Molecular Cloning of the cDNA Encoding Human Deoxyribonuclease II*

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Toshihiro Yasuda, Haruo Takeshita, Reiko Iida‡, Tamiko Nakajima, Osamu Hosomi, Yoshimitsu Nakashima, and Koichiro Kishiš

From the Department of Legal Medicine, Gunma University School of Medicine, Gunma 371, Japan and the ‡Department of Legal Medicine, Fukui Medical School, Fukui 910-11, Japan

A rapid amplification of cDNA ends method, using degenerate oligonucleotides based upon the N-terminal amino acid sequence of human hepatic deoxyribonuclease II (DNase II), allowed a novel cDNA encoding DNase II to be constructed from thyroid gland RNA. The composite nucleotide sequence (1593 bases) included an open reading frame of 1080 bases, which encoded a single polypeptide of 360 amino acids (signal peptide, 16; propeptide, 91; mature protein, 253). Although the sequence of DNase II showed no significant homology to other mammalian proteins, its cDNA structural organization resembled those of the lysosomal cathepsin families. The two parts of the cDNA corresponding to the propeptide and the mature protein were expressed in Escherichia coli, and the recombinant polypeptides thus obtained were strongly stained with an anti-DNase II antibody on Western blotting. DNase II is ubiquitously expressed in human tissues, and the DNase II gene (DNASE2) was assigned to chromosome 19.

Deoxyribonuclease II (DNase II), EC 3.1.22.1) is one of two distinct types of DNase present in mammalian tissues and body fluids. In the absence of metal ions, it hydrolyzes DNA to 3'-phosphoryl oligonucleotides under acidic conditions and has therefore been designated “acid DNase” (1). DNase II was isolated directly from lysosomes in the porcine spleen (2) and from rat and monkey livers (3, 4). DNase II activity is known to occur in various mammalian tissues (5-8). Although the enzymological properties of DNase II from different tissues and species are very similar, inconsistencies in the chemical properties of these enzymes (for example, with regard to molecular mass or subunit structure) have been recognized. Bernardi (1) suggested that the hog spleenic DNase II may have a dimeric structure composed of two similarly sized subunits, while the bovine hepatic enzyme appeared to consist of one 27-kDa polypeptide chain (9). However, the porcine hepatic and splenic DNase IIs have been demonstrated to consist of two non-identical subunits (2, 10).

One of the hallmarks of apoptosis or programmed cell death is the enzymatic internucleosomal cleavage of chromatin to yield an electrophoretic ladder pattern of DNA fragments. Several molecules that might be responsible for this endonucleolytic activity have been characterized from or detected in various sources (11). Barry and Eastman (12) demonstrated that a nuclear DNase II can mediate internucleosomal DNA digestion during apoptosis in Chinese hamster ovary cells following intracellular acidification. Furthermore, Torriglia et al. (13) suggested the specific involvement of DNase II in physiological nuclear degradation during lens fiber cell differentiation in chick embryos. However, although information on the cDNA encoding DNase II would allow the detailed elucidation of both the inherent structural properties of the enzyme and the intrinsic involvement of DNase II in apoptosis, no cDNA has as yet been cloned from any mammal.

In contrast to DNase I (EC 3.1.21.1), which has been studied extensively with regard to biochemical and human genetic aspects (14-17), DNase II has been less well characterized, particularly in human systems. Hitherto, DNase II has been only partially purified from gastric mucosa, uterine cervix (18), urine (7, 19), and lymphoblasts (20). We previously developed a sensitive and specific method for the quantitative detection of DNase II activity in human tissues and body fluids (7) and were consequently able to discover that human urinary and leukocyte DNase II shows genetic polymorphism with respect to its activity levels (21). The distribution of DNase II activity displays clear-cut bimodality in the Japanese population, and population and family studies found the activity levels to be controlled by two alleles, the dominant DNASE2*H and the recessive DNASE2*L, which are present at a single autosomal locus. These result in high and low activity, respectively. Differences in DNase II activity between various strains of mice have also been suggested to be determined by a single autosomal locus (22). Thus, it has been confirmed that DNase II is one of the limited number of enzymes that exhibits genetic polymorphism in its activity levels. However, it remains to be determined whether the genetic control over activity levels is due to variation in the structural or regulatory gene for DNase II. Clarification of the molecular basis for the genetic polymorphism of DNase II requires the structure of a cDNA for DNase II to be determined and analyzed.

