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Cloning and sequence analysis of cDNA for human renin precursor

(hypertension/aspartyl proteinase/sequence homology/recombinant DNA/kidney)

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ABSTRACT **The primary structure of human renin precursor has been deduced from its cDNA sequence. A library of cDNA clones was constructed from human kidney poly(A)$^+$ RNA by applying the vector/primer method of Okayama and Berg. The library was screened for human renin sequences by hybridization with the previously cloned mouse renin cDNA. Of the 240,000 colonies screened, 35 colonies that were positive for hybridization were isolated. Two recombinant plasmids containing long inserts of about 1,300 and 1,600 base pairs were selected for sequence analysis. The amino acid sequence predicted from the cDNA sequence shows that the human renin precursor consists of 406 amino acids with a pre and a pro segment carrying 20 and 46 amino acids, respectively. A high degree of sequence homology was found upon comparison of the mouse and human renins. Close similarities were also observed in the primary structures of renin and aspartyl proteinases that have known three-dimensional structures, suggesting a similar tertiary structure for renin.**

The renin–angiotensin system plays an important role in the regulation of blood pressure and electrolyte balance (1, 2). Activation of the system is initiated by the release of the enzyme renin from the kidney into the bloodstream, where it acts on its substrate to produce angiotensin I. The decapeptide angiotensin I is subsequently converted to angiotensin II, an octapeptide that causes marked vasoconstriction of arteriolar smooth muscle and stimulates aldosterone secretion in the adrenal cortex. Because the aspartyl proteinase renin is the initial and the rate-limiting component of the system, there has been considerable interest in the study of this enzyme, yet progress in elucidating the biochemical properties of renin, especially human renin, has been slow because of the limited supply of human kidneys and minute quantities of renin present in the kidney. Although the use of sophisticated methods of protein purification and characterization has circumvented these potential problems to some extent and improved the knowledge on renin significantly, precise identification of the structure of the renin molecule has not been achieved yet. For example, there are conflicting reports on the molecular weight and the amino acid composition of human renin (3–6).

Comparative biochemical studies have revealed that human renin is unique among other animal renins in its molecular properties such as M_r, pI, and antigenicity (3). The renin–substrate reaction is also species dependent (7); renin isolated from the kidneys of monkeys and humans reacts with angiotensinogens from all mammals, whereas renin from other mammals does not release angiotensin I from angiotensinogen of monkeys and humans. To establish a molecular and genetic basis for the differences in the biochemical properties as well as to determine the amino acid sequence of human renin, attempts were made to obtain full-length cDNA clones from poly(A)$^+$ RNA extracted from a surgically removed ischemic kidney in which the renin content was markedly increased due to the stenosis of the renal artery.

The results presented here describe the cloning and sequence analysis of human renin cDNAs. The entire 3′ noncoding sequence preceding the poly(A) tail, 1,218 nucleotides coding for renin precursor, and 42 nucleotides of the 5′ noncoding region were determined.

MATERIALS AND METHODS

Materials. Materials were obtained from the following sources: terminal deoxynucleotidyltransferase and T4 polynucleotide kinase from Takara Shuzo (Kyoto, Japan); *Escherichia coli* DNA polymerase I and *E. coli* DNA ligase from New England BioLabs; restriction enzymes from Takara Shuzo and New England BioLabs; *E. coli* RNase H from P-L Biochemicals; [γ-^{32}P]ATP (>5,000 Ci/mmol; 1 Ci = 3.7 × 10^{10} Bq) and [α-^{32}P]dCTP (≈3,000 Ci/mmol) from Amersham.

Construction of a Human Kidney cDNA Library. Total RNA, which originated from a kidney removed from a patient with severe renovascular hypertension causing renal ischemia, was extracted in 4 M guanidine thiocyanate buffer as described by Chirgwin *et al.* (8). Poly(A)$^+$ RNA was prepared by subjecting the total RNA preparation to oligo(dT)-cellulose column chromatography twice (9). A cDNA library was constructed by applying the method of Okayama and Berg (10), using 22 μg of poly(A)$^+$ RNA and 5.6 μg of vector/primer DNA. After transformation of *E. coli* HB101, the cells were plated on nitrocellulose filters on ampicillin-containing agar plates (11).

