Cloning, Expression, and Fatty Acid Regulation of the Human Δ-5 Desaturase*

(Received for publication, August 5, 1999, and in revised form, October 7, 1999)

HyeKyung P. Cho, Manabu Nakamura, and Steven D. Clarke‡

From the Program of Nutritional Sciences and the Institute for Cellular and Molecular Biology, The University of Texas-Austin, Austin, Texas 78712

Arachidonic (20:4(n-6)), eicosapentaenoic (20:5(n-3)), and docosahexaenoic (22:6(n-3)) acids are major components of brain and retina phospholipids, substrates for eicosanoid production, and regulators of nuclear transcription factors. One of the two rate-limiting steps in the production of these polyenoic fatty acids is the desaturation of 20:3(n-6) and 20:4(n-3) by Δ-5 desaturase. This report describes the cloning and expression of the human Δ-5 desaturase, and it compares the structural characteristics and nutritional regulation of the Δ-5 and Δ-6 desaturases. The open reading frame of the human Δ-5 desaturase encodes a 444-amino acid peptide which is identical in size to the Δ-6 desaturase and which shares 61% identity with the human Δ-6 desaturase. The Δ-5 desaturase contains two membrane-spanning domains, three histidine-rich regions, and a cytochrome b5 domain that all align perfectly with the same domains located in the Δ-6 desaturase. Expression of the open reading frame in Chinese hamster ovary cells instilled the ability to convert 20:3(n-6) to 20:4(n-6). Northern analysis revealed that many human tissues including skeletal muscle, lung, placenta, kidney, and pancreas expressed Δ-5 desaturase mRNA, but Δ-5 desaturase was most abundant in the liver, brain, and heart. However, in all tissues, the abundance of Δ-5 desaturase mRNA was much lower than that observed for the Δ-6 desaturase. When rats were fed a diet containing 10% safflower oil or menhaden fish oil, the level of hepatic mRNA for Δ-5 desaturase was much lower than that observed for the Δ-6 desaturase. When rats were fed a diet containing 10% safflower oil or menhaden fish oil, the level of hepatic mRNA for Δ-5 desaturase. When rats were fed a diet containing 10% safflower oil or menhaden fish oil, the level of hepatic mRNA for Δ-5 desaturase. When rats were fed a diet containing 10% safflower oil or menhaden fish oil, the level of hepatic mRNA for Δ-5 desaturase. However, the recent cloning of the Δ-6 desaturase revealed that the protein contains a cytochrome b5 domain which may allow the enzyme to function independently of cytochrome b5.

Studies based upon enzymatic activity suggest that the liver is the primary site for 20-carbon polyenoic fatty acid synthesis because the liver is the organ with the greatest amount of Δ-6 and Δ-5 desaturase activity (23, 24). In addition, dietary and hormonal studies indicate that the two enzymes may be coordinately regulated (25–28). For example, the enzymatic activity of both desaturases is reduced by diabetes, and this is associated with a reduced tissue content of 20:4(n-6) (27, 29, 30). Similarly, the hepatic activity of both desaturases is suppressed by fasting and induced by re-feeding carbohydrate (25, 27). However, molecular evidence for these conclusions has been lacking because the mammalian Δ-6 and Δ-5 desaturases had neither been cloned nor purified. Recently, we reported the cloning and expression of the human and mouse Δ-6 desaturase ORF (22). More importantly, Northern analysis of human tissues challenged the concept that the liver was the primary site for Δ-6 desaturase expression (22, 23, 24). Evidence from this study indicated that a clear understanding of the regulatory mechanisms governing the synthesis of biologically essential 20- and 22-carbon (n-6) and (n-3) fatty acids would require cDNAs for both desaturases. Therefore, our objective was to clone the mammalian Δ-5 desaturase and to utilize the cDNA in a comparative study of the tissue distribution and nutritional regulation of the Δ-6 and Δ-5 desaturases.

