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MOLECULAR CLONING OF HUMAN AMIDOPHOSPHORIBOSYLTRANSFERASE

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SUMMARY: The cDNA of human amidophosphoribosyltransferase (EC 2.4.2.14, ATase), which is the supposed regulatory allosteric enzyme of *de novo* purine nucleotide biosynthesis, has been cloned from human hepatoma (HepG2) cDNA library. The predicted open reading frame encodes a protein of 517 amino acids with a deduced molecular weight (Mr) of 57,398, which is consistent with the molecular mass of 56 kDa on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of the ATase subunit purified from human placenta. The derived amino acid sequence exhibits 93, 82, 41, 37, and 33% identity with the sequences of rat, chicken, *Bacillus subtilis*, *Escherichia coli*, and *Saccharomyces cerevisiae* ATases, respectively. Southern blot analysis suggested that the ATase gene exists as multiple copies. ATase mRNA (3.5 kb) is ubiquitously expressed in various human tissues. Comparison with rat and chicken ATases showed that two cysteine residues for an iron-sulfur cluster were conserved. Four consensus phosphorylation sites for cAMP-dependent protein kinase were found.

Amidophosphoribosyltransferase (EC 2.4.2.14, ATase) catalyzes the irreversible reaction between 5-phosphoribosyl-1-pyrophosphate (PRPP), glutamine, and water to form 5β-phosphoribosyl-1-amine, glutamate and inorganic pyrophosphate. This is the first reaction committed to the *de novo* purine nucleotide synthetic pathway and is considered to be a rate-limiting step (1). ATases have been shown to be an allosteric protein existing in two interconvertible forms in human and mouse; a small active form and a large inactive species (2,3). The ATases of *Bacillus subtilis*, rat, and human are oxygen-labile iron-sulfur proteins (4-6). Genes or cDNA

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sequences encoding ATase have been cloned from *Escherichia coli* (7,8), *B. subtilis* (9) *Saccharomyces cerevisiae* (10), chicken (11), and rat (12). Patterson *et al.* (13) reported that human ATase cDNA was isolated by functional complementation of *S. cerevisiae ade4* mutation. Zalkin *et al.* (14,15) suggested that they cloned human ATase cDNA. However, the nucleotide or amino acid sequence of human ATase cDNA has not been reported.

Because ATase is a rate-limiting enzyme of the *de novo* purine nucleotide synthesis, the aberration of this enzyme may be related to the urate overproduction in hyperuricemia or gout. Cloning of a human ATase cDNA is essential for characterization of the enzyme, screening the ATase gene abnormalities in patients with hyperuricemia and gout, and understanding the mechanism of cell proliferation.

In this paper, we report the determination of Mr of a purified human placental ATase subunit in spite of its extreme oxygen lability, and the determined sequence of the human ATase cDNA.

MATERIALS AND METHODS

Purification of Human ATase

All procedures were done at 4°C unless otherwise stated. All phosphate buffers were supplemented with 5 mM $MgCl_2$, 1 mM ethylenediaminetetraacetic acid (EDTA, pH 7.4) and 2.5 mM dithiothreitol (DTT), and flushed with N_2 gas.

Step I: Freshly delivered human placenta (~500 g) was homogenized with 500 ml of 25 mM KPi, pH 7.4 containing 50 mM KCl and protease inhibitors (final concentrations; 0.8 mM phenylmethanesulfonyl fluoride, 0.005 mg/ml aprotinin, 0.5 μg/ml antipain, 0.5 μg/ml chymostatin, 0.5 μg/ml pepstatin, 0.2 μg/ml α_2-macroglobulin, and 0.2 μg/ml leupeptin) by a blender. The homogenate was centrifuged at 10,000 x *g* for 45 min. The supernatant was collected (760 ml).

Step II: The crude extract was loaded onto a column (6 x 22 cm) of DEAE-cellulose (DE 52 from Whatman) equilibrated with 25 mM KPi, pH 7.4 containing 50 mM KCl. After washing with 700 ml of the above buffer, the activity was eluted with 1,200 ml of 25 mM KPi, pH 7.4 with 300 mM KCl.