Identification of Human Renin cDNA Clones. The human kidney cDNA library was screened by colony hybridization using a nick-translated *Acc* I/*Rsa* I fragment (358 base pairs) from renin cDNA of a mouse submandibular gland (12). Prehybridization was performed at 55°C in 50 mM Tris·HCl, pH 7.6/10 mM EDTA/1 M NaCl/0.2% polyvinylpyrrolidone/0.2% Ficoll/0.2% bovine serum albumin/60 μg of denatured *E. coli* DNA per ml. Hybridization was carried out at 55°C for 18 hr in the same buffer containing a ^{32}P-labeled probe (1 × 10^8 cpm/μg). Filters were washed six times with an excess of 0.3 M NaCl/0.03 M sodium citrate/0.1% $NaDodSO_4$ at 37°C.

The clones with renin cDNA insert were selected for DNA sequence analysis by the method of Maxam and Gilbert (13).

All of the cloning procedures were conducted in accordance with the guidelines for research involving recombinant DNA molecules issued by the Ministry of Education, Science and Culture of Japan.

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Blot Hybridization Analysis. The poly(A)$^+$ RNAs from human liver and infarcted kidney were denatured with glyoxal (14) and electrophoresed on 1.5% agarose gel. The separated RNAs were transferred to diazobenzyloxymethyl-paper according to the procedure of Alwine *et al.* (15). The diazo paper was prehybridized at 42°C for 24 hr in 50% (vol/vol) formamide/0.75 M NaCl/0.075 M sodium citrate/50 mM sodium phosphate buffer, pH 7.0/0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin/1% glycine/0.2% $NaDodSO_4$/100 μg denatured salmon sperm DNA per ml. The paper was hybridized at 42°C for 24 hr in the same solution except that glycine was omitted and that the solution contained a ^{32}P-labeled probe (nick-translated *Ava* II fragment containing nucleotides 170–1,127 from clone pHRn321). After the hybridization, the paper was washed at 42°C in 0.45 M NaCl/0.045 M sodium citrate/0.1% $NaDodSO_4$ and subjected to autoradiography.

RESULTS

Construction, Selection, and Characterization of Human Renin cDNA Clones. Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography and used for cDNA synthesis and cloning. Initial extraction of total RNA was carried out by the guanidine thiocyanate method of Chirgwin *et al.* (8) to isolate intact RNA. From 26 g of an infarcted human kidney, approximately 66 mg of RNA was obtained. Passage of the total RNA over an oligo(dT)-cellulose column gave a 5% yield of poly(A)$^+$ RNA. The presence of intact renin mRNA in this preparation was confirmed by cell-free translation of the poly(A)$^+$ RNA in the presence of [^{35}S]methionine. Immunoprecipitation of translation products with antirenin antibody and analysis of the radioactive precipitates by $NaDodSO_4$/polyacrylamide gel electrophoresis and fluorography revealed one major specific band with a M_r of 45,000 (data not shown).

A library of cDNA clones was constructed from the human renal poly(A)$^+$ RNA by using the vector/primer method of Okayama and Berg (10), which provides a highly efficient means for obtaining full-length cDNAs. Initially, the library was screened for human renin sequences by hybridization with a 358-base-pair *Acc* I/*Rsa* I restriction fragment obtained from the previously cloned mouse renin cDNA (12). Of the 50,000 colonies screened, 6 colonies positive for hybridization were isolated. Recombinant plasmids from the selected colonies were prepared, digested with various restriction enzymes, and electrophoresed in agarose gels to determine the size. The insert, which contained 800–1,300 base pairs, seemed unable to cover the entire coding sequence since mRNA coding for a M_r 45,000 protein is expected to have at least 1,400 nucleotides, including 5′ and 3′ noncoding sequences; in fact, this was proved to be the case by later sequence analysis (Fig. 1) and blot hybridization analysis (Fig. 3). To isolate longer cDNAs, therefore, 190,000 colonies were further screened by using the *Taq* I (390)/*Taq* I (630) restriction fragment (Fig. 1) excised from the longest human renin cDNA insert (pHRn011) cloned above, and 29 clones positive for hybridization were recovered. Size analysis of their inserts indicated that one recombinant plasmid, pHRn321, contained an insert with about 1,600 base pairs. This clone and the pHRn011 cDNA clone were subjected to sequence analysis.