* This work was supported by the National Institutes of Health Grant DK 53872 and by the sponsors of the M. M. Love Chair in Nutritional, Cellular, and Molecular Sciences at the University of Texas-Austin (to S. D. C.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: 117 GEA, The University of Texas at Austin, Austin, TX 78712. Tel.: 512-232-1537; Fax: 512-471-5630; E-mail: stevedclarke@mail.utexas.edu.

1 The abbreviations used are: ORF, open reading frame; PCR, polymerase chain reaction; CHO, Chinese hamster ovary; bp, base pair(s); kb, kilobase pair(s).
EXPERIMENTAL PROCEDURES

Cloning of the Human Δ-5 Desaturase cDNA—A BLAST search of the human genomic data base identified a gene (GenBank™ accession number AC004770) with exon sequences that displayed high homology to the nucleotide sequence of human Δ-5 desaturase (GenBank™ accession number AF126799). Using the genomic sequence, a candidate open reading frame of the putative human Δ-5 desaturase was cloned by a forward primer containing the candidate translation start codon (5'-CGCTGGCGAGCCGAGCTGATGGA-3') and a reverse primer containing a possible translation stop codon (5'-TTATGGTTGAAGATGACATG-3'). The primers were utilized to screen by PCR amplification a human adipos-derived retinal cDNA library (Marathon-Ready cDNA; CLONTECH). The amplification conditions consisted of an initial denaturation step of 94 °C for 1 min, followed by 5 cycles of 94 °C for 10 s and 70 °C for 4 min and finally by 40 cycles of 94 °C for 10 s and 68 °C for 4 min. The resulting DNA product was cloned into the cytomegalovirus promoter expression vector pCDNA3.1 (Invitrogen) by blunt ligation using the EcoRI restriction site. In-frame orientation was confirmed by dideoxy chain termination sequencing, and the predicted amino acid sequence of the ORF was performed using MacDNASIS pro (Hitachi).

Expression of the human Δ-5 Desaturase—The functionality of the Δ-5 desaturase was established by stably transforming CHO cells with the human Δ-5 desaturase expression plasmid. CHO cells were cultured in Kaighn's modified Ham's F-12 medium supplemented with 10% fetal bovine serum. When the cells reached 80% confluence, they were transfected using LipofectAMINE, and Plus reagent (Life Technologies, Inc.) (22). After the transfection period, the cells were replated, and stable transformed cells were also treated with 200 μg/ml G418 (Geneticin, Life Technologies, Inc.). The functionality of the expressed peptide was ascertained by the changes in amount of fatty acid product, i.e. 20:4(n-6). Fatty acid content and composition of the CHO cells was determined by saponifying the cellular lipids, and subsequently methylaing the fatty acids with boron trifluoride in methanol (Sigma) (22). Methylated fatty acids were separated and quantified by gas chromatography using a fused silica glass capillary column (30 m × 0.53 mm internal diameter, Omegawax 250; Supelco) (22). Heptadecanoic acid was added at the time of saponification as an internal standard.

Human Tissue Distribution and Dietary Fat Regulation of Δ-5 Desaturase—Human tissue distribution of the Δ-5 and Δ-6 desaturases was determined by Northern blot analysis using a human tissue blot containing RNA per lane that was purchased from CLONTECH. All tissues were histologically normal, and cause of death was generally sudden trauma. The probes for Δ-5 desaturase and Δ-6 desaturase were a 204- and a 202-bp fragment, respectively. The fragments were radiolabeled with [32P]dCTP by PCR amplification using the human Δ-5 and Δ-6 desaturase cDNAs as templates: 5'-GAAATACAAGCTGCGAAGCCATAAG-3' and 5'-CCTGAAGCTTACTGAGCAGC-3' as forward and reverse primers for the Δ-5 desaturase probe; and 5'-GCGAACACTTAAGCATAC-3' as the forward primer and 5'-GAGAGTGAAGGGGCGACAAG-3' as a reverse primer for the Δ-6 desaturase probe. The Δ-5 and Δ-6 desaturase probes corresponded to the sequences of 312 to 514 and 310 to 514 nucleotides downstream of the ATG for each ORF. These regions were located downstream of the cytosome b5 domain of each protein and prior to the first histidine-rich region of each desaturase. The respective regions shared only 26% nucleotide sequence identity. The specific activity of each probe was 2.2 × 10^6 dpm per μg of DNA. The Northern membranes for Δ-5 and Δ-6 desaturase were exposed to BIOMAX MS film (Eastman Kodak) for 18 h.

