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Cloning and Sequencing of Mouse Complementary DNA for Heme Oxygenase-2

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A mouse cDNA encoding a human homologue of heme oxygenase-2 (HO-2) was isolated. The deduced protein contains 315 amino acids and has a calculated molecular mass of 35.8 kDa. The nucleotide sequence is 85.6% identical and the amino acid sequence 87% identical to those of the human protein. The corresponding mRNA is present in brain and testis, but not in ovary, kidney, liver, or spleen.

Key words: cloning, HO-2, mouse.

It has long been known that heme oxygenase (HO: metabolizes heme to biliverdin, free iron, and CO) is a key enzyme in heme catabolism, but recently it was also found to act as an oxidative stress protein, producing carbon monoxide (CO) (*1*), which has similar actions to those of nitrogen monoxide (NO). Separate genes encode two isoforms of HO, and one of the isoforms, HO-1, is expressed mainly in peripheral organs including the liver. But the other isoform, HO-2, is present throughout the brain, with high expression in hippocampal CA1 pyramidal cells (*2*), and is also present in testicular germ cells (*3, 4, cf.* Ref. *5*). There is evidence that CO may participate in olfactory response (*2*) and smooth muscle relaxation (*6*). Then recently, CO has been considered as a candidate retrograde messenger in the hippocampus, based on experiments showing that metalloporphyrin (ex. Znpp) inhibitors of HO are capable of blocking the induction of long-term potentiation in hippocampal slices (*7, 8*). The HO-2 isoform is mainly present in the brain, so HO-2 may play an important role in hippocampal long-term potentiation (*9*). Moreover, HO-2 is also expressed during the development of testicular germ cells and also may have an important role in sperm development. Then we tried to isolate a mouse HO-2 cDNA clone using a human HO-2 probe. Here, we report the cloning and sequencing of a mouse HO-2 cDNA.

HO-2 is highly expressed in testicular germ cells, so an oligo(dT)-primed cDNA library prepared from mouse testes (donated by Dr. H. Nojima) was used to clone the mouse HO-2 cDNA. This cDNA library (2×10^6 clones) was screened with a human HO-2 cDNA probe (donated by Dr. S. Shibahara). One clone was obtained on the screening, which was a full-length mouse HO-2 cDNA clone. The sequence of this cDNA was determined by the dideoxy necleotide chain termination method (*10*). The total nucleotide sequence is shown in Fig. 1, together with the deduced amino acid sequence. The longest open leading frame was 945 bp, comprising bases 133-1077 and encoding 315 amino acids. The expected molecular mass of HO encoded by the cDNA was 35.8 kDa. In the 3′-untranslated region, a putative polyadenylation signal, AATAAA, is located 14-19 upstream of the poly(A^+) tract. A search for homology with other mamalian HO-2 genes revealed that the mouse HO-2 gene is 87.0, 85.1, and 95.2% identical to those of human, rabbit, and rat, respectively, in amino acid sequence [Fig. 2; *cf.* Refs. *11-13*) and 85.6% identical to the human HO-2 gene in nucleic acid sequence. And the heme oxygenase signature (LLVAHAYTR) is conserved in this amino acid sequence. We conclude, therefore, that we have cloned a mouse homologue of human HO-2 cDNA.

Northern blot analysis was carried out using our mouse HO-2 cDNA as a probe (Fig. 3). A major transcript of about 1.35 kb was found in testis and brain, but not in ovary, liver, kidney, or spleen. In spite of the many observations on HO-2, its physiological role remains to be clarified. The cDNA clone for mouse HO-2 may constitute a powerful tool for answering this question.

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GCCACCTCCCCTTTGACCACCTTGGGTTGCCCTCTTAACAAAGTTGGCCTTACCAAGGAG    60
CAGTCATCTTGGATTGTATAATTTGAATGAGCCAAGGACCAGAGTGAGGGCAGCACAAAC   120

TACTCAGCCACAATGTCTTCAGAGGTGGAGACCTCGGAGGGGGTAGATGAGTCAGAGAAG   180
            M  S  S  E  V  E  T  S  E  G  V  D  E  S  E  K      16

AACTCTATGGCACCAGAAAAGGAAAACCATACCAAAATGGCAGACCTTTCTGAGCTCGAG   240
N  S  M  A  P  E  K  E  N  H  T  K  M  A  D  L  S  E  L  E     36

CCGGAAGGGACCAAGGAAGCACATGACCGAGCAGAAAATACCCAGTTTGTCAAAGACTTC   300
P  E  G  T  K  E  A  H  D  R  A  E  N  T  Q  F  V  K  D  F     56