Step III: The DE fraction (1,085 ml) was made 30% in ammonium sulfate, stirred for 30 min and then centrifuged at 10,000 x *g* for 20 min. The supernatant was made 55% in ammonium sulfate, stirred for 30 min, and again centifuged at the same speed as above. The precipitate was dissolved in 25 mM KPi, pH 6.7, and the solution (75 ml) was applied to a column (6 x 25 cm) of Sephadex G-75, previously equilibrated with the above buffer. The colored fraction was collected (165 ml).

Step IV: The Step III fraction was applied to a column (4 x 7.7 cm) of hydroxyapatite (BioGel HT) previously equilibrated with 25 mM KPi, pH 6.7. The column was washed with 100 ml of the above buffer. The enzyme activity was eluted with a linear gradient (total 600 ml) of 25 mM KPi, pH 6.7 to 300 mM KPi, pH 7.4. Fractions containing the activity were collected (130 ml).

Step V: The first HA fraction was made 55% in ammonium sulfate, stirred for 30 min and then centrifuged at 12,000 x *g* for 20 min. The precipitate was dissolved in 25 mM KPi, pH 7.4, and the solution was applied to a column (2.5 x 90 cm) of Ultrogel AcA 34 (LKB), previously equilibrated with 25 mM KPi, pH 7.4. The column was eluted at a flow rate of 40 ml/h.

Step VI: The Ultrogel fraction (78 ml) was applied to a column (1.5 x 15 cm) of AffiGel Blue (BioRad) equilibrated with 25 mM KPi, pH 7.4. The column was washed with 100 ml of the above buffer, and then eluted with 500 mM KPi, pH 7.4.

Step VII: The AffiGel Blue fraction (55 ml) was made 55% in ammonium sulfate. The solution was stirred for 30 min and centrifuged at 12,000 x *g* for 20 min. The

precipitate was dissolved in 10 mM KPi, pH 6.7, and the solution (5 ml) was dialyzed against 2 liters of the above buffer for 12 h. The dialyzed fraction was centrifuged at 10,500 x *g* for 10 min to remove undissolved salts, and the supernatant was applied to a column (1.0 x 15 cm) of AMP-Sepharose (5'-AMP-Sepharose 4B from Pharmacia) previously equilibrated with 10 mM KPi, pH 6.7. The column was washed with 90 ml of the above buffer, and then the enzyme activity was eluted with 10 mM KPi, pH 6.7 containing 1 M KCl. The eluted fractions containing the activity were collected (11.5 ml), and then dialyzed against 3.5 liters of 50 mM KPi, pH 7.4 for 5.5 h.

Step VIII: The dialyzed solution from Step VII was applied to a column (1.0 x 10 cm) of hydroxyapatite, previously equilibrated with 50 mM KPi, pH 7.4. The column was washed with 50 ml of the above buffer, and then eluted with a linear gradient (total 60 ml) of 50 mM KPi, pH 7.4 containing from 0 to 1 M KCl. The tubes containing the activity were grouped into two fractions, Fr. A (9.0 ml, 817 fold) and Fr. B (17 ml, 2304 fold), on the basis of their specific activities. Fr. B was passed through a filter (Millex-HA, 0.45 μm, from Millipore) to remove undissolved salts, and concentrated to 136 μl using Centricon-30 (Amicon).

Step IX: One hundred microliters of concentrated Fr. B were injected to gel filtration HPLC (at room temperature) of G3000SW followed by G4000SW columns at a flow rate of 1 ml/min with 25 mM KPi, pH 7.4. A_{280} was monitored and one-ml fraction was collected. The peak fraction was applied to SDS-PAGE.

Assay of ATase Activity, Protein Assay, and SDS-PAGE

ATase activity was assayed as the PRPP-dependent conversion of [^{14}C]glutamine to [^{14}C]glutamic acid; the standard reaction mixture (50 μl) containing 50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 2.5 mM DTT, 5 mM PRPP (Sigma), 5 mM [^{14}C]glutamine (880 dpm/nmol) and enzyme. Preparations of Step VI through Step VIII were assayed in the reaction mixture supplemented with BSA (finally 0.5 mg/ml). Glutamic acid was separated from glutamine by high voltage paper electrophoresis using the borate buffer system (pH 9.0), after applying 20 μl from 50 μl mixture. Protein was determined by the BioRad standard assay using catalase as the standard. Microassay was applied to preparations of Step VII and VIII.

