analysis revealed an ≈1,250-nt PDesat-Tn Δ^{11}Z mRNA that is consistent with the spatial and temporal distribution of Δ^{11}-desaturase enzyme activity. Genetic transformation of a desaturase-deficient strain of the yeast *Saccharomyces cerevisiae* with an expression plasmid encoding PDesat-Tn Δ^{11}Z resulted in complementation of the strain's fatty acid auxotrophy and the production of Δ^{11}Z-unsaturated fatty acids.**

Acyl-CoA Δ^9Z-desaturases occur ubiquitously in the animal and fungal kingdoms, where they play essential roles in fatty acid metabolism (for review, see ref. 1) and the regulation of cell membrane fluidity in response to temperature fluctuations (2, 3). These non-heme iron-containing enzymes typically use saturated 16- and 18-carbon fatty acyl thioesters of CoA (palmitoyl-CoA and stearoyl-CoA, respectively) as substrates (4) and catalyze the NADH- and oxygen-dependent removal of hydrogen atoms from carbon atoms 9 and 10 to form the Z double bond of palmitoleic and oleic acids, respectively (5).

Early biochemical investigations of acyl-CoA Δ^9-desaturases of vertebrates showed that the enzyme is an integral membrane protein of the endoplasmic reticulum and that only a small portion of it, containing the iron-containing catalytic center, is exposed to the cytoplasm (5–7). Investigations of the enzyme's functional association with other proteins and membrane lipids showed that the active desaturase is a complex of three proteins, of which two are components of the NADH-dependent electron transport system of the endoplasmic reticulum, NADH-cytochrome *b5* reductase (a flavoprotein) and cytochrome *b5* (a hemoprotein) (8–12).

Characterization of a stearoyl-CoA Δ^9-desaturase cDNA isolated from rat liver revealed an encoded 358-aa apoprotein with >60% hydrophobic residues (13), consistent with the finding of earlier biochemical studies. Subsequent characterizations of additional vertebrate acyl-CoA desaturase cDNAs from mouse (14, 15), carp (3), and humans (16) showed extensive sequence similarity (identity plus conservative substitutions exceeding 90%, excluding the initial 40–60 amino-terminal amino acids) among the encoded proteins. Two cDNAs that encode proteins having significant sequence similarity to vertebrate acyl-CoA Δ^9-desaturases also have been isolated recently from the tick *Amblyomma americanum* and from *Drosophila melanogaster* (17).

The palmitoyl-CoA Δ^9-desaturase (*OLE1*) of the yeast *Saccharomyces cerevisiae* (18) also has been deduced from its encoding cDNA and has been shown to be a protein of 510 amino acids (251 hydrophobic) with a predicted molecular mass of ≈57 kDa (19). Optimal alignment of the *S. cerevisiae* OLE1 amino acid sequence with that of the rat stearoyl-CoA Δ^9-desaturase revealed a 257-aa region having 36% identity and 60% similarity (19). The 113-aa carboxyl terminus of the yeast OLE1 protein, which has no structural correlate among characterized animal desaturases, has regions of high sequence identity to cytochrome *b5* and has been shown to be essential for desaturase function (20). This cytochrome *b5*-like carboxyl-terminal extension is also present in the orthologous OLE1 desaturase of the oleaginous yeast, *Cryptococcus curvatus* (21). The functional equivalence of the *S. cerevisiae* and rat desaturases was shown by complementation of the desaturase-deficient *ole1* strain's unsaturated fatty acid (UFA) auxotrophy with a plasmid encoding the rat desaturase (19). Further molecular genetic investigations of the heterologously expressed rat desaturase identified eight histidine residues occurring in highly conserved sequence motifs that are essential for catalytic function (22).

The existence of a family of desaturases in the Lepidoptera possessing unusual catalytic properties initially was suggested by the elucidation of the chemical structures of the diverse UFA derivatives that are species-specific constituents of lepidopteran sex pheromones (reviewed in refs. 23–25). Subsequent studies of pheromone biosynthetic pathways revealed the basis of this molecular diversity arising from variations in

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Abbreviation: UFA, unsaturated fatty acid.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF035375).