Nucleotide Sequence Analysis. The sequences of two different clones were determined to verify the absolute fidelity of the cDNA sequence as a copy of the mRNA. The insert from pHRn011 and pHRn321 was analyzed with a series of restriction enzymes. Fig. 1 shows the restriction map and sequence analysis strategy. The complete nucleotide sequence of the longer clone (pHRn321) determined by the chemical degradation method is shown in Fig. 2. The entire sequence was obtained from both the message and complementary strands. There was complete agreement in the nucleotide sequences obtained from the overlapping areas. The insert contained 1,459 nucleotides and the length of the poly(A) tail was not included in this determination. The DNA sequence of the shorter insert, pHRn011, was found to be identical with that of the corresponding region of pHRn321. The cDNA insert of pHRn321 contained 42 nucleotides in the 5′ noncoding region, 1,218 nucleotides in the coding region, and 199 nucleotides in the 3′ untranslated region preceding the poly(A) tail. From this information it was possible to analyze most of the structure of human renin mRNA. The 5′ and 3′ untranslated regions of the mRNA share many of the features already noted in other eukaryotic mRNA sequences; for example, the nucleotide sequence around the initiation codon is consistent with the sequence $\begin{smallmatrix}A\\C\end{smallmatrix}$-N-N-A-U-G-G, which has been shown to be most favored for eukaryotic initiation sites (18). The 3′ untranslated region contains, in the expected position [20 nucleotides upstream from the poly(A) tail], the hexanucleotide 5′-A-A-U-A-A-A-3′ which could function as a signal for poly(A) addition or termination of transcription (19).

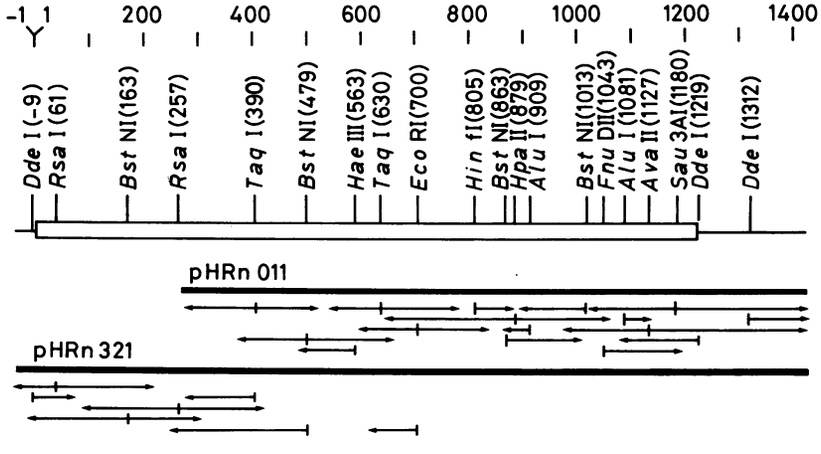

FIG. 1. Sequence analysis strategy of the human renin cDNA clones. For the isolation of clone pHRn011, see the text. For the isolation of clone pHRn321, cDNA library was rescreened by hybridization at 68°C with the nick-translated *Taq* I fragment containing nucleotides 390–630 from clone pHRn011. The sequence corresponding to the coding region is indicated by the open box. Only the positions of the relevant restriction sites are indicated (for the nucleotide numbers, see Fig. 2). The poly(dA)·poly(dT) and poly(dG)·poly(dC) tails are not included in the map. The horizontal arrows indicate the direction and extent of sequence determination. The sites of 5′-end-labeling are indicated by short vertical lines on the arrows.

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                                   -42 AACCTCAGTGGATCTCAGAGAGAGCCCCAGACTGAGGGAAGC   -1

   1  ATG GAT GGA TGG AGA AGG ATG CCT CGC TGG GGA CTG CTG CTG CTG CTC TGG GGC TCC TGT   60
      Met Asp Gly Trp Arg Arg Met Pro Arg Trp Gly Leu Leu Leu Leu Leu Trp Gly Ser Cys
      1                      {-60}       10                     {-50}           20

  61  ACC TTT GGT CTC CCG ACA GAC ACC ACC ACC TTT AAA CGG ATC TTC CTC AAG AGA ATG CCC  120
      Thr Phe Gly Leu Pro Thr Asp Thr Thr Thr Phe Lys Arg Ile Phe Leu Lys Arg Met Pro
                             {-40}       30                     {-30}           40

 121  TCA ATC CGA GAA AGC CTG AAG GAA CGA GGT GTG GAC ATG GCC AGG CTT GGT CCC GAG TGG  180
      Ser Ile Arg Glu Ser Leu Lys Glu Arg Gly Val Asp Met Ala Arg Leu Gly Pro Glu Trp
                             {-20}       50                     {-10}           60