SDS-PAGE was done as previously described (16). Molecular mass of standard proteins are as follows; bovine serum albumin 68 kDa, ovalbumin 43 kDa, glyceraldehyde-3-phosphate dehydrogenase (GAPDHase) 36 kDa, and carbonic anhydrase 29 kDa.

Screening of λ Phage Library and Nucleotide Sequencing

A phage λgt11 human hepatoma (HepG2) cDNA library (Clontech) was screened. The plasmid pRAT107 (F14) containing a PCR-amplified DNA fragment spanning the entire coding region of rat ATase (12) was radiolabeled with [α-^{32}P]dCTP (3000 Ci/mmol; Amersham) by a megaprime system (Amersham) and was used as a hybridization probe. Positively hybridizing plaques were plaque-purified and subcloned into pUC19. DNA sequencing was carried out using the Taq Dye Primer Cycle Sequencing Core Kit (401112) in the ABI 373A DNA sequencer. Cloned pUC19 was used as a template.

Southern Blot Analysis, Northern Blot Analysis, and Detection of mRNA by the Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Method

Five microgram aliquots of DNA isolated from human peripheral white blood cells were digested by the indicated restriction enzymes. Digested DNA samples were subjected to 0.7% agarose gel electrophoresis and transferred to nitrocellulose membrane (Schleicher & Schuell) by the method of Southern (17). Cloned phages λHAT4 and λHAT5 were subcloned into pUC19 and plasmids, pHAT4ER and pHAT5RE, were constructed. DNA fragments of fHAT4NN (864 bp, 1049-1912) and fHAT5SH (480 bp, 42-521) were cut out from pHAT4ER and pHAT5RE, respectively, and were used as hybridization probes.

Various human tissues were obtained at autopsy. Total RNA was isolated by the acid guanidinium thiocyanate-phenol-chloroform method (18), and poly(A)$^+$RNA

was separated from the total RNA using Oligotex^{TM}-dT30 (Roche). Twenty micrograms of total RNA or five micrograms of poly(A)$^+$RNA was electrophoresed and transferred onto nylon membrane (Hybond N; Amersham). The DNA fragment fHAT4NN was used as a hybridization probe. pHcGAP, a probe for human GAPDHase, was obtained from ATCC. Because ATase message was far less than that of GAPDHase, the image of ATase was amplified by Fuji BAS2000.

One microgram of total RNA treated with DNase was reverse transcribed as previously described (19). The oligomer oRB494 (5'-ATCGAGTCGACACACATCCAA-CCCTACCAG-3'; the under lined letters indicate non-matched bases to create a restriction site) was used as a primer for reverse transcription. A set of primers of oRB459 (5'-ATCGTTATGGGAAGATCGCTGTGG-3') and oRB460 (5'-ATCGTTTTGAT-CCTGGCTACCCAG-3') was used for amplification by PCR. Thirty cycles of amplification at 94, 50 and 72°C for 1,1 and 2 min, respectively, were carried out in the Program Temp Control System PC700 (Astec). The amplified DNA fragments were subjected to 8.0% polyacrylamide gel electrophoresis and visualized with ethidium bromide staining. The amplified fragment from splenic total RNA was cloned into pUC19 and the sequence was determined.

RESULTS

Mr of an ATase Subunit and Cloning of Human ATase cDNA

The purification scheme based on the enzyme activity gave rise to about 2,300-fold purification with a yield of about 3% from the crude extract, which resulted from centrifugation at 11,000 x *g*. As shown in Fig. 1, the molecular mass of the human ATase subunit determined by SDS-PAGE was 56 kDa.

Approximately 840,000 plaques were screened and 6 positively hybridizing phages were obtained. Three of them encompassing 1-1482 (λHAT5), 262-2167 (λHAT4), and 1072-2145 (λHAT1) of the finally cloned sequence were analyzed. The entire coding region of human ATase cDNA was covered by them.

