Two ornithine decarboxylase mRNA species in mouse kidney arise from size heterogeneity at their 3' termini

(androgen action/ornithine decarboxylase genes/polyadenylation/blot hybridization/DNA sequencing)

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ABSTRACT Ornithine decarboxylase (OrnDCase; L-ornithine carboxy-lyase, EC 4.1.1.17) mRNA present in mouse kidney comprises two species with molecular sizes of ≈ 2.2 and ≈ 2.7 kilobases (kb). cDNA clones prepared from murine kidney OrnDCase mRNA were used to determine the reason for the size heterogeneity of these mRNAs. Two of the cDNA clones (pODC16 and pODC74) that differed at the 3' termini were isolated and sequenced. DNA sequencing indicated that each cDNA had a poly(A) tail; however, pODC74 was 429 nucleotides longer than pODC16 at the 3' end and contained two AATAAA signals for poly(A) addition. That the longer cDNA corresponded to the larger mRNA was confirmed by hybridization of a unique *Pst* I/*Pst* I fragment from the 3' terminus of pODC74 only to the 2.7-kb OrnDCase mRNA. The two cDNAs did not represent full-length copies of OrnDCase mRNAs and were 1199 (pODC16) and 1204 base pairs (bp) (pODC74) long. There were five mismatches in their 759-bp-long overlapping nucleotide sequence, suggesting that the 2.2- and 2.7-kb OrnDCase mRNAs may be products of two separate, yet very similar, OrnDCase genes. Androgen regulation of the accumulation of these two OrnDCase mRNAs appeared to occur coordinately, as testosterone administration brought about comparable increases in their concentrations in mouse kidney.

Ornithine decarboxylase (OrnDCase; L-ornithine carboxy-lyase, EC 4.1.1.17) catalyzes the conversion of ornithine to putrescine and is the first and apparently rate-controlling enzyme in polyamine biosynthesis (1–3). In the murine kidney, OrnDCase activity and the enzyme protein concentration are increased by androgen treatment with relatively rapid kinetics, with a maximal induction at 18–24 hr after steroid administration (4, 5). In addition to enhancing enzyme synthesis, androgen administration increases OrnDCase concentration through prolonging the biological half-life of the enzyme protein (5, 6). To better understand the mechanisms regulating OrnDCase synthesis, workers in our own (7) and other laboratories (8–10) have prepared cDNA clones for the mRNA encoding OrnDCase. When these cDNAs were used to identify OrnDCase mRNA by hybridization after agarose gel electrophoresis, two mRNA species of ≈ 2.2 and ≈ 2.7 kilobases (kb) were found (7, 10). The nucleotide sequence and deduced amino acid sequence of the shorter OrnDCase mRNA have been recently published (11, 12). We report here the nucleotide sequence of the 3' ends of the two OrnDCase mRNA species in mouse kidney. From the sequences and from RNA blot hybridization data using the unique 3'-terminal probe from one of the clones, we conclude that the size heterogeneity of the two OrnDCase mRNAs is due to the dissimilar lengths of their 3' noncoding regions. In addition,

DNA sequence data suggest that they could be products of two similar but separate OrnDCase genes.

MATERIALS AND METHODS

Animals. Mature male and female NCS [randomly bred strain Rku:NCS(s) SPF] and 129/J mice were from The Rockefeller University and The Jackson Laboratory, respectively. The animals were treated for 7 days with testosterone-containing Silastic implants releasing either 40 or 200 μ g of testosterone per day.

OrnDCase cDNAs. The preparation and cloning of the two OrnDCase cDNAs (pODC16 and pODC74) have been described (7, 13). The plasmids were propagated in *Escherichia coli* strain LE 392 and purified by a modification of the method of Ish-Horowicz and Burke (14) followed by CsCl density gradient centrifugation.

End-Labeling and Sequencing of cDNAs. Plasmids carrying OrnDCase cDNAs were digested overnight with the appropriate restriction enzymes, ethanol-precipitated, and labeled at the 3' ends with [α - 32 P]dNTPs using the Klenow fragment of DNA polymerase I, or by the terminal deoxyribonucleotidyltransferase-catalyzed addition of cordycepin 5'-[α - 32 P]triphosphate. Labeled fragments were isolated by electrophoresis on 6% polyacrylamide gels under non-denaturing conditions, recovered from the gel slices by electroelution, and ethanol-precipitated. Isolated fragments were recut, purified by electrophoresis, and sequenced by the chemical degradation method of Maxam and Gilbert (15), as modified by Catterall *et al.* (16). Each sample was subjected to electrophoresis on 20%, two 10% and 8% polyacrylamide sequencing gels with 7 M urea/90 mM Tris-HCl/90 mM borate/2 mM EDTA buffer, pH 8.3, and autoradiographed at -70°C using Kodak XAR-5 film.

