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rapid communication

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Abstract A higher content of C16:1 n-10 has recently been reported in the preputial gland of mice with a targeted disruption of the gene encoding stearoyl-CoA desaturase 1 (SCD1$^{-/-}$ mice) when compared with wild-type mice. This result has provided the first physiological evidence for the presence and regulation of a palmitoyl-CoA Δ6-desaturase in mammals. To investigate the putative involvement of the known Δ6-desaturase (FADS2) in this process, COS-7 cells expressing rat Δ6-desaturase were incubated with C16:0. Transfected cells were able to synthesize C16:1 n-10, while nontransfected cells did not produce any C16:1 n-10. Evidence is therefore presented that the rat Δ6-desaturase, which acts on the 18- and 24-carbon fatty acids of the n-6 and n-3 series, is also able to catalyze palmitic acid Δ6-desaturation.—Guillou, H., V. Rioux, D. Catheline, J-N. Thibault, M. Bouriel, S. Jan, S. D'Andrea, and P. Legrand. **Conversion of hexadecanoic acid (palmitic acid, C16:0) to hexadecenoic acid (C16:1 n-10) by rat Δ6-desaturase. *J. Lipid Res.* 2003.** 44: **450–454.**

Supplementary key words desaturation • saturated fatty acids • FADS2 • animal

The presence of a palmitoyl-CoA Δ6-desaturase has recently been described in mice (1). This unexpected Δ6-desaturase activity was found in mice deficient for stearoyl-CoA desaturase 1 (SCD1$^{-/-}$ mice). However, this palmitoyl-CoA Δ6-desaturase activity has not yet been ascribed to any known desaturase gene.

The Δ6-desaturase (FADS2) has been cloned in several mammalian species (2, 3). In a previous work (4), we reported that recombinant rat Δ6-desaturase expressed in COS-7 cells acts on 18- and 24-carbon fatty acids of the n-3 and n-6 series in polyunsaturated fatty acid (PUFA) biosynthesis. This result, consistent with experiments performed in yeast (5) and the PUFA biosynthetic pathway initially proposed by Sprecher and coworkers (6), illustrated the broad chain length specificity of the Δ6-desaturase.

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In the present work, we investigated the putative involvement of FADS2 protein in the Δ6-desaturation of palmitic acid. We established that C16:0 is desaturated to C16:1 n-10 by COS-7 cells when expressing rat Δ6-desaturase. Moreover, coexpression of both Δ6-desaturase and SCD1 resulted in both Δ6- and Δ9-desaturation of palmitic acid. Therefore, this study reports for the first time that a single gene encodes a mammalian Δ6-desaturase that acts on one saturated fatty acid in addition to act on PUFAs.

MATERIALS AND METHODS

Chemicals

Fetal calf serum (FCS) was purchased from Perbio (Bezons, France). Solvents were purchased from Fischer Scientific (Elancourt, France). Fatty acids and other reagents were from Sigma (St Quentin Fallavier, France). Radiolabeled [1-^{14}C]palmitic acid was purchased from Perkin Elmer Life Sciences (Paris, France). The anti-rat liver Δ9-desaturase serum was a generous gift from Dr J. Ozols (7).

Plasmid construction for expression of rat Δ6- and Δ9-desaturases

The plasmid constructed for expression of rat Δ6-desaturase in mammalian cells (referred to as pCMV/Δ6) has already been described (4). A plasmid coding for rat Δ9-desaturase (SCD1) was constructed for expression in mammalian cells and is referred to as pcDNA3/Δ9. From the published (8) rat SCD1 sequence (GenBank accession number J02585), oligonucleotide primers were designed to PCR amplify the entire coding sequence with its stop codon using the high fidelity *Pfu* polymerase from Promega (Lyon, France). The forward primer (5′-CAATGGATCC**ATG**CCGGCCCACATGC-3′) included the translation start codon (in bold) and *Bam*HI restriction site (underlined). The reverse primer (5′-CGTGCTCGAGC**TCA**GCTACTCTTGT-

Abbreviations: FCS, fetal calf serum; GC, gas chromatography; MS, mass spectrometry; pCMV/Δ6, rat Δ6-desaturase open reading frame inserted in pCMV vector; pCDNA3/Δ9, rat Δ9-desaturase open reading frame inserted in pcDNA3 vector.