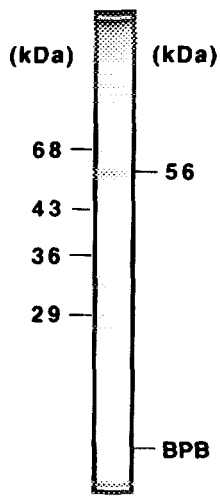

Figure 1. **SDS-PAGE of human placental ATase.** The purified human placental ATase was subjected to 10% SDS-PAGE and visualized with silver staining.

Nucleotide and Amino Acid Sequences

The 2,167 bp nucleotide sequence of the cDNA clone encoding human ATase and the deduced amino acid sequence are shown in Fig. 2. There is a single open reading frame starting at the first AUG, encompassing positions 122-124, and ending with a UAG at nucleotides 1673-1675. The deduced amino acid sequence encoded by this open reading frame is a peptide chain of 517 amino acids with the calculated Mr of 57,398.

Southern Blot Analysis, Northern Blot Analysis, and RT-PCR

Genomic DNA isolated from human peripheral white blood cells was digested, respectively, by one of 12 endonucleases with 6 base recognition sequences: *Bam*HI, *Bcl*I (*Fba*I), *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Eco*T22I, *Hin*dIII, *Nhe*I, *Pst*I, *Pvu*II, and *Sac*I. The genomic Southern blot prepared from these digests was probed with fHAT4NN and fHAT5SH. As shown in Fig. 3, multiple bands were observed in all lanes, suggesting that the ATase gene exists as multiple copies in the human genome (similar data with fHAT5SH, not shown).

Twenty micrograms of total RNAs or five micrograms of poly(A)$^+$RNAs from various human tissues and Lu65 were blotted onto nylon membrane and were probed with ^{32}P-labeled fHAT4NN. As shown in Fig. 4, kidney, pancreas, liver, lung, testis, stomach, and Lu65 expressed 3.5 kb ATase mRNA.

All 10 tissues examined by RT-PCR at its saturating phase, including colon, small intestine, heart, lung, liver, spleen, pancreas, kidney, stomach, and testis, expressed ATase mRNA (data not shown). Sequence analysis confirmed that the sequence of amplified 193 bp fragment from splenic total RNA was identical with that of the human ATase cDNA (data not shown).

DISCUSSION

In spite of the oxygen-sensitive lability of the iron-sulfur structure of human ATase (6), it was highly purified by a factor of about 2,300. Using this purified preparation, the molecular mass of the ATase subunit was estimated as 56 kDa on SDS-PAGE. This molecular mass is consistent with the molecular masses of chicken and rat ATase (11,12,21), and suggests that two molecular forms of human ATase correspond to dimer and tetramer forms (2).

The determined sequence contains a single open reading frame of 1,551 bp encoding a protein of 517 amino acids with a deduced Mr of 57,398. The entire derived amino acid sequence of human ATase exhibited 93, 82, 41, 37, and 33% identity with those of rat, chicken, *B. subtilis*, *E. coli*, and *S. cerevisiae* ATases,

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Figure 2. Nucleotide sequence of human ATase cDNA and the deduced amino acid sequence. The nucleotide sequence is arbitrarily numbered from the nucleotide at the 5'-end. The amino acid sequence shown by one letter is numbered from the N-terminus of the active mature ATase. Asterisks mark the four conserved consensus phosphorylation sites for cAMP-dependent protein kinase (Arg-X-X-Ser/Thr). DDBJ, EMBL and GenBank accession number is D13757.

