EXPERIMENTAL STUDY

Structural organization and chromosomal localization of the human type II deiodinase gene

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Abstract

Objective: The selenoenzyme type 2 iodothyronine 5′-deiodinase (DII) catalyzes the conversion of thyroxine into its active form tri-iodothyronine (T3), modulating thyroid hormone homeostasis in a local, tissue-specific manner. The amphibian, rodent and human cDNAs encoding this enzyme have been recently cloned and expressed. At present, little information regarding the genomic structure of mammalian DII is available.

Design and methods: The complete structure, including intron–exon junctions, of the human DII (hDII) gene was obtained by long PCR and rapid amplification of cDNA ends (RACE). Chromosomal assignment of the hDII gene was performed by fluorescence in situ hybridization using a highly specific probe.

Results and conclusions: Our data demonstrated that hDII is a single copy gene located on chromosome 14, position 14q24.3. The gene spans over 15 kb, and the 7 kb transcript is encoded by three exons of 149 bp, 273 bp and 6.6 kb separated respectively by two 274 bp and 7.4 kb introns. A restriction map of the hDII gene is also reported. These data will help in further studies of the role of DII in the maintenance of peripheral thyroid hormone homeostasis.

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Introduction

The prohormone thyroxine (T4), which represents the main product of the thyroid gland, is converted within the gland and at a target tissue level into its active form tri-iodothyronine (T3), or into its inactive metabolite, reverse T3 (rT3) by the action of a family of seleno-proteins, the deiodinases (1). Like other selenoenzymes, the deiodinases are characterized by the presence of a selenocysteine in the catalytic domain. The selenocysteine is encoded by a UGA codon which, in the presence of a specific element in the 3′ untranslated region, the selenocysteine insertion sequence (SECIS), interacts with a selenocystein-tRNA and other components of the translational machinery (2) allowing the incorporation of the selenocysteine instead of being read as a stop codon. Mutations of critical nucleotides in the SECIS element virtually abolish the incorporation of selenocysteine and the enzymatic activity (3).

The three known isoforms of deiodinase play different physiological roles; while the thyroid hormone inactivating pathway (5′-deiodination) is mostly catalyzed by type 3 deiodinase and to some extent by type I deiodinase, the activating pathway (5′-deiodination) is catalyzed by type 1 and 2 deiodinases (4). The former is expressed primarily in liver and kidney and appears to be an important factor in the maintenance of circulating T3 levels. The expression of type 2 deiodinase (DII) is tissue specific and highly regulated. Its activity has been demonstrated in brown adipose tissue, brain, pituitary gland and placenta (1) where T3 produced by DII is utilized locally, thus generating a sort of autocrine pathway (4). The expression and activity of DII are stimulated by the hypothyroid state and in brown adipose tissue by adrenergic stimuli suggesting an important role in the thermoregulation via the interaction with the uncoupling proteins (5). Interestingly, DII is expressed in human but not rat thyroid, and its activity is stimulated in patients with Graves’ disease, probably via the thyroid-stimulating hormone (TSH) receptor signaling pathway (6). The stimulation of DII activity by the hypothyroid state and its inhibition by high levels of thyroid hormones, most likely through a post-translational mechanism (7).
demonstrate that this enzyme plays a critical role in the maintenance of local thyroid hormone homeostasis. Some authors hypothesized that a DII deficit could result in some rare forms of pituitary thyroid hormone resistance (8).

A 1.9 kb cDNA containing the open reading frame of human DII (hDII) has been recently cloned and expressed (9). Northern blot analysis demonstrated that the full length transcript is approximately 7 kb with a wider-than-expected pattern of expression. In fact, the message has also been detected in skeletal muscle, myocardium and thyroid (10). More recently we localized the hDII gene on chromosome 14q24.3 by radiation hybrid screening and demonstrated that the coding region spans two exons separated by a 7.4 kb intron (11). The chromosomal assignment of hDII has also been confirmed by fluorescence in situ hybridization (12). Recently, Leonard and coworkers presented data from well designed experiments against the actual functionality of the transcripts encoding mammalian DIs (13). However, very recently the full-length cDNAs of hDII and mouse DII have been cloned and expressed (14, 15), clearly demonstrating that mammalian DIs are indeed selenoenzymes. While the genomic organization and chromosomal localization of the human type 1 and 3 deiodinase genes have been reported (16, 17), the genomic structure of the hDII gene has still to be completely determined. In this report we present the complete structure of the hDII gene and its chromosomal localization which was determined by fluorescence in situ hybridization.

Materials and methods

Genomic clones

Two overlapping genomic P1 clones containing the entire hDII gene (clones 12258 and 12259) were purchased from Genome System (St Louis, MO, USA); the screening was performed using, as a probe, a 325 bp PCR product whose sense primer 5'-ACCCAGCTAATCTAGTTCTATCTTGCTG3' is located on exon 1 and antisense primer 5'-CTTTCATTTCAAGCAC-3' is located within the previously described intron (11). The clones were digested with HindIII, BamHI and EcoRI (Boehringer, Monza, Italy) and subcloned in pBluescript KS II (Stratagene, La Jolla, CA, USA). Sequence analysis of the subclones was performed on both strands using an automated sequencer (ABI, Foster City, CA, USA).