 181  AGC CAA CCC ATG AAG AGG CTG ACA CTT GGC AAC ACC ACC TCC TCC GTG ATC CTC ACC AAC  240
      Ser Gln Pro Met Lys Arg Leu Thr Leu Gly Asn Thr Thr Ser Ser Val Ile Leu Thr Asn
                         {-1}{1}         70                     {10}            80

 241  TAC ATG GAC ACC CAG TAC TAT GGC GAG ATT GGC ATC GGC ACC CCA CCC CAG ACC TTC AAA  300
      Tyr Met Asp Thr Gln Tyr Tyr Gly Glu Ile Gly Ile Gly Thr Pro Pro Gln Thr Phe Lys
                         {20}            90                     {30}            100

 301  GTC GTC TTT GAC ACT GGT TCG TCC AAT GTT TGG GTG CCC TCC TCC AAG TGC AGC CGT CTC  360
      Val Val Phe Asp Thr Gly Ser Ser Asn Val Trp Val Pro Ser Ser Lys Cys Ser Arg Leu
                         {40}            110                    {50}            120

 361  TAC ACT GCC TGT GTG TAT CAC AAG CTC TTC GAT GCT TCG GAT TCC TCC AGC TAC AAG CAC  420
      Tyr Thr Ala Cys Val Tyr His Lys Leu Phe Asp Ala Ser Asp Ser Ser Ser Tyr Lys His
                         {60}            130                    {70}            140

 421  AAT GGA ACA GAA CTC ACC CTC CGC TAT TCA ACA GGG ACA GTC AGT GGC TTT CTC AGC CAG  480
      Asn Gly Thr Glu Leu Thr Leu Arg Tyr Ser Thr Gly Thr Val Ser Gly Phe Leu Ser Gln
                         {80}            150                    {90}            160

 481  GAC ATC ATC ACC GTG GGT GGA ATC ACG GTG ACA CAG ATG TTT GGA GAG GTC ACG GAG ATG  540
      Asp Ile Ile Thr Val Gly Gly Ile Thr Val Thr Gln Met Phe Gly Glu Val Thr Glu Met
                         {100}           170                    {110}           180

 541  CCC GCC TTA CCC TTC ATG CTG GCC GAG TTT GAT GGG GTT GTG GGC ATG GGC TTC ATT GAA  600
      Pro Ala Leu Pro Phe Met Leu Ala Glu Phe Asp Gly Val Val Gly Met Gly Phe Ile Glu
                         {120}           190                    {130}           200

 601  CAG GCC ATT GGC AGG GTC ACC CCT ATC TTC GAC AAC ATC ATC TCC CAA GGG GTG CTA AAA  660
      Gln Ala Ile Gly Arg Val Thr Pro Ile Phe Asp Asn Ile Ile Ser Gln Gly Val Leu Lys
                         {140}           210                    {150}           220

 661  GAG GAC GTC TTC TCT TTC TAC TAC AAC AGA GAT TCC GAG AAT TCC CAA TCG CTG GGA GGA  720
      Glu Asp Val Phe Ser Phe Tyr Tyr Asn Arg Asp Ser Glu Asn Ser Gln Ser Leu Gly Gly
                         {160}           230                    {170}           240

 721  CAG ATT GTG CTG GGA GGC AGC GAC CCC CAG CAT TAC GAA GGG AAT TTC CAC TAT ATC AAC  780
      Gln Ile Val Leu Gly Gly Ser Asp Pro Gln His Tyr Glu Gly Asn Phe His Tyr Ile Asn
                         {180}           250                    {190}           260

 781  CTC ATC AAG ACT GGT GTC TGG CAG ATT CAA ATG AAG GGG GTG TCT GTG GGG TCA TCC ACC  840
      Leu Ile Lys Thr Gly Val Trp Gln Ile Gln Met Lys Gly Val Ser Val Gly Ser Ser Thr
                         {200}           270                    {210}           280

 841  TTG CTC TGT GAA GAC GGC TGC CTG GCA TTG GTA GAC ACC GGT GCA TCC TAC ATC TCA GGT  900
      Leu Leu Cys Glu Asp Gly Cys Leu Ala Leu Val Asp Thr Gly Ala Ser Tyr Ile Ser Gly
                         {220}           290                    {230}           300