The impact of type of dietary fat on the hepatic expression of Δ-5 and Δ-6 desaturase was determined by Northern analysis using total hepatic RNA (30 μg per lane) that was extracted from male Harlan Sprague Dawley rats (n = 5/group) which had been fed for 5 days a high glucose, fat-free diet supplemented with 10% (w/w) triolein (99% purity, Sigma), safflower oil (65% 18:2(n-6)), or menhaden fish oil (35% 20:5(n-3) and 22:6(n-3)) (31). Total RNA was extracted by the phenol-guanidium isothiocyanate method (22, 32). The probe for Δ-5 desaturase was the 202-bp human fragment described above, and the Δ-6 desaturase probe was the 167-bp fragment described previously (22). The specific activity of the Δ-5 and Δ-6 desaturase probes were 4.2 × 10^6 and 5.4 × 10^6 dpm/μg of DNA, respectively. Because of the low abundance of the Δ-5 desaturase relative to Δ-6 desaturase, the hepatic Northern blots were exposed for 40 h to BIOMAX MS film (Eastman Kodak) and 11 h for the Δ-5 desaturase and Δ-6 desaturase transcripts, respectively.

RESULTS AND DISCUSSION

Cloning and Structural Characteristics of the Human Δ-5 Desaturase—As part of our efforts to identify and characterize the human genomic sequence for the recently reported Δ-5 desaturase cDNA (22), we conducted a BLAST search of the human genome. The search revealed that the 1332-bp Δ-6 desaturase ORF sequence and the entire 3'-untranslated region of the Δ-6 desaturase transcript was distributed along 12 predicted exons within a 39,000-bp region of chromosome 11 (GenBank™ accession number AC004770). Further review of the surrounding sequences within this region of chromosome 11 revealed the presence of a 14,348-bp stretch DNA that was approximately 11,000-bp downstream of the first exon that contained the initiation codon for the Δ-6 desaturase ORF. Within the 14,348-bp sequence were 12 predicted exons which appeared to contain a 1332 nucleotide ORF that was in reverse orientation to the Δ-6 desaturase ORF. The candidate desaturase ORF displayed 75% nucleotide homology with the human Δ-6 desaturase. The striking homology between the human Δ-6 desaturase sequence and the downstream desaturase sequence led us to hypothesize that the new gene may encode either a Δ-6 desaturase isoform (33), or the human Δ-5 desaturase.