5' RACE

The 5' untranslated region of hDII was characterized by 5' rapid amplification of cDNA ends (RACE) using a commercially available kit (Life Technologies, San Giuliano Milanese, Italy) according to the manufacturer’s instructions. Briefly, 750 ng placental RNA was reverse transcribed using the antisense primer 5'-AACCAGCTAATCTAGTTCTATCTTGCTG3' within exon 2; the cDNA was treated with RNase H, purified and tailed with an oligonucleotide (5' RACE abridged anchor primer). The PCR was then performed using the antisense primer 5'-TTTTCATTTCACAGCCTATGAGC-3' localized at −394 bp from the ATG and a sense primer (abridged universal amplification primer) with varying concentrations of denaturing agent and Advantage GC cDNA Taq polymerase (Clontech, Palo Alto, CA, USA) according to the manufacturer's instructions. The PCR program consisted of an initial denaturation of 94°C for 2 min, followed by 35 cycles of 55°C for 30 s, 72°C for 4 min, 94°C for 20 s, and a final extension step of 72°C for 10 min. Ten microliters of the PCR products were electrophoresed on a 1% agarose gel, blotted and hybridized with 5'-TTTACCTTTCTGTTCTATG3' [32P]-radiolabeled probe. The blot was then washed at high stringency and autoradiography was performed. The hybridizing band was electroeluted, reamplified, subcloned in pCR II (Invitrogen, San Diego, CA, USA) according to the manufacturer’s instructions and sequenced on both strands.

3' RACE

The characterization of the 3' untranslated region was carried out with a commercially available 3' RACE kit (Life Technologies) according to the manufacturer’s instructions. Briefly, 750 ng placental RNA was reverse transcribed with Superscript RT II using oligo-(dT) adapter antisense primer according to the manufacturer’s instructions; the cDNA was then amplified using an antisense primer (abridged universal amplification primer) and 5'-TGAGTTAGAAAAATTTGATCGATA CACACATACAT3' sense primer located 175 bp 3' to the end of clone Z44085 (9) with varying concentrations of denaturing agent and Advantage GC cDNA Taq polymerase (Clontech) according to the manufacturer’s instructions. The PCR program consisted of an initial denaturation of 94°C for 2 min, followed by 35 cycles of 55°C for 30 s, 72°C for 4 min, 94°C for 20 s, and 55°C for 1 min and a final extension step of 72°C for 10 min. Ten microliters of the PCR products were then electrophoresed on a 1% agarose gel, blotted and hybridized with a 5'-TATTCCTAGCTGCTGGGGAGGT3' [32P]-radiolabeled probe whose sequence is 27 bp 3' to the RACE sense primer. The blot was then washed at high stringency and autoradiography was performed. The hybridizing 1.1 kb band was then electroeluted, reamplified, subcloned into pCR II (Invitrogen) according to the manufacturer’s instructions and partially sequenced. A second round of RACE using 5'-GCACATTCCAAGCTTTATCTCTTAAAATTCCACC3' sense primer located at the 3' end of the 1.1 kb RACE product using the same conditions previously described was then performed. The PCR program consisted of an initial denaturation of 94°C for 2 min, followed by 35 cycles of
60 °C for 1 min, 72 °C for 3 min, 94 °C for 1 min, and a final extension step of 72 °C for 10 min. Ten microliters of the PCR products were electrophoresed on a 1% agarose gel, blotted and hybridized with [32P]-radiolabeled 5′-ACTGGGGAAAAGGATGATGG3′ probe whose sequence is located 82 bp 3′ to the RACE primer. The blot was washed at high stringency and autoradiography was performed. The 1.3 kb hybridizing band was electroeluted, reamplified, subcloned into pCR II-TOPO according to the manufacturer’s instructions (Invitrogen) and sequenced on both strands. The sequence was submitted to GenBank, accession number AF123661.

**Long PCR**

Long PCR on 20 ng genomic DNA and 5 μl placental cDNA (see above) was performed using the sense primer 5′-GCATGCTGACCTCAGAGGGACTGCGCTGCGTCTGG3′ whose sequence is located on exon 1 and the antisense primer 5′-GCACACATAAGCCTAGCGACCAATATGATAAA CACTC3′ located at the 3′ end of clone 23964 (GenBank accession number AF007144, see Results and Discussion for details) with Takara LA Taq (Takara, Shiga, Japan) according to the manufacturer’s instructions. The PCR program consisted of an initial denaturation of 94 °C for 4 min, followed by 14 cycles of 98 °C for 20 s, 68 °C for 20 min, followed by 16 cycles of 98 °C for 20 s, 68 °C for 20 min (15 s auto-elongation each cycle), and a final extension step of 72 °C for 10 min. The resulting 13.2 kb genomic and PCR product was subcloned into pCR II Topo (pCR II hDII) (Invitrogen) according to the manufacturer’s instructions.