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a discrete number of conserved enzymatic steps involving synthesis of saturated fatty acids from acetate and acetyl-CoA, desaturation, limited chain-shortening by β-oxidation, and reduction of the terminal functional group (23–25). The number of unique regio- and stereospecific desaturation mechanisms discovered in these pathways, including Z9, E9, Z10, Z11, E11, Z14, and E14 mechanisms (23–27), indicates that the evolution of novel functional properties among pheromone desaturases has played a significant role in generating the remarkable diversity of chemical structures used as species-specific mate recognition signals in this large taxonomic group (25).

Biochemical studies of Δ^{11}-desaturases from pheromone glands of *Trichoplusia ni* (28) and *Spodoptera littoralis* (29) showed that these enzymes have many similarities with the ubiquitous metabolic acyl-CoA Δ^9-desaturases. In this report, we describe the isolation from the *T. ni* pheromone gland of a cDNA encoding a protein that is homologous to animal and fungal acyl-CoA Δ^9-desaturases, the spatial and temporal occurrence of its corresponding mRNA at the level of Northern blot analysis, and its expression in an *ole1*-containing yeast strain resulting in complementation of the strain's UFA auxotrophy and the formation of Z11-UFAs.

MATERIALS AND METHODS

RNA Isolations. Pheromone glands were dissected from the abdomens of adult *T. ni* females reared in captivity, and total RNA was extracted as described (31, 32). RNA was isolated by the same procedure from dissected fat bodies of larvae (both sexes) and adult females and from abdominal muscle of adult females from which pheromone glands had been removed previously. Poly(A)$^+$ RNA was selected from total RNA by using either oligo(dT) cellulose (Ambion, Austin, TX) or oligo(dT) paramagnetic beads (Dynal, Great Neck, NY).

Isolation of Pheromone Desaturase cDNAs. First-strand oligo(dT)-primed cDNA was made from poly(A)$^+$ RNA (Superscript kit, GIBCO/BRL) according to the manufacturer's protocol. A pheromone gland cDNA library was made in λZAP (Stratagene) according to the manufacturer's protocol. A second library used has been described (32). A hybridization probe was made by using a 550-bp segment of the *T. ni* Δ^{11}-desaturase cDNA in a PCR-based procedure (33, 34). Degenerate primers were designed to encode histidine-rich sequence motifs conserved in acyl-CoA Δ^9-desaturases of rat (13) and yeast (19) as follows (where [N] is all four bases): 5′-d9d1 = 5′-CCCCA[T/C]C[G/A][N]CT[G/C]TGG[T/A]C[N]CA-3′; and 3′-d9d2 = 5′-CCCTCTAGA[G/A]TG[G/A][G/A][T/A]A[G/A]TT[G/A]TG[G/A][T/A]A-3′. Primers were incubated with 100 ng of cDNA template and *Taq* polymerase (Perkin–Elmer), and PCR was performed for 55 cycles of 94°C for 2 min, 48°C for 1 min, and 68°C for 1 min. Parallel reactions were performed by using the cloned rat and yeast acyl-CoA desaturase cDNAs as templates (kindly provided by Philipp Strittmatter, Univ. of Connecticut Health Center, Farmington, CT and Charles Martin, Rutgers Univ., Piscataway, NJ, respectively). The PCR product was digested with *Xba*I to produce a sticky end at the 3′-d9d2 terminus and was ligated into a plasmid linearized by digestion with *Xba*I and *Sma*I. *Escherichia coli* strain XL1-Blue (Stratagene) was transformed with the ligation reaction, and insert-containing plasmids obtained from the resultant clones were sequenced by using an automated sequencer (Applied Biosystems).

The cloned PCR product was labeled with digoxigenin (Genius kit, Boehringer Mannheim) according to the manufacturer's protocol and was hybridized under standard conditions to plaque lifts of the cDNA libraries. Probe-positive λZAP clones were isolated, and plasmids were obtained by the manufacturer's automatic excision protocol for sequencing as above.