 901  TCT ACC AGC TCC ATA GAG AAG CTC ATG GAG GCC TTG GGA GCC AAG AAG AGG CTG TTT GAT  960
      Ser Thr Ser Ser Ile Glu Lys Leu Met Glu Ala Leu Gly Ala Lys Lys Arg Leu Phe Asp
                         {240}           310                    {250}           320

 961  TAT GTC GTG AAG TGT AAC GAG GGC CCT ACA CTC CCC GAC ATC TCT TTC CAC CTG GGA GGC 1020
      Tyr Val Val Lys Cys Asn Glu Gly Pro Thr Leu Pro Asp Ile Ser Phe His Leu Gly Gly
                         {260}           330                    {270}           340

1021  AAA GAA TAC ACG CTC ACC AGC GCG GAC TAT GTA TTT CAG GAA TCC TAC AGT AGT AAA AAG 1080
      Lys Glu Tyr Thr Leu Thr Ser Ala Asp Tyr Val Phe Gln Glu Ser Tyr Ser Ser Lys Lys
                         {280}           350                    {290}           360

1081  CTG TGC ACA CTG GCC ATC CAC GCC ATG GAT ATC CCG CCA CCC ACT GGA CCC ACC TGG GCC 1140
      Leu Cys Thr Leu Ala Ile His Ala Met Asp Ile Pro Pro Pro Thr Gly Pro Thr Trp Ala
                         {300}           370                    {310}           380

1141  CTG GGG GCC ACC TTC ATC CGA AAG TTC TAC ACA GAG TTT GAT CGG CGT AAC AAC CGC ATT 1200
      Leu Gly Ala Thr Phe Ile Arg Lys Phe Tyr Thr Glu Phe Asp Arg Arg Asn Asn Arg Ile
                         {320}           390                    {330}           400

1201  GGC TTC GCC TTG GCC CGC TGAGGCCCTCTGCCACCCAGGCAGGCCCTGCCTTCAGCCCTGGCCCAGAGCTGGA 1273
      Gly Phe Ala Leu Ala Arg
                         {340}406

1274  ACACTCTCTGAGATGCCCCTCTGCCTGGGCTTATGCCCTCAGATGGAGACATTGGATGTGGAGCTCCTGCTGGATGCGT 1352

1353  GCCCTGACCCCTGCACCAGCCCTTCCCTGCTTTGAGGACAAAGAGAATAAAGACTTCATGTTCAC
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FIG. 2. Nucleotide and corresponding amino acid sequence of plasmids pHRn321 and pHRn011 encoding human renal preprorenin. Nucleotides are numbered in the 5′-to-3′ direction, beginning with the first residue of the ATG triplet encoding the initiator methionine, and the nucleotides on the 5′ side of residue 1 are indicated by negative numbers. The deduced amino acid residues are indicated below the nucleotide triplets. The mature polypeptide begins at the Leu residue labeled {1}. The numbers in the braces refer to amino acid positions within the mature renin. The single and double arrowheads indicate the probable ends of leader sequence and prosequence, respectively, as predicted by comparison with the mouse renin precursor (16) and human prepepsinogen (17). The two active-site aspartic acid residues, potential *N*-glycosylation sites, and the A-A-T-A-A-A sequence within the 3′ untranslated region are underlined.

Predicted Amino Acid Sequence of Human Renin. The amino acid sequence deduced from the nucleotide sequence analysis is shown in Fig. 2. At the NH_2 terminus is a sequence of hydrophobic amino acids characteristic of the signal peptides found in the precursors of many secreted proteins. The two adjacent basic residues, Lys-Arg at position 65–66, may represent the

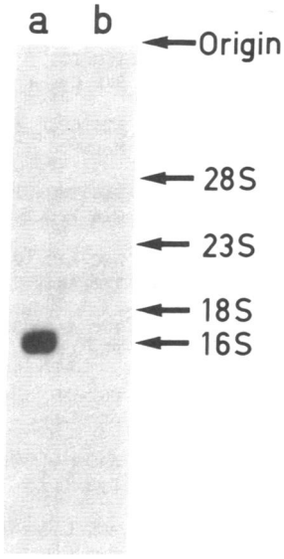

FIG. 3. Blot hybridization analysis of liver and kidney mRNA. Lane a was obtained with 10 μg of poly(A)$^+$ RNA from human kidney, whereas lane b had corresponding quantities of human liver poly(A)$^+$ RNA. The positions of the size markers (*E. coli* and human ribosomal RNAs) are indicated.