Using the genomic sequence information for the putative desaturase, 1350-bp cDNA was cloned from a human retina cDNA library using PCR amplification screening. Consistent with the predicted exon sequences found in chromosome 11, sequence analysis of the cDNA obtained from PCR screening of the retina library confirmed that the apparent desaturase transcript shared 75% nucleotide homology with the human Δ-6 desaturase. An ATG translation initiation codon was identified within the cDNA; and a TAA termination codon was found 1335 nucleotides downstream of the initiation site. The size of the ORF and the 444 amino acid peptide predicted by the ORF was identical to the human Δ-6 desaturase (Fig. 1A). Moreover, the amino acid sequence predicted by the ORF indicated that the desaturase candidate possessed 61% amino acid identity and 75% similarity to the human Δ-6 desaturase (22). The predicted amino acid sequence of the putative desaturase also revealed that the peptide contained all of the structural features that are characteristic of the human Δ-6 desaturase. For example, a hydropathy profile of the candidate desaturase revealed the presence of two membrane-spanning domains that are characteristic of membrane-associated proteins (34). In addition, three histidine-rich regions which may function as non-heme iron binding sites aligned almost perfectly with the same histidine-rich regions located in the Δ-6 desaturase (Fig. 1A). Most importantly, the desaturase candidate peptide contained a cytochrome b5 domain that resided in the hydrophilic N terminus (Fig. 1B), and this region was identical to the cytochrome b5 domain of human Δ-6 desaturase (22). It has been argued that the cytochrome b5 domain allows cytochrome b5 reductase to transfer electrons to the catalytic domain of the desaturase and thereby eliminate the need for cytochrome b5 reductase per se (35), but this conclusion remains to be demonstrated for the mammalian Δ-5 and Δ-6 desaturases. Although these structural features suggested that the desaturase may be a Δ-6 desaturase isoform of the retina, the histidine-rich and cytochrome b5 domains have also been reported to be components of the Δ-5 desaturase of the fungus M. alpina and the nematode Caenorhabditis elegans (36, 37). Moreover, the ap-
FIG. 1. Comparison of the predicted amino acid sequences and hydropathic patterns for human \( \Delta-5 \) and \( \Delta-6 \) desaturases. A, depicts a comparison of the amino acid sequences for human \( \Delta-5 \) desaturase and \( \Delta-6 \) desaturase (22) predicted by the nucleotide sequences for the respective ORFs. Identical amino acids are paired by vertical lines and conserved amino acids are matched by colors. The cytochrome \( b_5 \) domain is underlined. Transmembrane domains are shown in shaded areas, and three histidine-rich regions are in bold. B, depicts the hydropathic patterns for DSD (top) and D6D (bottom) as predicted by the Kyte-Doolittle method. Bars, the transmembrane regions; boxed \( H \), positions of histidine-rich regions; boxed Cyto.b5, location of a cytochrome \( b_5 \)-like domain.

parent ORF for the newly identified human desaturase possessed 54% nucleotide homology with the nucleotide sequence of Mortierella alpina and C. elegans. Thus it was possible that the desaturase candidate may in fact be the human \( \Delta-5 \) desaturase rather than a \( \Delta-6 \) desaturase isoform.

Expression of \( \Delta-5 \) Desaturase in CHO Cells—To determine whether the newly cloned desaturase was \( \Delta-5 \) or \( \Delta-6 \) desaturase, CHO cells were stably transformed with an expression plasmid containing the ORF of the putative desaturase. CHO cells that had been stably transformed with the candidate desaturase ORF were incubated with the \( \Delta-6 \) and \( \Delta-5 \) desaturase substrates, 18:3\( \alpha-3 \) and 20:3\( \alpha-6 \), respectively. Cellular fatty acid analysis revealed that the stably transformed cells did not convert 20:3\( \alpha-6 \) to 20:4\( \alpha-6 \) (data not shown), but they readily converted 20:3\( \alpha-6 \) to the \( \Delta-5 \) desaturase product, 20:4\( \alpha-6 \) (Fig. 2). In fact, cells expressing the desaturase ORF converted over 12% of the 20:3\( \alpha-6 \) to 20:4\( \alpha-6 \) during the 24 h period of incubation. In contrast, the nontransformed cells displayed no increase in total cellular content of 20:4\( \alpha-6 \) during this same time period. In addition, CHO cells expressing the desaturase ORF displayed a 3-fold increase in the content of a very long chain polyunsaturated fatty acid (>20-carbons). The identity of the fatty acid has not yet been established, but its elution pattern suggests that it is an elongation product of 20:4\( \alpha-6 \).

These functional data demonstrate that the ORF of the putative human desaturase indeed encodes the human \( \Delta-5 \) desaturase.