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                                        TCGGGAAGGAAGAGTCTGTCGCGAGCGCGAGCTT   34
CTGAGCTCGACGGGCCGAGCTGGCAGCTGGTTGGTGCTTACACCTTGGCCGCAGCGGCAG   94

GTCCTTCCACGTGCTTTCGGCGGCGACATGGAGCTGGAGGAGTTGGGGATCCGAGAGGAA  154
                                  M  E  L  E  E  L  G  I  R  E  E   -1

TGTGGCGTGTTCGGGTGCATCGCCTCAGGAGAGTGGCCCACGCAGCTGGATGTACCGCAT  214
 C  G  V  F  G  C  I  A  S  G  E  W  P  T  Q  L  D  V  P  H    20

GTGATCACTCTGGGACTCGTGGGGCTGCAGCACCGGGGTCAGGAGAGTGCTGGTATTGTG  274
 V  I  T  L  G  L  V  G  L  Q  H  R  G  Q  E  S  A  G  I  V    40

ACTAGTGATGGGAGTTCGGTGCCAACATTCAAATCACACAAGGGAATGGGTCTTGTAAAT  334
 T  S  D  G  S  S  V  P  T  F  K  S  H  K  G  M  G  L  V  N    60

CACGTCTTTACTGAAGACAATTTGAAAAAATTATATGTTTCAAATCTTGGAATTGGACAC  394
 H  V  F  T  E  D  N  L  K  K  L  Y  V  S  N  L  G  I  G  H    80

ACCAGGTATGCCACCACAGGAAAATGTGAACTAGAAAATTGTCAGCCCTTCGTTGTTGAA  454
 T  R  Y  A  T  T  G  K  C  E  L  E  N  C  Q  P  F  V  V  E   100
    *  *  *  *
ACACTTCATGGGAAGATAGCTGTGGCACATAATGGCGAATTGGTAAATGCTGCTCGATTA  514
 T  L  H  G  K  I  A  V  A  H  N  G  E  L  V  N  A  A  R  L   120

AGGAAAAAGCTTCTGCGTCATGGTATTGGTCTGTCTACAAGTTCTGATAGTGAAATGATT  574
 R  K  K  L  L  R  H  G  I  G  L  S  T  S  S  D  S  E  M  I   140

ACCCAGTTACTGGCGTATACCCCTCCTCAGGAACAAGATGACACCCCAGACTGGGTAGCC  634
 T  Q  L  L  A  Y  T  P  P  Q  E  Q  D  D  T  P  D  W  V  A   160

AGGATTAAAAACTTGATGAAGGAAGCACCCACAGCATACTCCCTGCTTATAATGCACAGA  694
 R  I  K  N  L  K  K  E  A  P  T  A  Y  S  L  L  I  M  H  R   180

GATGTTATTTATGCAGTACGAGATCCTTATGGAAATCGTCCCTTATGCATTGGTCGTCTT  754
 D  V  I  Y  A  V  R  D  P  Y  G  N  R  P  L  C  I  G  R  L   200

ATTCCAGTGTCTGATATAAATGACAAAGAGAAAAAAACATCAGAAACAGAAGGATGGGTG  814
 I  P  V  S  D  I  N  D  K  E  K  K  T  S  E  T  E  G  W  V   220

GTGTCTTCAGAATCTTGTAGCTTCTTATCTATTGGTGCAAGATATTACCGTGAAGTCTTG  874
 V  S  S  E  S  C  S  F  L  S  I  G  A  R  Y  Y  R  E  V  L   240

CCTGGAGAAATTGTGGAAATATCCAGACACAATGTCCAAACTCTTGATATTATATCAAGG  934
 P  G  E  I  V  E  I  S  R  H  N  V  Q  T  L  D  I  I  S  R   260

TCTGAAGGAAACCCAGTGGCTTTTTGTATCTTTGAATATGTTTATTTTGCAAGACCAGAC  994
 S  E  G  N  P  V  A  F  C  I  F  E  Y  V  Y  F  A  R  P  D   280
                                                  *  *  *


AGTATGTTCGAAGACCAAATGGTTTATACAGTAAGATACCGTTGTGGCCAGCAGCTAGCG 1054
 S  M  F  E  D  Q  M  V  Y  T  V  R  Y  R  C  G  Q  Q  L  A   300
 *
ATTGAAGCACCTGTGGATGCAGATTTGGTTAGCACTGTTCCAGAATCTGCTACGCCTGCT 1114
 I  E  A  P  V  D  A  D  L  V  S  T  V  P  E  S  A  T  P  A   320