Isolation of Renal RNA and Gel Blot Hybridizations. Total RNA was isolated from murine kidney by the lithium chloride/urea method (17) and enriched for poly(A)-containing RNA by oligo(dT)-cellulose chromatography (18). RNA samples (4–6 μ g) were fractionated on 1% agarose gels containing 2.2 M formaldehyde (19), transferred to nitrocellulose filters, and hybridized with radioactive probes (20). The probes used for hybridization were isolated from pODC74 or pODC16 by preparative gel electrophoresis on 6% polyacrylamide gels after cleavage with appropriate restriction enzymes, and labeled with [α - 32 P]dCTP by nick-translation. The terminal *Pst* I/*Pst* I fragment of pODC74 (from the cloning site to the first *Pst* I site at the 3' end) was the unique 3' probe, whereas the *Hpa* II/*Hha* I fragment of pODC74 or the 5' *Pst* I/*Pst* I fragment of pODC16 were the probes representing overlapping sequences (see Fig. 1). Quantitation of the signals was achieved by excision of the

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Abbreviations: OrnDCase, ornithine decarboxylase; kb, kilobase(s); bp, base pair(s).

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areas corresponding to the OrnDCase mRNAs from the nitrocellulose filters followed by measurement of their radioactivity in Liquiscint (National Diagnostics, Somerville, NJ) as the scintillation fluid.

Materials. Restriction enzymes, the Klenow fragment of DNA polymerase I, terminal deoxyribonucleotidyltransferase, and urea were purchased from Bethesda Research Laboratories; radioisotopes were from New England Nuclear; oligo(dT)-cellulose was a product of Collaborative Research (Lexington, MA); reagents for polyacrylamide gel electrophoresis were obtained from Bio-Rad; and testosterone was from Steraloids (Wilton, NH). Other chemicals were purchased from either Sigma or Fisher and were of the highest purity grade available.

RESULTS

In previous studies, mouse kidney OrnDCase mRNA has been observed to be composed of two species with approximate molecular sizes of 2.2 and 2.7 kb (7, 10, 13). During the characterization of OrnDCase cDNAs, we identified two cDNAs (pODC16 and pODC74) with dissimilar 3' termini and reasoned that they could represent cDNAs originating from the two OrnDCase mRNA species. To study this possibility, we isolated a number of fragments from the plasmids carrying OrnDCase cDNA sequences and used them as hybridization probes in RNA gel blot analysis. As shown in Fig. 1, the most 3' sequence of pODC74 (*Pst* I/*Pst* I fragment) hybridized only to the 2.7-kb OrnDCase mRNA, whereas fragments isolated from overlapping regions of pODC16 and pODC74 hybridized to both 2.2- and 2.7-kb mRNAs. Accumulation of the two OrnDCase mRNAs was regulated by androgen treatment. The data presented in Fig. 2 indicate that testosterone treatment of female mice increased the accumulation of the 2.2- and 2.7-kb OrnDCase mRNAs in a parallel fashion; the concentration of each mRNA was 3- and 7-fold higher than in intact males at daily testosterone doses

of 40 and 200 μ g, respectively. The relative changes measured for the accumulation of the 2.7-kb mRNA were independent of the hybridization probe used (Fig. 2B). For this particular study, control female levels are not shown, because the low hybridization signals precluded accurate radioactivity measurements from the excised nitrocellulose filters. However, our previous results (7) and a long exposure of the filters have indicated that the two OrnDCase mRNAs are constitutively expressed in kidneys of intact female mice.

To determine the extent of the differences in the 3' termini of the two OrnDCase cDNAs, to ensure that both cDNAs (pODC16 and pODC74) had poly(A) tracts, and, perhaps, to gain information about how the 2.7-kb OrnDCase mRNA arose, we sequenced the two OrnDCase cDNAs by the chemical degradation method (15). The sequencing strategy is shown in Fig. 3. pODC16 contained an 1199-base-pair (bp) cDNA insert corresponding to the 3' end of the 2.2-kb OrnDCase mRNA. Like the sequence previously reported for OrnDCase cDNA (11, 12), pODC16 had a 329-nucleotide-long 3' noncoding region containing an AATAAA signal 18 nucleotides upstream from the poly(A) addition site (Fig. 4). Our sequence differed from that reported by Gupta and Coffino (11) at nucleotides 1, 87, 108, 381, 402, 567, 894, and 895 of pODC16. Six of the mismatches fell in the coding region with the change of an A to a T at nucleotide 1 of pODC16, causing a change in amino acid sequence from the reported arginine to tryptophan; and the change of a T to a G at nucleotide 87, resulting in a glutamic acid rather than aspartic acid. The other four differences at nucleotides 108, 381, 402, and 567 in the coding region were silent.

pODC74 contained a 1204-bp insert corresponding to the 3' end of the 2.7-kb OrnDCase mRNA (Fig. 4). In the region of overlap between pODC16 and pODC74, they differed in five nucleotides at positions 517, 567, 894, 895, and 1058. Two of these mismatches fell in the coding region; the one at nucleotide 517 (C to T) resulted in a tyrosine to histidine change in pODC74, while the other mismatch was silent.