In the present study, we purified the DNase II from human liver and determined its N-terminal amino acid (aa) sequence. This was used as the basis of a polymerase chain reaction (PCR)-based cloning strategy for the construction of a cDNA coding for human DNase II. We report herein the molecular cloning process and the complete nucleotide sequence of DNase II cDNA. We also describe the bacterial expression of this...
cDNA and the chromosomal assignment of the gene coding for DNase II (DNASE2). Finally, we present evidence that DNase II is ubiquitously expressed in human tissues. This is the first report of the cloning and characterization of a cDNA for mammalian DNase II.

EXPERIMENTAL PROCEDURES

Materials and Biological Samples—Superscript II RNA H− reverse transcriptase (RT), RNHase H, Tag DNA polymerase, a dNTP mixture, 5′- and 3′-rapid amplification of cDNA ends (RACE) systems, and an oligo(dT) primer were obtained from Life Technologies, Inc. All other chemicals used were of reagent grade or the purest grade available commercially. All the oligonucleotide primers used in this study were synthesized and purchased from Life Technologies, Inc. An antibody against human DNase II was produced by injecting a mixture of human urinary DNase II, purified essentially as described previously (7), and Freund's complete adjuvant into a Japanese white rabbit. The antibody reacted strongly with the enzyme on SDS-polyacrylamide gel electrophoresis (PAGE). Furthermore, the enzymatic activity of both the purified enzyme and human urine was completely inhibited by the antibody. The antibody showed no reactivity toward human DNase I. A specific antibody against human DNase I was prepared according to a previously described method (15). Seventeen different kinds of human tissues were obtained from eight individuals (between 20 and 30 years of age) who had died from traumatic shock or loss of blood with no pathological changes. The samples were collected within about 20 h of death and stored at −80 °C until use.

DNase II Activity Assay—DNase II activity was determined by a single radial enzyme diffusion (SRED) method as described previously (7, 21). One unit of DNase II activity was defined as an increase of 1.0 in the absorbance at 260 nm. Levels of DNase II activity in each tissue sample were determined as follows (23). The human tissues were cut into small pieces, washed with cold saline to remove excess blood, homogenized using an Ultra-turrax (IKA-WERK, Staufen, Germany) in 1–2 ml 50 mM Tris (pH 8.3), 1.5 mM MgCl2, 0.01 mM dithiothreitol, 0.2 mM each of 25). First, the DNA fragment corresponding to the 3′-end region of the cDNA and the chromosomal assignment of the gene coding for the N-terminal Amino Acid Sequence Analysis of the Purified Human DNase II—A whole liver (ca. 1200 g) was obtained at autopsy from a 57-year-old woman 15 h after death due to loss of blood and was stored at −80 °C until use. DNase II was purified from the liver according to a modification of a previously described method (7). Details of the purification will be published elsewhere. The purified DNase II was subjected to SDS-PAGE in 12.5% gel according to the method of Laemmli (24). After the electrophoretic run, the protein was transferred onto an Immobilon-P™ membrane (Millipore, Bedford, MA) by electroblotting. The portion of the membrane carrying the enzyme was directly subjected to automatic Edman degradation using a protein sequencer (model 477A, Applied Biosystems, Foster City, CA) according to the manufacturer's instructions.

Construction of a cDNA Encoding Human DNase II—Total RNA was separately extracted from the thyroid gland and spleen of a 48-year-old man, obtained at autopsy 12 h after death due to loss of blood, by the acid guanidinium thiocyanate-phenol-chloroform extraction method (25). First, the DNA fragment corresponding to the 3′-end region of the human DNase II cDNA was obtained by the 3′-RACE method (26) using a 3′-RACE system. The synthesis of the first strand of the total cDNA was performed using the RT reaction as follows. After denaturation at 70 °C for 10 min, total RNA (about 2 μg) was incubated with 200 units of Superscript II at 42 °C for 45 min in a reaction mixture (20 μl) comprising 20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM MgCl2, 0.5 mM each dNTP, 10 mM dithiothreitol, and 500 mM potassium adipate primer (Life Technologies, Inc.), 5′-GGGACGCGCTGACATCTACATCTATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
Experimental Procedures.