Northern Blot Analysis. Northern blots of poly(A)+ RNA isolated from various tissues were hybridized with digoxigenin-labeled probes as described (35). The equivalence of RNA loadings was assessed by spectrophotometric measurement at A_{260}/A_{280}, methylene blue staining of RNA on the blot, and quantitation of β-actin sequences by reverse transcription–coupled PCR (Superscript kit, GIBCO/BRL). A 784-bp digoxigenin-labeled hybridization probe was made in a PCR (33 cycles of 95°C for 1 min, 56°C for 1 min, and 72°C for 1.5 min) containing the PDesat-TnΔ^{11}Z cDNA, the specific primers 5′-d11–1 = 5′-GAAGCTCGCACGATGAC-3′ and 3′-d11–3 = 5′-CTAATTCTGCTGTGCGGTAATCCCATG-GAAAGAC-3′, 400 μM dATP, 400 μM dCTP, 400 μM dGTP, 260 μM dTTP, 140 μM alkali-labile digoxigenin-dUTP (PCR DIG labeling mixture, Boehringer), and *Taq* Polymerase (Perkin–Elmer). Reverse transcription–coupled PCRs containing the β-actin primers 5′-βact = 5′-ATGTG[C/T]AA[A/G]GC[N]GG[N]TT[T/C]GC-3′ and 3′-βact = 5′-GGNGC[A/G/T]AT[A/G/T)AT[C/T]TT[A/G/T]AT[C/T]TTCAT-3′, derived from the invariant amino acid sequences MCKAGFA and MKIKIIAP (36), respectively, were done as follows: 35 cycles of 94°C for 2 min, 60°C for 1 min, and 72°C for 1 min.

Plasmid Construction and Yeast Transformation. A cDNA fragment containing the 349-aa ORF of the PDesat-TnΔ^{11}Z cDNA and flanked by *Bam*HI and *Sac*I restriction sites at its 5′ and 3′ ends, respectively, was obtained by PCR by using specific primers complementary to the 20-nt terminal sequences of the ORF. This product was digested with *Bam*HI and *Sac*I and was subcloned into a plasmid derivative of YEp352/OLE4.8 in a procedure identical to that used to construct a functional yeast/rat desaturase gene (19). The final plasmid, designated YEpOLEX-CLR7, contains the 349-aa PDesat-TnΔ^{11}Z-encoding ORF ligated via a four-codon linker in-frame with and downstream from the 5′ end of the OLE1 ORF encoding the first 27 amino acids of the yeast Δ^9-desaturase. Sequences flanking the chimeric ORF consisted of the promoter and terminator elements of the OLE1 gene contained on the original YEp352/OLE4.8 plasmid. The latter and the *S. cerevisiae* strain L8–14C (*MAT*α, *ole1*Δ::*LEU2*, *leu2–3*, *leu2–112*, *trp1–1*, *ura3–52*, *his4*) (19) used in this investigation were generously provided to us by Charles Martin.

Before transformation, L8–14C cells were grown to $\approx 1 \times 10^7$ cells/ml at 30°C in yeast extract/peptone/dextrose medium (2% Bacto-peptone, 1% yeast extract, 2% glucose) containing 1% Tergitol (Type Nonidet P-40, Sigma–Aldrich; to solubilize UFAs), 0.5 mM oleic acid, and 0.5 mM palmitoleic acid. The strain was transformed with the YEpOLEX-CLR7 plasmid DNA by a standard method (37) according to the manufacturer's protocol (alkali-cation yeast transformation kit, Bio 101) and was plated onto complete synthetic dextrose medium containing 1% Tergitol (Type Nonidet P-40, Sigma–Aldrich; to solubilize UFAs), 0.5 mM oleic acid, and 0.5 mM palmitoleic acid and lacking uracil.

Functional Assays. To test for genetic complementation of the *ole1* auxotrophy by the YEpOLEX-CLR7 plasmid, URA$^+$ transformant colonies were selected and patched onto complete (yeast extract/peptone/dextrose) medium lacking supplemental UFAs and were incubated at 30°C for 24–48 hours. The transformants then were grown to $\approx 1 \times 10^7$ cells/ml at 30°C in yeast extract/peptone/dextrose medium and were washed and extracted with chloroform/methanol (3:1). After solvent evaporation, the residue was treated with 0.5 M KOH/methanol, and the resulting fatty acid methyl esters were analyzed by GC/MS (HP 5970). The positions of double bonds in unsaturated 16- and 18-carbon fatty acids were confirmed by analysis of the degradation products of their dimethyl disulfide adducts (38).