Fig. 1. Gel blot hybridization analysis of OrnDCase mRNAs with specific probes. Poly(A)-containing RNA was isolated from kidneys of androgen-treated NCS mice by the lithium chloride/urea method and oligo(dT)-cellulose chromatography. Duplicate samples (6 μ g of RNA) were subjected to electrophoresis on a 1% agarose gel containing 2.2 M formaldehyde and were transferred to nitrocellulose. The indicated fragments were cleaved from pODC16 and pODC74, isolated by polyacrylamide gel electrophoresis, nick-translated, and used as hybridization probes. Fragments from λ phage DNA cleaved with *Hind*III served as molecular size markers.

FIG. 2. Androgen-regulation of OrndCase mRNA species. Renal RNA was isolated from kidneys of intact male mice (M) (129/J strain) and from female animals treated for 7 days with Silastic implants releasing either 40 μ g (T-40) or 200 μ g (T-200) of testosterone per day. Duplicate samples of polyadenylated RNA (4 μ g per lane) were fractionated on agarose gels and RNA was transferred to nitrocellulose as described in the text and in the legend to Fig. 1. The most 5' *Pst* I/*Pst* I fragment from pODC16 was used as the hybridization probe in A, whereas the most 3' *Pst* I/*Pst* I fragment from pODC74 was the hybridization probe in B. Exposure times were 7 hr at room temperature and 16 hr at -70°C for A and B, respectively. The numbers above each panel show the mean radioactivity on the nitrocellulose containing the 2.7-kb OrndCase mRNA bands. The corresponding information for the 2.2-kb mRNA is shown by numbers below lanes in A.

Only one of the mismatches altered a restriction site in our two cDNAs; a *Sau*3A site was present in pODC16 at nucleotides 564–567 but it was absent in pODC74. This difference was confirmed by an appropriate restriction enzyme digestion (data not shown). pODC74 contained the same polyadenylation signal as pODC16 at 312 nucleotides from the translation termination codon; however, there was another AATAAA signal 422 nucleotides downstream resulting in a 3' noncoding region 748 nucleotides long for pODC74.

DISCUSSION

The present study indicates that at least two OrndCase mRNA species are expressed and coordinately regulated by androgens in murine kidney. Under our experimental conditions, the longer OrndCase mRNA (2.7 kb) was always present in a lower concentration than the 2.2-kb mRNA, a finding previously observed for both murine kidney (7, 10, 13) and other mouse tissues or cell lines (8, 9). The cDNA clones corresponding to the two mRNAs differed by 419 bp in their

3' nontranslated regions, which was in good agreement with the estimated size difference for the 2.2- and 2.7-kb OrndCase mRNAs in the renal tissue (7, 13). That size heterogeneity at the 3' termini of the two OrndCase mRNAs is the major reason for their dissimilar molecular sizes was further shown by hybridization of the mRNAs with a unique 3'-terminal fragment of pODC74. However, since our cDNA clones were not full-length copies of the mRNAs, we could not rule out the possibility that some size heterogeneity also exists at the 5' ends of OrndCase mRNAs, as would be the case when different promoters are used.

There are numerous reports to indicate that a single gene can encode multiple mRNAs. The possible mechanisms involve the use of different promoters (21–23), the presence of multiple polyadenylation/termination signals (24–27), and alternative splicing that leads to inclusion or deletion of exons and introns (24, 25, 28–31). The 3'-nontranslated region of pODC74 contained two potential polyadenylation signals (AATAAA), and utilization of the second AATAAA signal for poly(A) addition seems to be the reason for the

FIG. 3. Sequencing strategy of the two OrndCase cDNAs. Arrows indicate the direction of DNA sequencing that was performed by the chemical degradation method (15). All fragments were labeled at the 3' end and the tail of an arrow represents the position of the radioactive nucleotide. □, pBR322; —, OrndCase cDNA sequences.

FIG. 4. Comparison of the nucleotide sequences and deduced amino acid sequences of pODC16 and pODC74. The numbers on left start from the first nucleotide of pODC16. The sequence of pODC16 is shown by the upper lines. —, No mismatch between the two sequences; ●, mismatch between pODC16 and pODC74 (a change from C to T at nucleotide 517 results in a tyrosine codon in pODC74).

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