site of proteolytic cleavage upon the conversion of prorenin to mature renin; the presence of prorenin was suggested by the finding that human kidney contains inactive renin that can be activated by trypsin. The molecular weight (M_r = 37,200; sugar residues not included) calculated from the predicted sequence is in agreement with that determined by Yokosawa *et al.* (3) for the purified protein. The mature protein is composed of 340 amino acid residues: Ala, 16; Arg, 10; Asp, 17; Asn, 12; Cys, 6; Glu, 16; Gln, 12; Gly, 34; His, 6; Ile, 22; Leu, 28; Lys, 15; Met, 8; Phe, 19; Pro, 13; Ser, 33; Thr, 30; Trp, 3; Tyr, 17; and Val, 23.

Codon utilization for human renin is not random and exhibits a marked preference for codons ending with G or C. For example, while 20 codons for valine end in G or C, only four end in A or T; similarly for leucine, the codons with G or C in the third position occur 8 times more frequently than the codons ending in A or T. Such a nonrandom codon usage has been observed in other animal genes (20).

Identification and Size Determination of Renin mRNA by Blot Hybridization Analysis. Takahashi and Tang (21) have recently shown that bovine cathepsin D and renin from the mouse submandibular gland are unexpectedly similar in their amino acid sequences. Therefore, to confirm that the cloned pHRn321 is complementary to renin mRNA and not to cathepsin D and to determine the size of human renin mRNA, cloned cDNA was used as a probe to hybridize to mRNA isolated from human kidney, a major source of renin, and from the liver, an abundant source of cathepsin D. As anticipated, the probe hybridized only to kidney mRNA (Fig. 3). On the basis of its migration in a denaturing gel system, it was estimated that the sequence of mature human renin mRNA is 1,600 nucleotides long. A protein with 406 amino acid residues requires 1,218 bases for its coding sequence. The renin mRNA must, therefore, have about 380 noncoding bases, including the 3′-poly(A) tail.

DISCUSSION

Emphasis was placed on the study of human renin, which is involved in the pathogenesis of several forms of hypertension. Human kidney renin is a key enzyme in the control of blood pressure as well as water and electrolyte balance. Its nucleotide and amino acid sequences had not been analyzed previously. The selection of transformants containing sequences corresponding to human renin was greatly facilitated by the availability of the defined cDNA of the mouse submandibular renin, which has previously been shown to cross-hybridize with human genomic DNA. Since the primary sequence of human renin was unknown, the final confirmation of the identity of the clones was obtained by determining the sequences of the cDNA inserts and comparing the derived amino acid sequences with the published sequences of the renin cDNA of the mouse submandibular gland (16). A considerable degree of homology was thus found between the amino acid sequences of the two enzymes, as discussed below.

Recently the primary structure of the renin of the mouse submandibular gland has been determined independently by Misono *et al.* (22) and Panthier *et al.* (16) through chemical sequence analysis of purified protein and sequencing of a cDNA clone, respectively. Comparison of the sequence of human renin with these published sequences (Fig. 4) suggests that certain regions of renin are highly conserved between the two species, particularly the regions corresponding to the functional domains that contain catalytically important aspartic acid residues, such as Phe-Asp-Thr-Gly-Ser at amino acid positions 37–41 (numbering refers to mature human renin) and Val-Asp-Thr-Gly (residues 225–228). The overall homology for the amino acid sequences of renin isolated from human kidney and mouse submandibular gland, including the signal peptides, is 68%, while the nucleotide sequences show a 76% homology. The major difference observed is that, in contrast to the renin from the mouse submandibular gland, the renin from human kidney contains