RESULTS AND DISCUSSION

Rationale for Homology Probing Strategy. The discovery that pheromone desaturases have significant biochemical similarities to the ubiquitous metabolic acyl-CoA Δ^9-desaturases (28, 29) led us to hypothesize that both classes of enzymes are related by descent from a common ancestral gene and that structural domains that are conserved among metabolic acyl-CoA Δ^9-desaturases from phylogenetically distant species also will be present in the lepidopteran pheromone gland desaturases. To test this hypothesis, we used a PCR-based homology probing methodology that used degenerate primers encoding two highly conserved, histidine-rich sequence motifs that have been implicated in iron binding and catalytic function of acyl-CoA Δ^9-desaturases (22).

The cDNA templates used in PCR reactions were synthesized from mRNA isolated from the pheromone glands of *T. ni*, which was chosen for this investigation for the following reasons: (*i*) The pheromone desaturase of this species is one of only two biochemically well characterized pheromone desaturases (28, 29); (*ii*) the unsaturated products present in the *T. ni* pheromone gland are all derivatives of a Z11 desaturation mechanism that is present only in the pheromone gland (28); (*iii*) the *T. ni* pheromone gland is a relatively large discrete eversible sac that can be neatly dissected away from other cuticular structures and fatty tissues; and (*iv*) the temporal induction of pheromone biosynthetic enzymes and protein expression patterns in the pheromone gland of this species have been characterized (30), enabling us to use a developmental stage in which pheromone desaturase mRNA levels are most likely to be at their highest levels.

Isolation of the PDesat-TnΔ^{11}Z cDNA from *T. ni* Pheromone Gland. The experimental procedures that we used resulted in the cloning of a full length cDNA with an ORF of 1,047 nt encoding the 349-aa protein designated PDesat-TnΔ^{11}Z (Fig. 1). The 560-bp product obtained in the initial PCRs containing the degenerate primers 5′-d9d1 and 3′-d9d2 and *T. ni* pheromone gland cDNA was labeled and hybridized to two independently prepared pheromone gland cDNA libraries. An extremely high percentage (>2%) of clones was labeled, consistent with a high abundance of the corresponding mRNAs in the pheromone glands from which the libraries were made. Several probe-positive clones were isolated and sequenced. All cDNAs longer than 1.2 kilobases contained an ORF of 1,047 nt encoding a 349-aa protein. Several sequence polymorphisms were observed among the clones, with the most common ones occurring at positions 22 (Val or Met), 58 (Cys or Ser), 70 (Ser, Thr or Ala), and 315 (Lys or Glu). The protein representing the consensus amino acid sequence shown in Fig. 1 has a predicted molecular mass of 40,240 Da and a pI of 9.12. It is possible that translation could be initiated at a second ATG occurring in-frame only three codons downstream from the ATG initiating the ORF, which would result in a slightly smaller protein. These two inferred PDesat-TnΔ^{11}Z polypeptides are similar in size to one of about a dozen proteins, the expression of which is associated with the onset of maximal pheromone biosynthetic capability in the *T. ni* pheromone gland (30).