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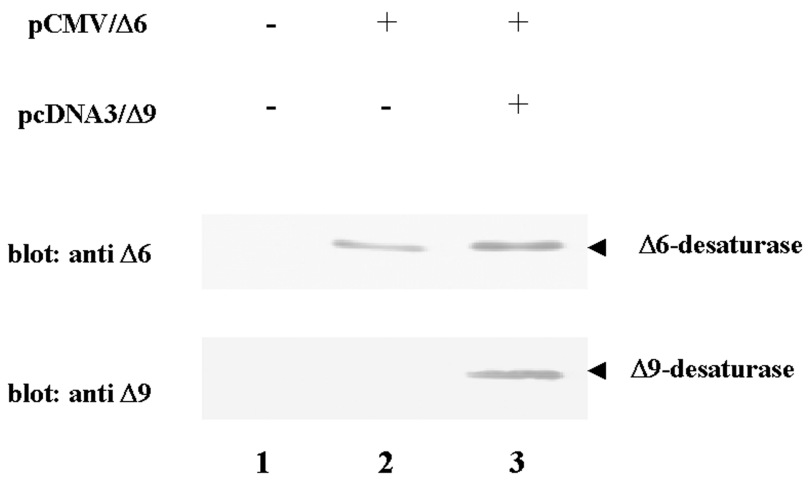

Fig. 1. Detection of rat Δ6- and Δ9-desaturases in transfected COS-7 cells by Western blot analysis. COS-7 cells were not transfected (lane 1), transfected with rat Δ6-desaturase opening frame inserted in pCMV vector (pCMV/Δ6) (lane 2) or transfected with both pCMV/Δ6 and rat Δ9-desaturase opening frame inserted in pcDNA3 vector (pcDNA3/Δ9) (lane 3). COS-7 total cell homogenates (2 μg of protein) were resolved by SDS-PAGE and blotted. The blot was immunoprobed with anti-rat Δ6-desaturase sera or anti-rat Δ9-desaturase sera. Detection was performed using chemifluorescence (ECL Plus detection system).

GGCT-3′) contained the translation stop codon (in bold) and *Xho*I restriction site (underlined). The PCR product amplified from rat liver cDNA was treated with *Bam*HI and *Xho*I before cloning into pcDNA3 (Invitrogen, San Diego, CA). The integrity of the construct was confirmed by DNA sequencing.

Expression of rat Δ6- and Δ9-desaturases in COS-7 cells

Rat Δ6- and Δ9-desaturases were expressed by transiently transforming COS-7 cells with pCMV/Δ6 and pcDNA3/Δ9. COS-7 cells were routinely maintained at about 50% confluence and were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% FCS, 50 IU/ml penicillin, and 50 μg/ml streptomycin. The cells were split 1 day before transfection to 30% confluence and transfected the following day by using the Easyject Plus electroporator (Equibio, Monchelsea, UK). Briefly, 10^6 COS-7 cells in 0.8 ml of DMEM were mixed with 30 μg of purified plasmid, electroporated at 250 V and 1,500 μF with unlimited resistance, and seeded on a 10 cm dish (Falcon, AES, Combourg, France) con-