Table I  Distribution of DNase II in human tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. of samples</th>
<th>Range (units/g wet weight)</th>
<th>Activity*</th>
<th>DNase II gene transcriptb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrum</td>
<td>8</td>
<td>1.0–7.5</td>
<td>2.7 ± 1.9</td>
<td>+</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>8</td>
<td>1.6–4.9</td>
<td>2.4 ± 1.0</td>
<td>+</td>
</tr>
<tr>
<td>Pituitary gland</td>
<td>7</td>
<td>12.0–53.0</td>
<td>24.0 ± 7.3</td>
<td>+</td>
</tr>
<tr>
<td>Submaxillary gland</td>
<td>7</td>
<td>4.4–7.7</td>
<td>5.3 ± 1.4</td>
<td>+</td>
</tr>
<tr>
<td>Thyroid gland</td>
<td>6</td>
<td>12.0–62.0</td>
<td>30.0 ± 16.0</td>
<td>+</td>
</tr>
<tr>
<td>Parotid gland</td>
<td>5</td>
<td>2.3–7.5</td>
<td>4.2 ± 1.8</td>
<td>+</td>
</tr>
<tr>
<td>Thymus</td>
<td></td>
<td></td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>Heart</td>
<td>8</td>
<td>1.9–7.0</td>
<td>4.2 ± 2.0</td>
<td>+</td>
</tr>
<tr>
<td>Lung</td>
<td>6</td>
<td>7.1–21.0</td>
<td>12.0 ± 5.1</td>
<td>+</td>
</tr>
<tr>
<td>Stomach</td>
<td>8</td>
<td>3.0–11.0</td>
<td>6.7 ± 2.0</td>
<td>+</td>
</tr>
<tr>
<td>Liver</td>
<td>6</td>
<td>8.8–30.0</td>
<td>20.0 ± 5.8</td>
<td>+</td>
</tr>
<tr>
<td>Pancreas</td>
<td>7</td>
<td>5.2–10.0</td>
<td>9.5 ± 3.6</td>
<td>+</td>
</tr>
<tr>
<td>Kidney</td>
<td>8</td>
<td>3.3–21.0</td>
<td>9.5 ± 6.0</td>
<td>+</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>6</td>
<td>6.2–110</td>
<td>34.0 ± 36.0</td>
<td>+</td>
</tr>
<tr>
<td>Spleen</td>
<td>8</td>
<td>6.7–18.0</td>
<td>12.0 ± 4.2</td>
<td>+</td>
</tr>
<tr>
<td>Small intestine</td>
<td>8</td>
<td>2.3–8.6</td>
<td>4.7 ± 2.0</td>
<td>+</td>
</tr>
<tr>
<td>Large intestine</td>
<td>6</td>
<td>1.7–8.6</td>
<td>4.4 ± 1.4</td>
<td>+</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>5</td>
<td>9.2–78.0</td>
<td>28.0 ± 22.0</td>
<td>NT</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations of triplicate determinations for each tissue, derived from different individuals. ND, not determined.

+ = amplification of a 762-bp DNase II-specific fragment by RT-PCR using primers 6 and 7. NT, not tested.

from human liver were subjected to SDS-PAGE and detected by immunoblotting using anti-human DNase II antibody (28).

Chromosomal Assignment of the Human DNase II Gene—Genomic DNAs extracted from a panel of 20 different cloned human or rodent (hamster or mouse) hybrid cell lines were obtained from BIOS Laboratories (New Haven, CT). A set of two primers, 5 and 6, was used to amplify the human DNase II-specific DNA fragment from a panel of somatic cell hybrids. About 50 ng of DNA was subjected to PCR under the same amplification conditions as above, except that the annealing temperature used was 60°C. The products were separated on 1.5% (w/v) agarose gel and visualized by ethidium bromide staining. Using human DNA as a template, a 1.7-kilobase (kb) DNA fragment was specifically amplified by these primers. Upon Southern blotting, the human DNase II cDNA probe hybridized well with the fragment. Otherwise, these primers failed to demonstrate any specific products in either hamster or mouse DNAs. Therefore, the presence of the amplified 1.7-kb fragment upon PCR with these primers allowed unequivocal identification of the human component.

RESULTS AND DISCUSSION

Distribution of DNase II Activity in Human Tissues—No systematic examination of the tissue distribution of DNase II has so far been carried out in humans or other mammals. Under optimal assay conditions established on the basis of the catalytic properties of purified human urinary and hepatic DNase II, DNase II activity was determined in 17 different tissue extracts by the SRED method (7). As shown in Table I, the adrenal gland, thyroid gland, lymph nodes, and pituitary gland showed high activity. In contrast to mammalian DNase I (22, 29–32), all the tissue samples examined exhibited a significant level of activity. The activities detected in these tissues were completely abolished by anti-human DNase II antibody but not by anti-human DNase I. From these findings, it seems reasonable to conclude that the activity detected by the SRED method was indeed derived from DNase II. The tissue activity distribution and the amount of tissue available meant that the liver was the most suitable organ for the preparation of human DNase II.