Homology of PDesat-Tn Δ^{11}Z to Acyl-CoA Δ^9-Desaturases. Alignment of the PDesat-TnΔ^{11}Z amino acid sequence with those of rat (13) and yeast (19) acyl CoA Δ^9-desaturases (Fig. 1) shows that the deduced PDesat-TnΔ^{11}Z protein is similar in size to the acyl-CoA Δ^9-desaturase from rat and lacks the cytochrome *b5*-like carboxyl-terminal extension of the OLE1 desaturases of *S. cerevisiae* (20) and *C. curvatus* (21). The highest level of sequence conservation between PDesat-TnΔ^{11}Z and any acyl-CoA Δ^9-desaturase occurs in the region corresponding to the primary 560-bp PCR product delimited by amino acid positions 86 and 267 (for rat, 55% amino acid identity and 72% similarity; for yeast, 34% identity and 58% similarity). Of the 13 histidine residues occurring in the PDesat-TnΔ^{11}Z core domain, 11 are conservative, including all of those that are required for acyl-CoA Δ^9-desaturase catalytic function (22). Conservation outside the core domain is somewhat lower, particularly in the amino terminal domain, resulting in overall conservation values as determined by the BLAST 2.0 program (39) of 52% identity and 70% similarity vs. rat and 30% identity and 50% similarity vs. yeast. As with the rat and yeast acyl-CoA Δ^9-desaturases, the PDesat-TnΔ^{11}Z protein has a high percentage of hydrophobic residues (77%), and its Kyte–Doolittle hydrophobicity plot (40) is similar to those of the rat and yeast acyl-CoA Δ^9-desaturases (data not shown), consistent with conservation of the proposed acyl-CoA Δ^9-desaturase transmembrane topology (19).

Comparisons of the sequences of the PDesat-TnΔ^{11}Z core domain to the corresponding regions of two presumptive arthropod acyl-CoA Δ^9-desaturases (sequences not shown) reveal that the PDesat-TnΔ^{11}Z is about as similar to the fly acyl-CoA Δ^9-desaturase as it is to the tick acyl-CoA Δ^9-desaturase (for *D. melanogaster*, 61% identity and 85% similarity; for *A. americanum*, 61% identity and 80% similarity). Comparisons of the corresponding region of the rat acyl-CoA Δ^9-desaturase to the latter two sequences gave similar values (for *D. melanogaster*, 64% identity and 81% similarity; for *A. americanum*, 61% identity and 78% similarity). In the context of the similarity of all of these values, it is of interest to note that fossil evidence supports the divergence of lepidopteran and dipteran orders in the Permian Era between 240 and 280 million years ago (41) whereas recent molecular analyses support the divergence of insects and chelicerates (the latter including contemporary tick species) from a common ancestral form in the Precambrian Era >550 million years ago (42, 43).

PDesat-TnΔ^{11}Z mRNA Occurrence Coincides with Δ^{11}-Desaturase Activity. Northern blot experiments showed the presence of abundant levels of the PDesat-TnΔ^{11}Z transcript in poly(A)$^+$ RNA from pheromone glands of 48-hour posteclosion adult females (Fig. 2, lane 1), consistent with our finding of an extremely high percentage of clones containing PDesat-TnΔ^{11}Z sequences in the pheromone gland cDNA libraries made from the same developmental stage. The mobility of the PDesat-TnΔ^{11}Z transcript is indistinguishable from that of a transcript synthesized *in vitro* by using the linearized PDesat-TnΔ^{11}Z cDNA template and SP6 RNA polymerase (Fig. 2, lane 3). The latter finding is consistent with the interpretations, based on the sequence analysis presented above, that the largest PDesat-TnΔ^{11}Z cDNA clone isolated is either full length or nearly full length and that it encodes the entire PDesat-TnΔ^{11}Z protein. The PDesat-TnΔ^{11}Z transcript is not detected in loadings of similar amounts of poly(A)$^+$ RNA isolated from fat bodies of adults (Fig. 2, lane 5) and larvae (Fig. 2, lane 7). We performed additional Northern blots and reverse transcription–coupled PCR experiments (data not shown) designed to increase the sensitivity of detection (by manipulating probe strength and duration of signal integration) and to quantify the levels of transcripts detected relative to those encoding β-actin, respectively. These experiments revealed PDesat-TnΔ^{11}Z transcript in pheromone gland poly(A)$^+$ RNA from newly eclosed (0–12 hour) adult females at levels approximately two orders of magnitude lower than those present on a mass equivalent basis in poly(A)$^+$ RNA from pheromone glands of 48-hour posteclosion adult females but failed to detect PDesat-TnΔ^{11}Z transcript in poly(A)$^+$ RNA from adult thoracic muscle or fat bodies from larvae and adults. Reverse transcription–coupled PCR experiments performed on the RNA samples used for the Northern blots indicated that the maximal level of PDesat-TnΔ^{11}Z transcript present in pheromone glands of 48-hour posteclosion females is at least three orders of magnitude greater than the level of β-actin transcripts present in the same developmental stage. Previous investigations have shown that the Δ^{11}-desaturase