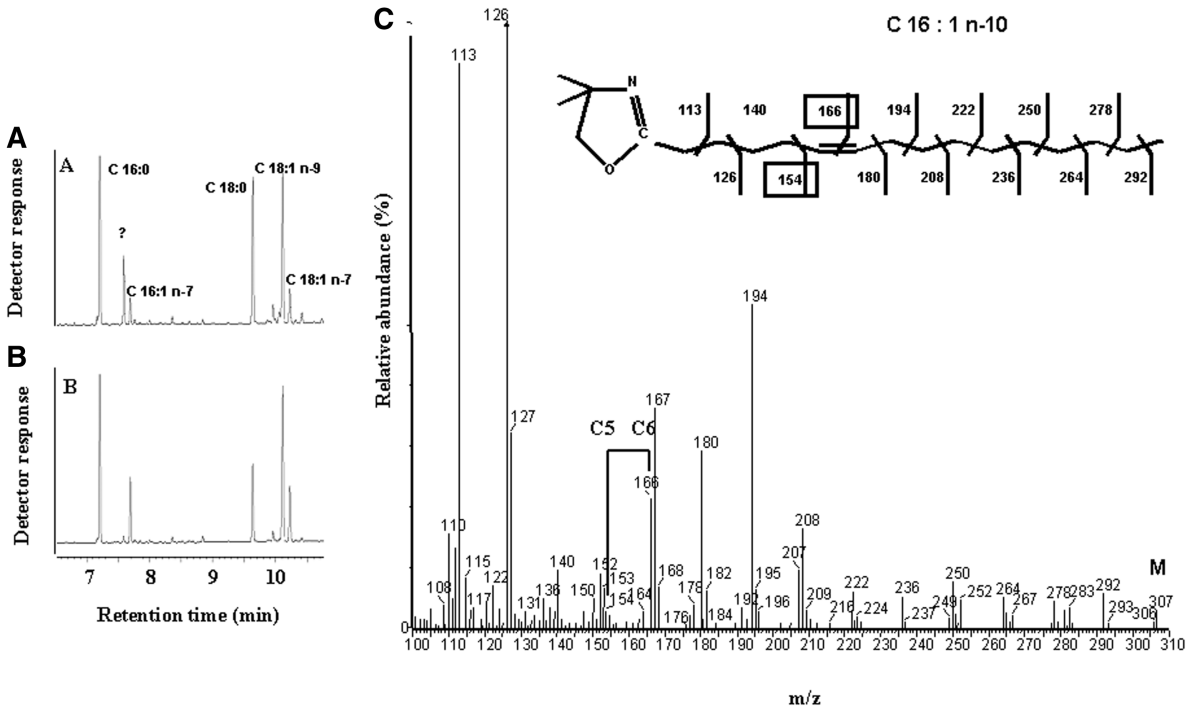

Fig. 2. Gas chromatography (GC) analysis of cellular fatty acid methyl esters from COS-7 cells incubated with C16:0 and expressing rat Δ6-desaturase (A) versus control COS-7 cells (B). GC-mass spectrometry (MS) identification of C16:1 n-10 (C). COS-7 cells transfected with pCMV/Δ6 (A) or nontransfected (B) were cultivated for 24 h with albumin-bound C16:0 (100 μM). The identity of each fatty acid peak is indicated above the respective peak. The unidentified peak was confirmed to be C16:1 n-10 by GC-MS analysis of 4,4-dimethyloxazoline derivatives (C).

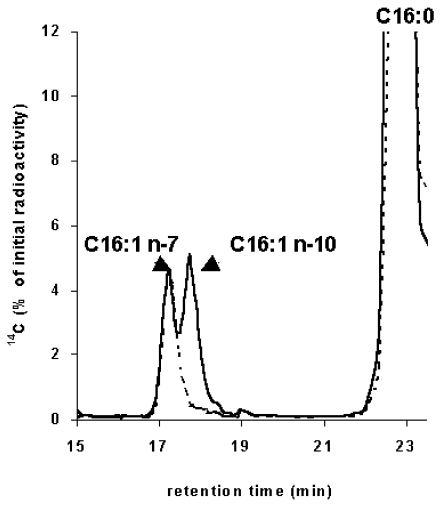

Fig. 3. HPLC analysis of cellular fatty acid naphthacyl esters from COS-7 cells incubated with [1-^{14}C]C16:0 and expressing rat Δ6-desaturase versus control COS-7 cells. COS-7 cells transfected with pCMV/Δ6 (large line) or nontransfected (dotted line) were cultivated for 24 h with albumin-bound [1-^{14}C]C16:0 (100 μM, 37 MBq/mmol). The identity of each fatty acid peak is indicated above the respective peak.

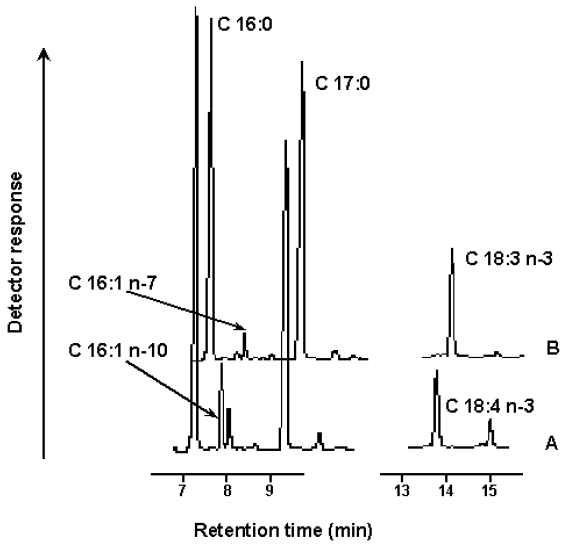

Fig. 5. GC analysis of cellular fatty acid methyl esters from COS-7 cells incubated with both C16:0 and C18:3 n-3 and expressing rat Δ6-desaturase (A) versus control COS-7 cells (B). COS-7 cells transfected with pCMV/Δ6 (A) or nontransfected (B) were cultivated for 24 h with albumin-bound fatty acids: 80 μM C16:0, 20 μM C18:3n-3. The identity of each fatty acid peak is indicated above the respective peak. C17:0 was added as an internal standard.

taining culture medium. For cotransfection of Δ6- and Δ9-desaturases, 30 μg of each plasmid was mixed before electroporation.