TTGAAAGGAAACATTAAGAAGGAGCTATTTAAGCTGGCCACCACTGCACTTTACTTCACA   360
L  K  G  N  I  K  K  E  L  F  K  L  A  T  T  A  L  Y  F  T     76

TACTCAGCCCTTGAGGAGGAAATGGACCGCAACAAGGACCACCCAGCCTTCGCCCCCTTA   420
Y  S  A  L  E  E  E  M  D  R  N  K  D  H  P  A  F  A  P  L     96

TATTTCCCCACGGAGCTACACCGGAAGGCAGCACTGATCAAGGACATGAAGTATTTCTTT   480
Y  F  P  T  E  L  H  R  K  A  A  L  I  K  D  M  K  Y  F  F    116

GGTGAAAACTGGGAGGAGCAGGTGAAGTGCTCTGAGGCTGCCCAGAAGTATGTGGATCGG   540
G  E  N  W  E  E  Q  V  K  C  S  E  A  A  Q  K  Y  V  D  R    136

ATTCACTATGTAGGGCAAAATGAGCCAGAGCTGCTGGTGGCCCATGCTTATACTCGTTAC   600
I  H  Y  V  G  Q  N  E  P  E  L  L  V  A  H  A  Y  T  R  Y    156

ATGGGGGACCTTTCAGGAGGCCAGGTACTGAAGAAGGTTGCCCAGAGGGCACTAAAACTT   660
M  G  D  L  S  G  G  Q  V  L  K  K  V  A  Q  R  A  L  K  L    176

CCCAGCACTGGGGAAGGGACCCAATTCTACCTGTTTGAGCATGTGGACAATGCCCAGCAA   720
P  S  T  G  E  G  T  Q  F  Y  L  F  E  H  V  D  N  A  Q  Q    196

TTCAAGCAGTTCTACCGCGCTAGAATGAATGCCTTGGACCTGAATTTGAAGACCAAAGAG   780
F  K  Q  F  Y  R  A  R  M  N  A  L  D  L  N  L  K  T  K  E    216

AGGATTGTGGAGGAGGCCAATAAAGCCTTTGAATATAACATGCAGATATTCAGTGAACTG   840
R  I  V  E  E  A  N  K  A  F  E  Y  N  M  Q  I  F  S  E  L    236

GACCAGGCTGGCTCCATGCTAGCAAGAGAAACCCTGGAGGATGGGCTCCCGGTACATGAT   900
D  Q  A  G  S  M  L  A  R  E  T  L  E  D  G  L  P  V  H  D    256

GGGAAGGGAGATATACGTAAATGCCCCTTTTATGCTGCTCAGCCAGACAAAGGTACACTA   960
G  K  G  D  I  R  K  C  P  F  Y  A  A  Q  P  D  K  G  T  L    276

GGAGGCAGAAACTGCCCCTTCCAGACAACCGTGGCTGTGCTGAGGAAGCCTAGCCTGCAG  1020
G  G  R  N  C  P  F  Q  T  T  V  A  V  L  R  K  P  S  L  Q    296

CTCATTCTGGCTGCCAGTGTGGCCTTGGTAGCTGGACTCTTGGCCTGGTACTACATGTGA  1080
L  I  L  A  A  S  V  A  L  V  A  G  L  L  A  W  Y  Y  M       315

AGGACCTGTCATGTTGTTTGCATCCTACCCCAAGTGACCTGTTCTATCCCCATCTCCACC  1140
TCTGCCTAAAACTACCACCTCAGGTGACTTTTTTTTTAATGCTGGGTTTGAGAAAACAAGC  1200
AACCAATAAAAGCCAGACACTAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA  1260
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Fig. 1. **Full length cDNA sequence of mouse HO-2.** The conserved heme oxygenase signature is double-underlined and the poly A singnal is sigle-underlined.

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mouse     1' MSSEVETSEGVDESEKNSMAP-EKENHTKMADLSELEPEGTKEAHDRAENTQFVKDFLKG
             **.*************.. .. ****.******** **********************
human     1* MSAEVETSEGVDESEKKNSGALEKENQMRMADLSELLKEGTKEAHDRAENTQFVKDFLKG
             **.**********.*.....  ***.******** ************.*********
rabbit    1  MSAEVETSEGVDEPEEKNFG----ENHIRMADLSELLKEGTKEAHDRAENTKFVKDFLKG
             ***************.** **_********* **********************
rat       1* MSSEVETSEGVDESENNSTAP-EKENHTKMADLSELLKEGTKEAHDRAENTQFVKDFLKG