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Ratdesat    .......... MPAHMLQEIS SSYTTTTTIT EPPSGNLQNG REK.......
T.ni        .......... .......... .......... .......... ..........
Ole1desat   MPTSGTTIEL IDDQFPKDDS ASSGIVDEVD LTEANILATG LNKKAPRIVN

                                                              35
Ratdesat    .......... MKKVPLYLEE DIRPEMREDI HDPSYQDEEG PPPKLE....
T.ni        .......... .MAVMAQTVQ ETATVLEEEA RTVTLVAPKT TPRKYK....
Ole1desat   GFGSLMGSKE MVSVEFDKKG NEKKSNLDRL LEKDNQEKEE AKTKIHISEQ

                                                                 75
Ratdesat    .......... YVWRNIILMA LLHVGALYGI TL.IPSSKVY TLLWGIFYYL
T.ni        .......... YIYTNFLTFS YAHLAALYGL YLCFTSAKWE TLLFSFVLFH
Ole1desat   PWTLNNWHQH LNWLNMVLVC GMPMIGWYFA LSGKVPLHLN VFLFSVFYYA

                       ******                                   125
Ratdesat    ISALGITAGA HRLWSHRTYK ARLPLRIFLI IANTMAFQND VYEWARDHRA
T.ni        MSNIGITAGA HRLWTHKTFK AKLPLEIVLM IFNSLAFQNT AITWAREHRL
Ole1desat   VGGVSITAGY HRLWSHRSYS AHWPLRLFYA IFGCASVEGS AKWWGHSHRI

                                                                 175
Ratdesat    HHKFSETHAD PHNSRRGFFF SHVGWLLVRK HPAVKEKGGK LDMSDLKAEK
T.ni        HHKYSDTDAD PHNASRGFFY SHVGWLLVKK HPDVLKYGKT IDMSDVYNNP
Ole1desat   HHRYTDTLRD PYDARRGLWY SHMGWMLLKP NPKYKARA.. .DITDMTDDW

                                                                 224
Ratdesat    LVMFQRRYYK PGLLLMCFIL PTLVPWYCWG ETFLHSLFVS TFLRYTLVLN
T.ni        VLKFQKKYAV PLIGTVCFAL PTLIPVYCWG ESWNNAWHI. ALFRYIFNLN
Ole1desat   TIRFQHRHYI LLMLLTAFVI PTLICGYFFN D.YMGGLIYA GFIRVFVIQQ

                                                  **** ***       274
Ratdesat    ATWLVNSAAH LYGYRPYDKN IQSRENILVS LGSVGEGFHN YHHAFPYDYS
T.ni        VTFLVNSAAH IWGNKPYDKS ILPAQNLLVS FLASGEGFHN YHHVFPWDYR
Ole1desat   ATFCINSMAH YIGTQPFDDR RTPRDNWITA IVTFGEGYHN FHHEFPTDYR

                                                                 321
Ratdesat    ASEYRWH.IN FTTFFIDCMA ALGLAYDRKK VSKAAV...L ARIKRTGDGS
T.ni        TAELGNNFLN LTTLFIDFCA WFGWAYDLKS VSEDII...K QRAKRTGDGS
Ole1desat   NA.IKWYQYD PTKVIIYLTS LVGLAYDLKK FSQNAIEEAL IQQEQKKINK

                                        349
Ratdesat    HKSS*..... .......... .......... .......... ..........
T.ni        SGVIWGWDDK DMDRDIKSKA NIFYAKKE.. .......... ..........
Ole1desat   KKAKINWGPV LTDLPMWDKQ TFLAKSKENK GLVIISGIVH DVSGYISEHP