N-terminal Amino Acid Sequence of Human DNase II—With regard to the aa sequence of mammalian DNase II, only one description of the aa sequence around an essential histidine residue of the porcine DNase II has so far been reported (33). In this study, about 20 µg of human DNase II was obtained from a whole liver, and its catalytic and immunological properties were found to closely resemble those of human urinary DNase II (7). When the isolated enzyme was subjected to SDS-PAGE followed by protein staining, a nearly single band was observed at a position corresponding to approximately 32 kDa on the gel. Edman degradation allowed the N-terminal aa sequence of this protein to be identified up to the 30th residue as follows: Ser-Ser-Met-Arg-Gly-His-Thr-Lys-Gly-Val-Leu-Leu-Asp-His-Asp-Gly-Gly-Phe-Trp-Leu-Val-His-Ser-Val-Pro-Asn-Phe-Pro. Comparison of this aa sequence with those in the SWISS-PROT protein sequence data base showed no significant homologies.

Molecular Cloning of a cDNA Encoding Human DNase II—Due to its high DNase II activity (as shown in Table I) and its wide availability among the human tissue samples collected, the thyroid gland was selected for the preparation of total RNA. Total RNA extracted from a thyroid gland sample was separately amplified by the 3′- and 5′-RACE methods (26) to construct a cDNA encoding human DNase II. Two partially overlapped degenerate oligonucleotides, primer-1 and -2, corresponding to the aa sequence from Asp-14 to Trp-20 and from Gly-17 to His-23, respectively, were prepared for nested PCR amplification. Following 3′-RACE of the total thyroid RNA with the gene-specific primer-1, PCR amplification was performed using the downstream primer-2. After cloning into pCRII vector, 40 independent clones were selected from the 3′-RACE cDNA library and sequenced to screen for a clone that would insert a cDNA encoding DNase II. Five clones (DN2–3R) with an insert 1.2 kb long had a nucleotide sequence that completely corresponded to the aa sequence from Ser-24 to Pro-30. Next, the 5′-end region of the cDNA was amplified by 5′-RACE. After reverse transcription of the thyroid RNA with primer-3 priming, nested PCR amplification using primer-5 followed by the upstream primer-4 yielded a unique 0.55-kb fragment. After subcloning, the clone (DN2–5R) in which the corresponding RACE product was inserted was selected and sequenced. The first ATG codon was found at position 73 of the sequence. Sequence analysis of each RACE product showed the composite sequence (1593 bp) to include an open reading frame of 1080 bp, along with portions of the 5′-untranslated (72 bp) and 3′-untranslated (441 bp) regions (Fig. 1). The open reading frame started at position 73 with an ATG initiation codon and ended with a TAA stop codon at position 1153. The sequence flanking the ATG codon at position 73 was completely consistent with that obtained from the spleen of the same individual.

Analysis of the Predicted DNase II Sequence—the aa sequence predicted by nucleotide analysis is shown in Fig. 2. Assuming that DNase II translation starts at the first ATG, the open reading frame coded for a protein of 360 aa, in which the presence of a stretch of hydrophobic aa close to the initial Met strongly suggested the presence of a signal peptide, which is also present in lysosomal enzymes. The predicted signal peptide exhibited the common characteristics found among signal
peptides: hydrophobic aa clusters in the interior (Leu-4 to Val-21) and a residue with a short side chain for cleavage by the signal peptidase (Ala-15). Human cathepsins B and D have been predicted to be cleaved after the Ala-X-Ala sequence (35, 36). Considering the "(−3, −1) rule" (37), i.e. the cleavage site of a signal peptide must contain residues with small neutral side chains in positions −1 and −3, corresponding to Ala-16 and Ala-14, respectively, we assumed Leu-17 to be the N terminus of DNase II. If cleavage does follow this pattern, the corresponding protein would be composed of 354 aa residues. However, the N-terminal aa sequence determined chemically up to the 30th residue from hepatic DNase II was found to be located in the portion corresponding to Ser-108 to Pro-138 as deduced from the cDNA data, and these sequences were exactly identical to each other. Although no smaller component was visible on SDS-PAGE analysis of the purified enzyme by protein staining, probably due to its low dye affinity (2, 10), a short polypeptide was predicted to represent the aa sequence from 17 to 107. However, immunostaining with the anti-human DNase II antibody following SDS-PAGE permitted the native enzyme isolated from human liver to resolve into two bands on the gel at respective positions corresponding to about 32 and 12 kDa (Fig. 3). Therefore, sequencing analysis of the cDNA revealed the structural organization of its primary translation product: a total of 360 aa residues containing a signal peptide (16 residues), a propeptide region (91 residues), and a mature protein region (253 residues) corresponding to regions 1 to 16, 17 to 107, and 108 to 360, respectively. So far, the chemical aspects of mammalian DNase II have remained ambiguous, as described in the Introduction. Liao et al. (2, 10) reported that the porcine hepatic and splenic enzymes consist of two non-identical chains, the α- and β-chains, of 35 and 10 kDa, respectively. Also, bovine splenic DNase II has been demonstrated to be a heterodimer of 30–31-kDa and 10-kDa subunits (12). In contrast, the monkey hepatic enzyme was suggested to be monomeric in the native state. Although the subunit structure was not known at the time, molecular masses of the native human DNase II ranging from 31 to 45 kDa have been estimated by gel filtration (7, 18–20), and these are compatible with the mass of 41 kDa predicted from the two-chain structure of the human enzyme revealed in the present study. The aa compositions of the propeptide and mature protein portions of human DNase II derived from the cDNA data agree well with those for the β- and α-chains of the porcine enzyme (10). The aa composition of the holoprotein was also in agreement with that for hog DNase II (33).