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       *  *  *  *
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 E  V  H  I  R  V  A  S  P  P  I  K  Y  P  C  F  M  G  I  N   420
             *  *  *  *
ATTCCTACAAAAGAAGAGCTCATTGCCAATAAACCAGAATTTGATCACCTTGCAGAATAT 1474
 I  P  T  K  E  E  L  I  A  N  K  P  E  F  D  H  L  A  E  Y   440

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 L  G  A  N  S  V  V  Y  L  S  V  E  G  L  V  S  S  V  Q  E   460

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 G  I  K  F  K  K  Q  K  E  K  K  H  D  I  M  I  Q  E  N  G   480

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 N  G  L  E  C  F  E  K  S  G  H  C  T  A  C  L  T  G  K  Y   500

CCTGTAGAATTAGAATGGTAGCTGGTAGGGTTGGATGTGTGTAGTTTCAAGATAGAAAGT 1714
 P  V  E  L  E  W ***                                          506

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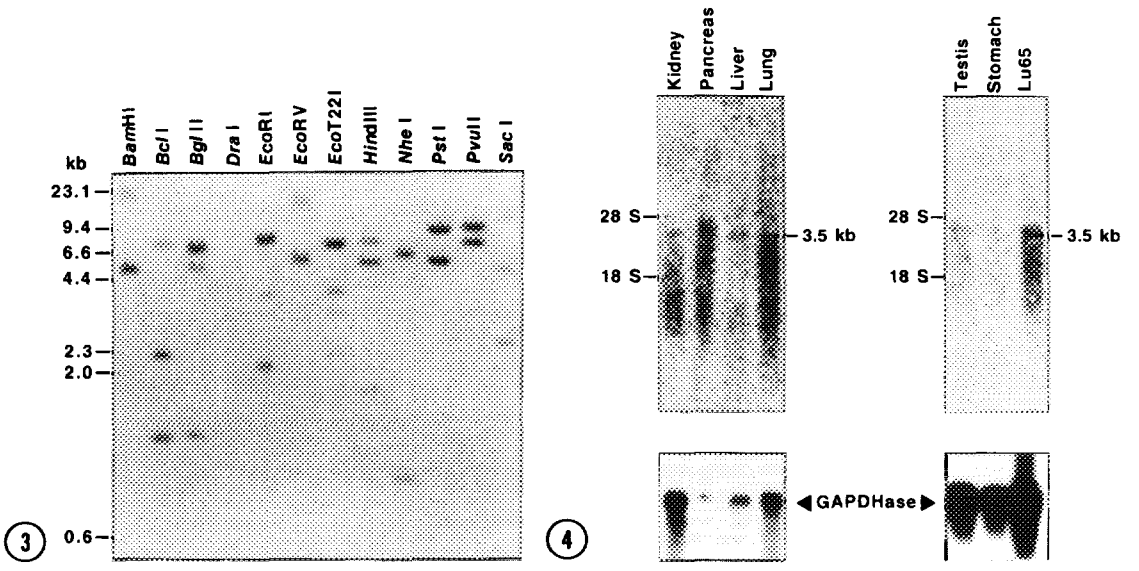

Figure 3. **Southern blot analysis.** Human genomic DNA (5 μg) was digested, respectively, by *Bam*HI, *Bcl*I (*Fba*I), *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Eco*T22I, *Hin*dIII, *Nhe*I, *Pst*I, *Pvu*II, and *Sac*I and subjected to electrophoresis on a 0.7% agarose gel. After Southern blotting, the nitrocellulose membrane was hybridized with probes fHAT4NN, which contain a 864 bp fragment from base pair 1048 to 1912 of the human ATase cDNA.

Figure 4. **Northern blot analysis.** Total RNAs (20 μg) isolated from human kidney, pancreas, liver and lung (left) or poly(A)$^+$RNAs (5 μg) isolated from human testis and stomach, and a human cell line Lu65 (right), were hybridized with the probe fHAT4NN. GAPDHase was used as an internal control.

respectively (7-12). A Cys^1-His^{100}-Asp^{29} catalytic triad was implicated in glutamine amide transfer in *E. coli* ATase (22). These catalytic residues were conserved as Cys^1-His^{110}-Glu^{35} in human ATase as well as in rat and chicken ATases (11,12). These results strongly suggest that the isolated clone encodes human ATase.