Ratdesat    .......... .......... .......... .......... ..........
T.ni        .......... .......... .......... .......... ..........
Ole1desat   GGETLIKTAL GKDATKAFSG GVYRHSNAAQ NVLADMRVAV IKESKNSAIR

Ratdesat    .......... ......
T.ni        .......... ......
Ole1desat   MASKRGEIYE TGKFF*
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FIG. 1. The PDesat-TnΔ^{11}Z consensus amino acid sequence (T. ni) compared with acyl-CoA Δ^9-desaturases of rat (Ratdesat) and yeast (Ole1desat). Numbering begins with the first methionine of the *T. ni* sequence. Identities in the aligned sequences are shown in bold, and nonidentities are shown in gray. Starred amino acids are the motifs to which 5′-d9d1 and 3′-d9d2 target primers were made.

activity of *T. ni* is limited to the pheromone gland of adult females whereas Δ^9-desaturase activity is present in other fatty tissues throughout development in both sexes (26, 31). Furthermore, investigations of the induction of pheromone bio-

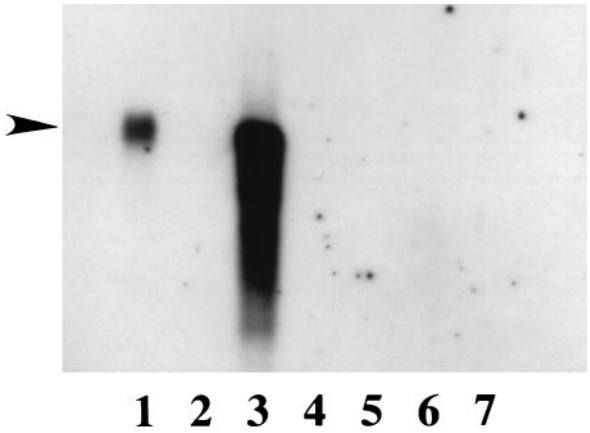

FIG. 2. Northern blot of *T. ni* poly(A)$^+$ RNAs isolated from various tissues and hybridized with the PDesat-TnΔ^{11}Z digoxygenin-labeled probe. Lanes: 1, adult (48 hours posteclosion) pheromone glands; 2, 4, and 6, no RNA; 3, *in vitro* synthesized PDesat-TnΔ^{11}Z transcript; 5, adult (48 hours posteclosion) fat bodies; 7, third instar larval fat bodies. RNA loadings were 3 μg/lane except for the positive control in lane 3, which was <50 ng. Exposure time for the film shown was 90 minutes. The position of the arrow corresponds to the mobility of an RNA of ≈1,250 nt and was interpolated from the relative mobilities of RNAs present in a molecular mass ladder (not shown).

synthetic enzymes during the functional development of the *T. ni* pheromone gland showed that the amount of Δ^{11}-desaturase activity in the pheromone gland increases by two to three orders of magnitude during the first 2 days after adult eclosion (31). The Δ^{11}-desaturase activity of *T. ni* is, thus, coincident with the tissue-specific occurrence, rapid induction, and high steady state levels of PDesat-TnΔ^{11}Z transcript in the pheromone glands of posteclosion females.

Functional Expression of PDesat-TnΔ^{11}Z in Yeast. The demonstration that the UFA auxotrophy of a desaturase-deficient *ole1* strain of *S. cerevisiae* can be complemented genetically with a plasmid encoding a rat stearoyl-CoAΔ^9-desaturase cDNA (19) indicates the functional conservation of the interactions between acyl-CoA desaturases and the electron transport protein components of the functional desaturase complex (i.e., cytochrome *b5* reductase and cytochrome *b5*). This, in conjunction with the experimental finding that the *ole1* strain's UFA auxotrophy can be rescued by supplementation of the growth medium with Δ^{11}-UFAs (18), led us to predict that transformation of the *ole1* strain with plasmids expressing the *T. ni* Δ^{11}- desaturase also would