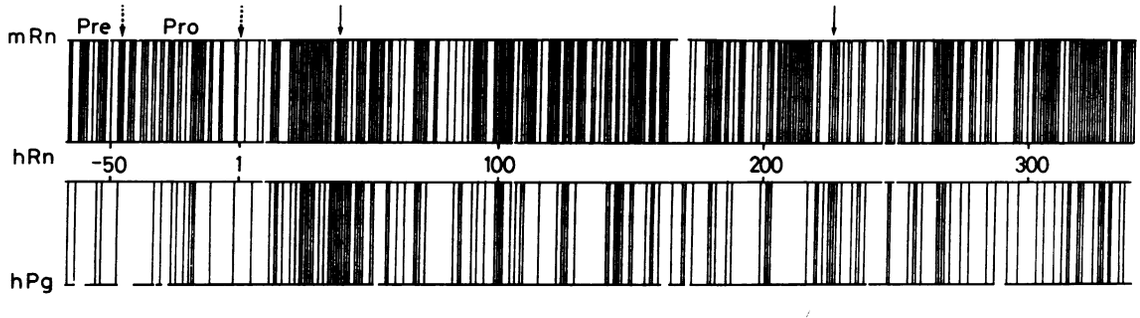

FIG. 4. Amino acid sequence homologies between human kidney renin (hRn), mouse submandibular gland renin (mRn), and human pepsinogen (hPg). The mouse renin and the human pepsinogen sequences are derived from Panthier *et al.* (16) and Sogawa *et al.* (17) publications, respectively. Positions of homology are indicated by vertical bars. The numbers refer to amino acid positions within the mature human renin. The blank spaces in the three sequences were introduced to maximize the homology. The percentage of homologous positions for the two comparisons are: hRn–mRn, 68%; and hRn–hPg, 34%. Broken arrows represent the sites of proteolytic cleavage upon the conversions of preprorenin to prorenin and of prorenin to mature renin. Arrows indicate the catalytically important residues: Asp-38 and Asp-226.

the amino acid sequence Asn-X-Thr (residues 5–7 and 75–77; numbering refers to the mature protein), which could be potential glycosylation sites.

X-ray crystallographic studies of aspartyl proteinases (22–25) have revealed unique structural features common to all the members of this family. The molecule is bilobal, with two approximately equal domains separated by a deep and long cleft that serves as the substrate binding site. Each domain has a similar folding of the peptide chain and forms a wall-like structure of mixed β-sheet surrounding the cleft. The two catalytically important aspartic acid residues are centrally located in the cleft. To obtain a rough image of the secondary and tertiary structures of human renin, we compared the primary structure of renin with that of other aspartyl proteinases, whose structures are well defined. The comparison revealed that (*i*) a considerable degree of homology exists between renin and other aspartyl proteinases (45–60%) and (*ii*) many of the identical residues are located in the surroundings of the cleft, particularly in the regions corresponding to the active site aspartates. It seems reasonable, therefore, to assume that the common structural features of aspartyl proteinases are also present in renin.

Knowledge of renin has recently become very detailed but confusing. Multiple forms of active renin have been extracted from kidneys of several species (26). The probability that these renins are the products of separate renin genes seems to be very low because all the human renin cDNAs cloned in this study yielded identical patterns of restriction fragments when digested with *Ava* II, *Rsa* I, or *Bst*NI and analyzed by electrophoresis. Therefore, the different forms of renin may result from posttranslational modifications or more likely represent a mere experimental artifact induced by limited proteolysis during the extraction and purification procedures. In support of this view, four cleavage sites with two basic amino acids (Lys^{250}-Arg^{251}, Lys^{293}-Lys^{294}, Arg^{321}-Lys^{322}, and Arg^{329}-Arg^{330}) are known to occur near the COOH terminus of mature renin. In addition to the active renins, inactive renins that can be activated by trypsin have been demonstrated in human kidneys and plasma. Biochemical characterizations have suggested that inactive renin in the kidney corresponds to prorenin, a biosynthetic precursor of renin. However, the relationship between the renal prorenin and the inactive material in plasma, which accounts for more than 80% of the total plasma renin, is not clear (for reviews see refs. 27–29). Attempts to show that inactive renin in plasma is a precursor of the circulating active renin have produced conflicting results, and currently available data do not allow one to determine whether the plasma inactive renin represents the prorenin secreted from the kidney or the previously active renin covalently combined with an inactivating protein. It is anticipated that the size and the amino acid sequence of the pro segment reported here may contribute to a more precise analysis of the exact nature of plasma inactive renin.

Recent studies that have demonstrated the presence of renin in several organs other than the kidney have modified the classic assumption that the renin–angiotensin system is a system of renal origin that regulates blood pressure. Especially, renin from the brain has drawn a great deal of attention because it is implicated in the regulation of numerous activities of the central nervous system (30, 31). Full-length renin cDNAs cloned here could serve as useful probes for the cloning of extrarenal renin cDNAs as well as for the analysis of the organization and expression of human renin genes.

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