**Chromosomal localization**

Chromosomal localization was determined by in situ hybridization on karyotypically normal human cells. Briefly, pCR II hDII was linearized with NotI and used as a probe for chromosomal localization that was carried out on metaphase spreads obtained by standard methods from short-term lymphocyte cultures from a healthy donor. The slides were pretreated with DNase-free RNase (100 μg/ml) in 2×SSC at 37 °C for 1 h. Chromosomal DNA was denatured by incubation in 70% formamide in 2×SSC for 2 min at 70 °C, quenched in ice-cold 70% ethanol and dehydrated through a 70%, 90% and 100% ethanol series. For each slide 300 ng dCTP-Cy3-labeled (Amersham Pharmacia Biotech, Uppsala, Sweden) was added to the slides.
Cologno Monzese, Italy) pCRII hDII and 20 ng bio-16 dUTP-labeled (Boehringer, Monza, Italy) p14.1 were mixed in the hybridization buffer (50% formamide in 2x SSC, dextran sulphate 10%), and denatured for 8 min at 80°C. Hybridization was carried out overnight at 37°C. Post-hybridization washes were performed at high stringency. The aphloid biotin-labeled sequences were detected with avidine–fluorescein isothiocyanate (FITC) (Vector, Burlingame, CA, USA) diluted 1:300. After counterstaining in 4'-6 diamidino-2-phenylindole (DAPI), fluorescent images were captured with a CCD camera (Photometrics 01) using IPLab software (Signal Analytic Corporation) and processed with a Macintosh Power Corporation) and processed with a Macintosh Power (Photometrics 01) using IPLab software (Signal Analytic Corporation) and processed with a Macintosh Power.[58x747]270

**Results and Discussion**

The 5’ untranslated region of hDII gene was characterized by 5’RACE. The presence of a 274 bp intron at −51 bp from the ATG was demonstrated by comparing the RACE product sequence with the one obtained from a genomic subclone. Interestingly, 83 base pairs 5’ to the splice junction are within the originally published brain cDNA clone (GenBank accession number Z44085) (9); this finding is compatible with either an alternative splicing mechanism or a cloning artifact. Two transcription start sites were mapped at −470/−474 bp from the ATG with primer elongation experiments and an RT-PCR performed using a sense primer located immediately 3’ to the transcription start site confirmed the presence of such intron (data not shown). The sequence of the 5’UT and 803 bp 5’ to the transcription start site has been submitted to GenBank, accession number AF079364. These data are in keeping with the very recent manuscript from Bartha and coworkers who demonstrated the presence of a transcription start site 708 bp 5’ to the ATG and an alternative splicing of the 5’ untranslated region in thyroid tissue (19). Northern blot analysis performed on placental RNA demonstrated a single 7 kb hybridizing band and no amplification was obtained by a PCR performed on placental cDNA using a sense primer located immediately 3’ to the −708 bp transcription start site, suggesting that a single transcript is present in this tissue (data not shown). An almost complete degree of homology, with the exception of a 32 bp deletion in the 274 bp intron, was observed by comparing our data with the recent GenBank submission, accession number AC007372, containing the entire hDII locus.

The characterization of the 3’ untranslated region was carried out with a commercially available 3’RACE kit in two sequential experiments using a gene walking strategy. The 1.3 kb PCR product resulting from the second experiment was subcloned and sequenced on both strands. The sequence was submitted to GenBank, accession number AF123661. A GenBank search using a 200 bp 3’ end sequence demonstrated 100% homology with a cDNA clone 23964 accession number AF007144. A computerized structure analysis with RNAdraw (20) on the last 300 bp of the 23964 clone demonstrated the presence of a putative SECIS element. Interestingly, the sequence of the hDII putative SECIS element shows a high degree of homology, 85%, with the mouse SECIS element (Fig. 1A).

The long PCR experiments on genomic DNA and placental cDNA resulted in a 13.2 kb genomic product and a 5.8 kb cDNA PCR product, thus demonstrating the continuity of the gene and the absence of large size introns in the 3’ end of the gene (Fig. 1B). The result was confirmed by Southern blot analysis (data not shown) and a restriction map of the hDII gene was obtained (Fig. 1C); the exon–intron splicing junctions are also reported (Fig. 1D).

Previous screening of two different radiation hybrid panels demonstrated that the hDII gene is located on chromosome 14, at q24.3 on a linkage integrated cytogenetic map (11). The result was also confirmed by fluorescence in situ hybridization (12). In situ hybridization of hDII on normal human cells using a gene-specific probe containing part of exon 1, the 7.4 kb intron and most of the exon 2 demonstrated unequivocally that hDII is a single copy gene (Fig. 2).

In conclusion, we now report the complete genomic
structure and chromosomal localization of the hDII gene. This data will provide further tools for the study of the molecular mechanisms of peripheral thyroid hormone metabolism in humans and possibly for molecular genetics studies of some forms of thyroid hormone resistance not due to mutations of the thyroid hormone receptor-β gene.

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References


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