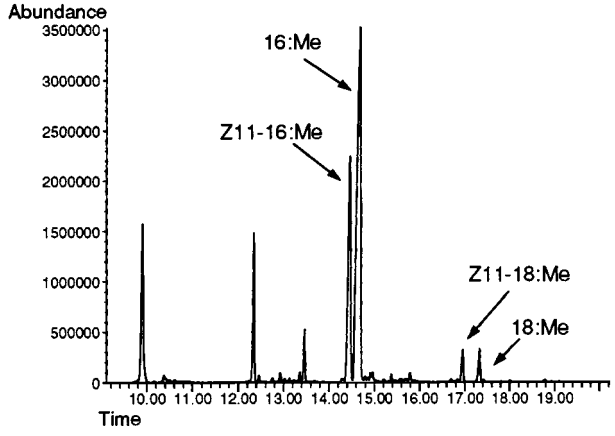

FIG. 3. GC/MS total ion spectrum of fatty acid methyl esters of the YEpOLEX-CLR7-transformed *ole1* strain. The profile of the whole cell lipid extract shows the resolution of the identified monounsaturated products by capillary GLC.

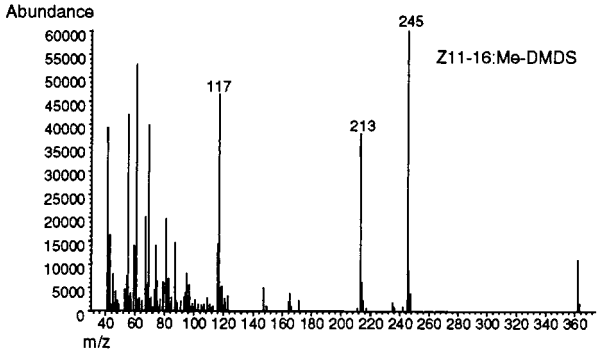

FIG. 4. Mass spectral confirmation of the double bond position of the 16-carbon unsaturated product of the YEpOLEX-CLR7-transformed *ole1* strain. The diagnostic *m/z* values in the MS scan of the methyl sulfide degradation products of the dimethyl disulfide adduct of Z11–16:Me are labeled.

meet the strain's nutritional requirement for UFAs. To test this prediction, we constructed the plasmid YEpOLEX-CLR7 and transformed the L8–14C strain containing a nonreverting *ole1* mutation with it. The YEpOLEX-CLR7 plasmid encodes a chimeric desaturase comprising a 27-aa amino terminal sequence fused via a short linker to the PDesat-TnΔ^{11}Z protein. Transcription of the mRNA is under the regulation of the OLE1 promoter, which is repressed by oleic acid and palmitoleic acid but not by Δ^{11}-UFAs (44, 45). When URA$^+$ transformant colonies were transferred to complete (yeast extract/peptone/dextrose) medium lacking supplemental UFAs, the transformants grew, indicating complementation of the *ole1* mutation by the yeast/PDesat-TnΔ^{11}Z fusion protein. Analysis of the UFAs present in the YEpOLEX-CLR7-transformed strain revealed both Z11–16 and Z11–18 fatty acids in a ratio of ≈10-to-1 and no detectable Z9–16 or Z9–18 fatty acids (Fig. 3). The structural identities of the UFAs of the transformants were verified by making dimethyl disulfide adducts and analyzing their distinct mass spectra. The dimethyl disulfide adduct of Z11–16 methyl ester produces three diagnostic fragments: two methyl sulfide fragments at *m/z* 117 and 245 and a degradation product of the latter at *m/z* 213 (Fig. 4), compared with two fragments at *m/z* 145 and 217 and a degradation product of the latter at *m/z* 185 for the Z9 isomer (data not shown).

Taken together, these findings unequivocally demonstrate that the PDesat-TnΔ^{11}Z protein is homologous to acyl-CoA Δ^9-desaturases occurring in species representing phylogenetically distant lineages and that it is the apoprotein component of the functional acyl-CoA Δ^{11}-desaturase complex of the *T. ni* pheromone gland. The diversity of catalytic specificities that occur among the pheromone desaturases provides a unique opportunity to investigate the mechanistic basis of the desaturation reaction. The present investigation provides the technical precedent for the isolation and characterization of additional pheromone desaturases, which, in conjunction with functional studies, should permit the identification of specific mutational events that gave rise to additional catalytic specificities.

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