Western blotting

Reduced protein samples were analyzed by SDS-PAGE and blotted onto nitrocellulose (Schleicher and Schuell, Dassel, Germany). Membranes were probed with anti-rat Δ6-desaturase or anti-rat Δ9-desaturase sera as previously described (4, 7). Rabbit antibodies were revealed with horseradish-peroxidase-conjugated sheep anti-rabbit IgG (Sigma). Peroxidase activity was revealed by following the procedure provided for the ECL Plus reagent detection system kit (Amersham Biosciences, Les Ulis, France).

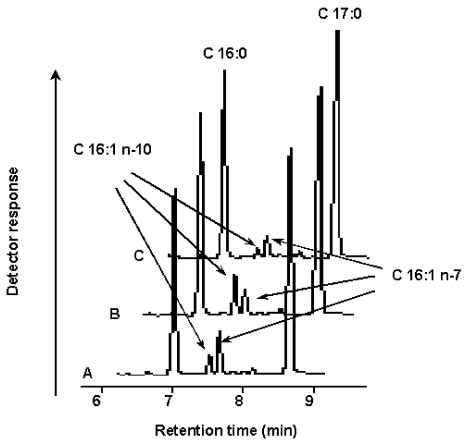

Fig. 4. GC analysis of cellular fatty acid methyl esters from COS-7 cells incubated with C16:0 and expressing both rat Δ6-desaturase and Δ9-desaturase (A), or only rat Δ6-desaturase (B), versus control COS-7 cells (C). COS-7 cells transfected with both pCMV/Δ6 and pcDNA3/Δ9 (A), with pCMV/Δ6 only (B), or nontransfected (C) were cultivated for 24 h with albumin-bound C16:0 (100 μM). The identity of each fatty acid peak is indicated above the respective peak. C17:0 was added as an internal standard.

Incubation of transfected COS-7 cells with fatty acid albuminic complexes

The activity of the expressed protein was investigated by incubating the transfected COS-7 cells with different fatty acid albuminic complexes. Fatty acid albuminic complexes were prepared as previously described (4, 9). The final fatty acid concentration of the incubation medium was 0.1 mM. When incubations were carried out with radiolabeled palmitic acid, the specific radioactivity was 37 MBq/mmol. At 3 h post-transfection, the incubation of COS-7 cells was initiated by replacing the culture medium with 10 ml of the fatty acid-containing medium per dish. Incubation was carried out for 24 h at 37°C in 5% CO_2 atmosphere.

Fatty acid analysis

COS-7 cells were washed twice with ice-cold PBS and scraped in PBS. Cellular lipids were extracted with hexane-isopropanol (3:2, v/v) as previously described (9). After saponification, fatty acids were either methylated or naphthacylated (4).

Fatty acid methyl esters were extracted with pentane and analyzed by gas chromatography (GC) using an Agilent Technologies 6890N (Bios Analytique, Toulouse, France) with a split injector (1:20) at 250°C and a bonded silica capillary column (30 m × 0.25 mm ID, BPX 70, SGE, Villeneuve-St-Georges, France) with a stationary phase of 70% cyanopropylpolysilphenylene-siloxane (0.25 μm film thickness). Helium was used as gas vector (average velocity 24 cm/s). The column temperature program started at 150°C, ramping at 2°C per min to 220°C and holding at 220°C for 10 min. The flame ionisation detector temperature was 250°C. Identification of the fatty acid methyl esters was based upon retention times obtained for methyl ester standards. A C16:1 n-10 methyl ester standard was prepared from *Thunbergia alata* seeds

that contain 80% C16:1 n-10 (10). To confirm C16:1 n-10 identity, fatty acids extracted from COS-7 cells were converted to 4,4-dimethyloxazoline derivatives (11) and analyzed by GC-mass spectrometry (GC-MS) in electron impact ionization mode (GC 8060 chromatograph coupled to a VG Platform II, Fisons Instruments, Altrincham, England). The column and gas vector were similar to those used for GC analysis.

Fatty acid naphthacyl esters were extracted and analyzed by HPLC as described earlier (4).