           60' NIKKELFKLATTALYFTYSALEEEMDRNKDHPAFAPLYFPTELHRKAALIKDMKYFFGEN
               ************************.************* *****.**.***.******
           61* NIKKELFKLATTALYFTYSALEEEMERNKDHPAFAPLYFPMELHRKEALTKDMEYFFGEN
               *****.**************************************** *****.**.***.******
           57* NIKKEIFKLATTALYFTYSALEEEMDRNKDHPAFAPLYFPMELHRKEALTKDMEYFFGEN
               ************************************************.******.******
           60* NIKKELFKLATTALYFTYSALEEEMDRNKDHPAFAPLYFPTELHRKEALIKDMEYFFGEN

          120' WEEQVKCSEAAQKYVDRIHYVGQNEPELLVAHAYTRYMGDLSGGQVLKKVAQRALKLPST
               *****.*..******.*****.**********************************
          121* WEEQVQCPKAAQKYVERIHYIGQNEPELLVAHAYTRYMGDLSGGQVLKKVAQRALKLPST
               *****.*********.*****.**********************************
          117* WEEQVQCSEAAQKYVERIHYIGQNEPELLVAHAYTRYMGDLSGGQVLKKVAQRALKLPST
               ************************************************************
          120* WEEQVKCSEAAQKYVDRIHYVGQNEPELLVAHAYTRYMGDLSGGQVLKKVAQRALKLPST

          180' GEGTQFYLFEHVDNAQQFKQFYRARMNALDLNLKTKERIVEEANKAFEYNMQIFSELDQA
               **********.*********.**********.*********************.*****
          181* GEGTQFYLFENVDNAQQFKQLYRARMNALDLNMKTKERIVEEANKAFEYNMQIFNELDQA
               **********.**************************************************
          177* GEGTQFYLFENVDNAQQFKQFYRARMNALDLNLKTKERIVEEANKAFEYNMQIFSELDQA
               ****************************..****************************
          181* GEGTQFYLFEHVDNAQQFKQFYRARMNALDLSMKTKERIVEEANKAFEYNMQIFSELDQA

          240' GSMLARETLEDGLPVHDGKGDIRKCPFYAAQPDKGTLGGRNCPFQTTVAVLRKPSLQLIL
               ** *********.********.********..***.*.*..***.*..*********.**
          241* GSTLARETLEDGFPVHDGKGDMRKCPFYAAEQDKGALEGSSCPFRTAMAVLRKPSLQFIL
               **  *.**.**  .********.****.***  . .*.*.*..***....**********.*
          237* GSAPASETVEDRIPVHDGKGDVRKCPYYAAGQVNGALEGSSCPFRAAMAVLRKPSLQLVL
               ***..*****************.**********.****.*..**************
          241* GSMLTKETLEDGLPVHDGKGDVRKCPFYAAQPDKGTLGGSNCPFRTAMAVLRKPSLQLIL


          300' AASVALVAGLLAWYYM
               **.***.*********
          301* AAGVALAAGLLAWYYMSTHHATPV
               **.***.*********
          297* AAAVALAAGLLAWYYM
               ****************
          301* AASVALVAGLLAWYYM
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Fig. 2. **Amino acid alignment of the HO-2 proteins of mouse, human, rabbit, and rat.** Identity of the HO-2 sequences is denoted by asterisks (*) and similarity of the sequences is denoted by dots (.). The search for sequences homologous to HO-2 was performed with the BLAST Program Network Service at the Institute of Chemical Research, Kyoto University, Kyoto.

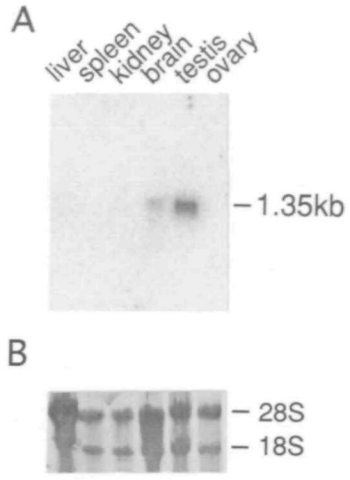

Fig. 3. **Northern blot analysis.** A: Total RNAs were extracted from mouse (ICR) ovary, testis, brain, liver, kidney, and spleen. Ten micrograms of RNA was separated by electrophoresis in a 1% agarose gel with formaldehyde and then transferred to a nylon membrane. The membrane was hybridized with the ^{32}P-labeled HO-2 cDNA probe and then washed with 0.1×SSC, 0.1% SDS at 55°C. Imaging was performed with a BAS2000 Fuji Imaging system. B: As a control, the filter was stained with methylene blue, and 28S and 18S rRNAs were detected.

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