ATases of *S. cerevisiae* and *E. coli* do not have a propeptide but those of *B. subtilis*, chicken, and rat have an 11-amino acid residue propeptides (7-12). The propeptide is conserved in human ATase and is identical to 10 of 11, 11 of 11, or 5 of 11 corresponding amino acid sequences of rat, chicken or *B. subtilis* ATases, respectively. Glu residues at positions -2 and -1 and Cys at 1, which are considered essential for the processing of the propeptide (23), are conserved in human ATase. The propeptide must be processed in rat, chicken, and *B. subtilis* to produce an active mature enzyme (11,12,23).

B. subtilis ATase contains a unique [4Fe-4S] iron-sulfur cluster (4) and rat and human ATases have characteristic properties of iron-sulfur proteins (5,6). The iron-sulfur cluster is essential for formation of the native structure and for propeptide processing in chicken (14). Mutational analyses have shown that 4 cysteine residues at positions 393, 445, 448, and 451 of *B. subtilis* ATase serve as ligands to the [4Fe-4S] cluster (24). Of these amino acids, only Cys^{427}, which corresponds to the Cys^{393} of *B. subtilis* ATase, is conserved in human, rat, and chicken ATases. Human ATase contains 14 cysteine residues but no Cys-X-X-Cys-X-X-Cys motif. Therefore, the

structure of human ATase may differ from *B. subtilis* ATase with regard to the iron-sulfur cluster. However, Cys^{415} and Cys^{488}, which may be ligands to the iron-sulfur cluster in chicken ATase (14), are conserved in human ATase at positions 415 and 495 as well as in rat ATase (12).

Human ATase has four consensus phosphorylation sites for cAMP-dependent protein kinase (Arg-X-X-Ser/Thr) (20) which are Arg^{82}-Tyr^{83}-Ala^{84}-Thr^{85}, Arg^{278}-Pro^{279}-Asp^{280}-Ser^{281}, Arg^{383}-Gly^{384}-Asn^{385}-Thr^{386}, and Arg^{405}-Val^{406}-Ala^{407}-Ser^{408}. The first three are conserved in all ATases cloned from *S. cerevisiae*, *E. coli*, *B. subtilis*, chicken, and rat. The last one is conserved in chicken and rat. Human ATase may share the presumed phosphorylation-related regulation with rat ATase. Rat liver ATase activity is speculated to be regulated by glucagon and/or cAMP (25,26) and the partially purified rat liver ATase is phosphorylated *in vitro* by a catalytic subunit of cAMP-dependent protein kinase (27). These circumstantial evidences imply that the cAMP-dependent phosphorylation of ATase may be a regulatory factor of ATase activity as has been suggested for carbamoyl phosphate synthetase II, the rate-limiting enzyme of pyrimidine biosynthesis (28).

The human ATase gene presumably exists as multiple copies in the genome, since all samples in the Southern blot analysis using 12 endonucleases with 6 base recognition sites, showed multiple bands. We identified an intronless ATase gene by PCR using human genomic DNA as a template (unpublished data). The intronless gene was supposed to be a pseudogene. However, there might be another very similar ATase gene which is expressed, as suggested (15).

In northern blot analysis, the relative abundance of ATase message was far less than that of GAPDHase probably due to the rate-limiting function of ATase, and the size of human ATase mRNA was shown to be 3.5 kb. Therefore, the isolated clone of 2,167 bp might be truncated at the 3' noncoding region of the gene. The last adenine stretch of 14 bp might have originated from the oligo(dT)-primer which was used in cDNA synthesis. RT-PCR analysis disclosed that all tissues examined expressed ATase, which is consistent with a housekeeping function of ATase.

The expression of the cloned gene, the functional complementation of ATase-deficient cells, and the immunological characterization of the protein expressed from cloned cDNA are underway.

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