The proteolytic conversion of a single-chain to a multi-chain enzyme is apparently a phenomenon universal to all the lysosomal enzymes (38). Although the C-terminal aa sequence of the propeptide remains to be determined, one of the cleavage sites in the DNase II molecule was identified as the Asp–Ser bond between positions 107 and 108. The processing of human asparaginylglucosaminidase has been found to involve an Asp–Thr bond (39). Cathepsins B and D appear to be synthesized as a proenzyme, procathepsin, which is activated, most probably in lysosomes, to single-chain cathepsin and subsequently to a two-chain enzyme. The native enzyme comprises a heavy and a light chain (35, 36), analogous to the heterodimeric structure of human DNase II. It has been suggested that asparaginyl bonds (Asn-X) may be an initial point in processing that is specific for lysosomal enzymes (38). The Asn residue at position 96 was found to be in the vicinity of the cleavage site of human DNase II. The proteolytic processing region might be located around the sequence from Asn-96 to Ser-108, which is in the hydrophilic region of the enzyme. Consequently, it is tempting to speculate that DNase II is synthesized as a proenzyme, pro-DNase II, and that following the release of a signal peptide, proteolytic processing transforms the single chain DNase II into the two-chain enzyme.

A His residue at the active site of porcine DNase II was reported to be involved in its catalytic function (33). Later, the aa sequence surrounding this His was determined to be Ala-Thr-Glu-Asp-His-Ser-Lys-Trp (11). The corresponding region in the human enzyme was found to run from Ser-291 to Trp-298 and to be composed of Ser-Thr-Glu-Asp-His-Ser-Lys-Trp; thus, this sequence is very well conserved. A search for protein modification sites with the predicted aa sequence using GENETYX-MAC (Software Development Co., Tokyo) revealed four potential N-glycosylation sites at positions 86, 212, 266, and 290, the first of these being located in the putative proregion of the enzyme. Such sites are thought to be necessary for post-translational mannosylation, which is important in channeling lysosomal enzymes into lysosomes (40). Additionally, two potential protein kinase C phosphorylation sites were found at positions 109 and 301, one potential tyrosine kinase phosphorylation site at position 233, and three potential N-myristoylation sites at positions 40, 261, and 323.

On comparison of the aa sequence of DNase II with that of other mammalian proteins, no homologous proteins could be found. However, the structural organization of the cDNA for DNase II, which was composed of three parts (a signal peptide, a short propeptide and a long mature protein) and contained a long 3′-untranslated region, was very similar to those of the cDNAs for cathepsins B and D (Fig. 1). Most notably, there was no significant sequence homology between DNases I and II.