RESULTS

Recombinant rat Δ6-desaturase and rat SCD1 expressed in COS-7 cells were analyzed by Western blot. **Figure 1** shows the result of Western blot analysis of total homogenates obtained from COS-7 cells transfected with pCMV/Δ6 or both pcDNA3/Δ9 and pCMV/Δ6 versus nontransfected cells. A single band (45 kDa) was detected in COS-7 cells expressing rat Δ6-desaturase (Fig. 1, lanes 2 and 3). A major (42 kDa) band was detected in COS-7 cells expressing rat SCD1 (Fig. 1, lane 3). These bands were not revealed in nontransfected COS-7 cells (Fig. 1, lane 1).

In order to investigate the putative involvement of rat FADS2 protein in the Δ6-desaturation of palmitic acid, COS-7 cells transfected with pCMV/Δ6 were grown for 24 h in culture medium supplemented with palmitic acid (C16:0). COS-7 cells transfected with pCMV/Δ6 produced a detectable amount of an unknown fatty acid (**Fig. 2A**) when compared with nontransfected COS-7 cells (Fig. 2B). GC-MS analysis allowed the identification of this fatty acid as C16:1 n-10, the Δ6-desaturated product of C16:0 (Fig. 2C). We therefore established that rat Δ6-desaturase expressed in COS-7 cells confers to these cells the ability to synthesize hexadecenoic acid (C16:1 n-10). Similarly, when radiolabeled palmitic acid was incubated with COS-7 cells expressing rat Δ6-desaturase, radiolabeled C16:1 n-10 was synthesized (**Fig. 3**).

When COS-7 cells were cotransfected with both pCMV/Δ6 and pcDNA3/Δ9 and grown for 24 h in culture medium supplemented with palmitic acid (C16:0), the amount of both C16:1 n-10 (2% of total fatty acids) and C16:1 n-7 was higher than in nontransfected (0.9% of total fatty acids) (**Fig. 4**). However, the C16:1 n-10 production was lower than in cells only transfected with pCMV/Δ6 (3.7% of total fatty acids) (Fig. 4).

Finally, when COS-7 cells transfected with pCMV/Δ6 were incubated with both palmitic acid and α-linolenic acid, the production of C16:1 n-10 was still observed while C18:3 n-3 was converted to C18:4 n-3 as well (**Fig. 5**).

DISCUSSION

This study was designed to address the possible role of the characterized rat Δ6-desaturase (2, 4) in the conversion of palmitic acid to hexadecenoic acid (C16:1 n-10). We provide evidence that the expression of rat Δ6-desaturase in COS-7 cells (Fig. 1) leads to a significant conversion of C16:0 to C16:1 n-10 when cells were incubated with C16:0 (Fig. 2, 3).

Our results show that the biosynthesis of C16:1 n-10 was limited when COS-7 cells expressed both rat Δ6- and Δ9-desaturases as compared with COS-7 cells expressing Δ6-desaturase only (Fig. 4). The presence of high amounts of C16:1 n-10 has initially been reported in the preputial gland of SCD1$^{-/-}$ mice (1). Consistent with this study, our present results suggest that C16:1 n-10 biosynthesis may occur in tissues with high Δ6-desaturase expression and low SCD1 expression.

In the presence of both C16:0 and C18:3 n-3, the latter being a well-characterized substrate for rat Δ6-desaturase (4), the Δ6-desaturation of palmitic acid was not abolished, while conversion of C18:3 n-3 to C18:4 n-3 was also observed (Fig. 5). This result assesses that a high level of Δ6-desaturase expression simultaneously allows the Δ6-desaturation of C16:0 and C18:3 n-3. However, Miyazaki and coworkers (1) suggested that the conversion of C16:0 to C16:1 n-10 could be performed by an enzyme distinct from FADS2. From our study, it appears unlikely that an additional enzyme desaturates C16:0 to C16:1 n-10. Indeed, in the FADS gene cluster (12) identified in mammals, FADS1 is a Δ5-desaturase (5, 13). Moreover, we recently isolated FADS3 gene cDNA from rat (GenBank accession number AJ494720). When transfection studies were conducted for expression of rat FADS3 in COS-7 cells, the protein encoded by this cDNA was not able to desaturate C16:0 to C16:1 n-10 (data not shown).

Therefore, we show that FADS2 acts as a palmitoyl-CoA Δ6-desaturase and present the first evidence that a mammalian gene (FADS2) encodes a desaturase that acts on both PUFAs and one saturated fatty acid.

The antibody to rat liver microsomal stearoyl-CoA desaturase 1 was a generous gift from Dr. Juris Ozols (University of Connecticut Health Center, Farmington, CT). The authors thank A. Leborgne and K. L. Cung for able technical assistance.

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