Tissue Distribution and Nutritional Regulation of \( \Delta-5 \) Desaturase mRNA—Northern analysis revealed that the human \( \Delta-5 \) desaturase is a single transcript that is approximately 4.4 kb in size (Fig. 3). This is slightly bigger than the 3.4- and 3.8-kb size of the human and rat \( \Delta-6 \) desaturase transcripts (22). Expression of \( \Delta-5 \) desaturase was greatest in the human liver (Fig. 3). Human heart, brain, and lung contained comparable levels of \( \Delta-5 \) desaturase mRNA, whereas low but detectable levels of expression were found in placenta, skeletal muscle, kidney, and pancreas (Fig. 3). A search of the human expressed sequence tag data base revealed that the human \( \Delta-5 \) desaturase was expressed in fetal liver, as well as the fetal spleen and heart, and in the placenta and pregnant uterus. A comparison of the relative abundance of \( \Delta-5 \) and \( \Delta-6 \) desaturase mRNA in various human tissues revealed that the level of \( \Delta-6 \) desaturase mRNA in all tissues was significantly greater than the amount of \( \Delta-5 \) desaturase mRNA (Fig. 3). This observation is particularly interesting because \( \Delta-6 \) desaturase is often considered the enzyme which catalyzes the rate-limiting step in the synthesis of 20- and 22-carbon polyunsaturated fatty acids (20, 25, 27). Regardless of which gene has the higher level of mRNA, the observation that many tissues express detectable levels of both \( \Delta-5 \) and \( \Delta-6 \) desaturase is consistent with the importance that the desaturase pathway plays in producing 20- and 22-carbon polyunsaturated fatty acids (e.g. 20:4\( \alpha-6 \)) for membrane structure and cell signaling.

In an earlier report, we noted that the hepatic abundance of \( \Delta-6 \) desaturase mRNA was relatively low and well below the amount found in brain (22). This was somewhat surprising because the liver has long been considered the primary site of \( \Delta-5 \) and \( \Delta-6 \) desaturase synthesis in vivo (25). However, the Northern blot of Fig. 3 reveals that the human liver may have a very high level of \( \Delta-6 \) desaturase. In fact, a calculation of the relative expression of the \( \Delta-5 \) and \( \Delta-6 \) desaturase mRNAs in liver versus brain revealed that the liver contains 4–5-times more \( \Delta-5 \) desaturase and 12–times more \( \Delta-6 \) desaturase than does the human brain. The relative difference in human liver expression of \( \Delta-6 \) desaturase between this report and our earlier work (22) may have two possible explanations. First, the
RNA for the earlier Northern (22) was prepared from a 35-year old male who had died of a trauma accident (CLONTECH). It is possible that aging reduces the level of hepatic expression of Δ-6 desaturase. Second and perhaps more likely is that the hepatic expression of the Δ-5 and Δ-6 desaturase genes is nutritionally regulated (Fig. 4; Ref. 22). It is very possible that the nutritional state and prior diet pattern varied between the two human donors. To resolve this conflict, the relative abundance of the Δ-5 desaturase transcripts.

A wide array of dietary studies indicate that, when animals are fed an essential fatty acid-deficient diet, the enzymatic activity of hepatic Δ-5 and Δ-6 desaturase increases 3-fold (25, 28). However, when the diet is supplemented with polyenolic fatty acids of the (n-6) and (n-3) families, these enzymatic activities are reduced (26, 28). Consistent with the enzymatic activity changes, we found that supplementing a high glucose diet was quantified in the rat brain (data not presented). Moreover, a 10-fold difference in mRNA content between liver and brain is consistent with the difference in Δ-6 desaturase enzymatic activity that reportedly exists between liver and brain (25).

Finally, an examination of the human genome data base revealed that the Δ-5 and Δ-6 desaturase genes are positioned in reverse sequence orientation to each other on chromosome 11 at 11q12.2–11q13. More importantly, the distance between the exon containing the translation start site for Δ-6 desaturase is approximately 11,000 bp from the exon that contains the translation initiation codon for Δ-5 desaturase. Although the specific promoters for the two desaturases have not yet been located, the proximity of the promoters opens the possibility that transcription of the Δ-5 and Δ-6 desaturase genes may be coordinately governed by regulatory sequences within the 11,000-bp region that are common to both genes. One additional interesting feature of the Δ-5 and Δ-6 desaturases is their location on chromosome 11. Chromosome 11 is linked to the obesity found in Pima Indians (38, 39). In light of this linkage, it is tempting to speculate that anomalies in the expression of Δ-5 and Δ-6 desaturase may play a role in the outcome or cause of the pathophysiologies associated with obesity, e.g. insulin resistance (40).

Acknowledgments—The authors thank Suzanne Barzee for the contribution to fatty acid analysis.