Production of Recombinant DNase II in E. coli—Two fragments, containing 295- and 782 bp, respectively, carrying the respective coding sequences for the propeptide and mature protein portions of human DNase II were inserted into the expression vector pQE-30. The resulting constructs, termed pQE-30/propeptide and pQE-30/mature protein, were transformed into E. coli and induced with IPTG. The IPTG-induced materials derived from the pQE-30/propeptide and pQE-30/mature protein constructs contained polypeptides of about 12 and 30 kDa, respectively, neither of which was present in materials isolated from non-induced cells. On Western blotting, bands corresponding to both the mature protein and the propeptide were strongly stained with anti-human DNase II.
antibody (Fig. 3). Furthermore, the N-terminal aa sequences of both polypeptides corresponded exactly to those deduced from DNase II cDNA. Therefore, it could finally be concluded that the cDNA isolated in this study encodes human DNase II and that this protein consists of two non-identical subunits, a propeptide and the mature enzyme, in the native state.

The isolated recombinant proteins were then subjected to the renaturation process used to recover active cathepsins B and O.

**Fig. 2.** Nucleotide and deduced amino acid sequences of human DNase II cDNA. Nucleotides are numbered in the 5'→3' direction, and the deduced amino acid residues are indicated (using the standard three-letter designations) under the corresponding nucleotide sequence. Amino acid position 1 was assigned to the first aa residue of the whole proDNase II polypeptide. The open and solid arrows indicate the ends of the putative signal peptide and propeptide, respectively. The putative polyadenylation and N-glycosylation signals are single-underlined and double-underlined, respectively.
incubated with IPTG for 4 h (lane 1) or 1 h (lane 2) or without IPTG for 4 h (lane 3), recombinant proteins were prepared from the cells and isolated by Ni-nitrilo-triacetic acid affinity chromatography. The recombinant proteins thus obtained were separately subjected to SDS-PAGE on 15% (A) or 12.5% (B) polyacrylamide gel. The native enzyme (about 1 μg) isolated from human liver was analyzed on 12.5% (C) gel in the same manner. The arrow indicates the positions of bromophenol blue used as a tracking dye. After electrophoretic, the proteins were stained with anti-human DNase II antibody (46). The cathode is at the top.

after production in E. coli (41, 42). Neither polypeptide exhibited endonucleolytic activity by itself, and no such activity was detected even in an equimolar mixture of the two polypeptides. It has been reported that once they are dissociated, the two polypeptides derived from porcine DNase II cannot be reassociated to regenerate DNase II activity (10). Our results are compatible with a previous suggestion that a single large pro-DNase II may be required to ensure proper folding before it is activated to generate the two-chain enzyme (10).

Distribution of the DNase II Gene Transcript in Human Tissues—The expression of DNase II-specific mRNA was verified by RT-PCR of total RNA extracted from 17 different human tissues as shown in Table I. Using a set of two primers, 6 and 7, a unique 762-bp fragment corresponding to the region amplified endonucleolytic activity by itself, and no such activity was observed only in hybrid cell lines containing human chromosome 19. DNase II has 0% discordance only with chromosome 19. DNase II gene is ubiquitously expressed in human tissues. It is considered that the mature protein could be amplified from the total RNA of all the human tissues examined (4). Thus, all tissues with significant levels of DNase II activity (Table I) yielded detectable amplified products specific for DNase II. Therefore, in contrast to DNase I, which shows tissue-specific expression of the gene (23, 30), our results indicate that the DNase II gene is ubiquitously expressed in human tissues. It is interesting that, although the biological significance remains to be clarified, high levels of enzyme activity were detected in endocrine tissues such as the pituitary, thyroid, and adrenal glands, probably due to high expression of the DNase II gene.

Chromosomal Assignment of the DNase II Gene—Among 20 different human × rodent hybrid cell lines, the 1.7-kb DNA fragment specific for the human DNase II gene was strongly observed only in hybrid cell lines containing human chromosome 19. DNase II has 0% discordance only with chromosome 19, and therefore we conclude that the DNase II gene (DNASE2) is located within human chromosome 19. We have previously confirmed, based on pedigree analysis of the genetic polymorphism of human DNase II, that sex linkage of the DNase II locus can be formally excluded (21), and this is consistent with the present results. Bruns et al. (49) and, later, Brook et al. (44) used electrophoretic techniques to analyze acid DNase expression in human × mouse somatic cell hybrid clones and suggested that a locus encoding acid DNase may be present on chromosome 19, proximal to q13. However, any relationship between this enzyme and DNase II remains to be elucidated. The gene for DNase I (DNASE1) has been assigned to 16p13.3 (45). Considering the lack of homology between their aa sequences, the differences in the tissue distribution of their gene expression, and their different chromosomal localizations, it seems plausible that two distinct type of DNase, DNases I and II, are present in mammals and that these may not have evolved from the same ancestral gene.

REFERENCES