

HUMAN PROSTATIC ACID PHOSPHATASE: cDNA CLONING, GENE MAPPING AND
PROTEIN SEQUENCE HOMOLOGY WITH LYSOSOMAL ACID PHOSPHATASE

Farida S. Sharief¹, Hansoo Lee², Mary M. Leuderman³, Ake Lundwall⁴,
Larry L. Deaven³, Ching-li Lee² and Steven S.-L. Li^{1,5}

¹Laboratory of Genetics, National Institute of Environmental Health Sciences,
National Institutes of Health, Research Triangle Park, North Carolina 27709

²Department of Diagnostic Immunology Research and Biochemistry, Roswell Park
Memorial Institute, Buffalo, New York 14263

³Life Sciences Division, Los Alamos National Laboratory,
Los Alamos, New Mexico 87545

⁴Department of Clinical Chemistry, University of Lund,
Malmo General Hospital, S-214 01 Malmo, Sweden

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The cDNAs encoding human prostatic acid phosphatase were cloned and characterized. The mRNAs contain 3' noncoding regions of heterogeneous sizes 646, 1887 or 1913 nucleotides. A dimer and a monomer of the conserved Alu-repeats are present in the longer 3' noncoding sequences. The complete sequence of 354 amino acids for the mature enzyme was determined by sequencing both cDNA and protein. Human prostatic and lysosomal acid phosphatases exhibit 50% sequence homology, including five Cys residues and two putative N-linked glycosylation sites. The Acp-3 gene coding for human prostatic acid phosphatase was mapped onto chromosome 3 in this investigation. The Acp-2 gene coding for lysosomal acid phosphatase has previously been located on chromosome 11, while the Acp-1 gene coding for red blood cell acid phosphatase is on chromosome 2.

Acid phosphatases (Acp; EC 3.1.3.2) are a group of enzymes capable of hydrolysing phosphomonoesters under acidic conditions, and can be differentiated according to their immunological properties, tissue distribution and subcellular location(1). Since Gutman et al. (1936) first reported an elevation of serum acid phosphatase activity in patients with metastatic prostate cancer (2), human prostatic acid phosphatase (PAP) has been used as a diagnostic marker for prostate cancer (3). The physiological function of PAP is not well understood, but recent reports suggested that PAP

⁵To whom all correspondence should be addressed.

Abbreviations: Acp, Acid phosphatase; PAP, prostatic acid phosphatase;
LAP, lysosomal acid phosphatase.

may function in vivo as a phosphotyrosyl-protein phosphatase (4). The PAP is synthesized under androgen regulation by epithelial cells of the prostate, and it is secreted into the seminal fluid (5). In order to study the protein structure-function and mechanism of androgen regulation, we have undertaken the cDNA cloning and protein sequencing of human PAP. In this report, we describe the cDNA cloning and gene mapping of human PAP as well as protein sequence homology between human prostatic and lysosomal acid phosphatases.

Materials and Methods

Protein sequencing:

Human PAP protein was purified (6) and cleaved with CNBr and/or Lys-C protease (7). The resulting peptides were separated by HPLC microbore RP-C8 column using a linear gradient of acetonitrile (0-70% in 60 min) in 0.1% trifluoroacetic acid (8). The amino-terminal sequences of the mature enzyme and purified peptides were determined by automatic micro-sequencer equipped with on-line HPLC and data module (Applied Biosystems, Inc.).

cDNA cloning and sequencing:

A human prostate λ gt11 cDNA expression library established from benign prostatic hyperplasia (9) was screened with rabbit antiserum against human PAP as previously described (10-12). The putative positive clones were purified to homogeneity using polyclonal and monoclonal antibodies against human PAP. The DNAs purified from the positive clones were analyzed by restriction endonuclease mapping and Southern blotting (13). The DNA fragments derived from EcoRI cleavage, as well as overlapping deletions constructed using an erase-a-base kit (Promega), were further subcloned into M13 mp18 phage (14), and the nucleotide sequences of the inserted DNAs were determined by the dideoxy chain termination method with the sequencing protocol modified to use deoxyadenosine 5-(α -35S)thiotriphosphate (15).

Gene mapping:

Human chromosomes were isolated and flow-sorted into 24 spots on nitrocellulose discs as previously described (16). These spot-blots and human genomic DNA blot were hybridized with ³²P-labeled PAP cDNA probe as indicated in Figure 1. The conditions for hybridization and washing have been described previously (13,17).

Results

Protein sequencing:

The amino-terminal sequence of 45 residues from the purified PAP protein was obtained by micro-sequencing. A total of 80 amino acids (residue nos. 44-65, 148-153, 203-219, 240-251, 254-265 and 298-308) was also sequenced from four CNBr peptides and two Lys-C peptides (Fig. 2A). The amino acid sequence of one CNBr peptide was found to overlap with the amino-terminal sequence of the PAP protein. Thus, the sequence of the first 65 amino acids from mature PAP enzyme was unambiguously established.

cDNA cloning and sequencing:

Six putative PAP cDNA clones were plaque-purified using polyclonal and monoclonal antibodies against PAP, and their inserted DNAs were shown to

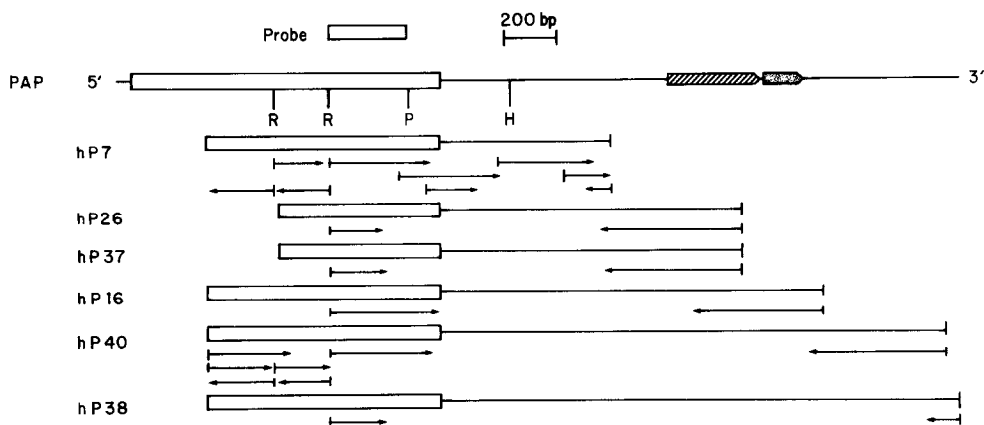


Fig. 1. Restriction endonuclease map and sequencing strategy of human PAP cDNA clones.

The restriction sites given (R, EcoRI; P, PstI and H, HindIII) are those used in nucleotide sequencing and preparation of hybridization probe. The direction and length of each sequencing run are indicated by arrows. The protein-coding sequence is denoted by open box. The dimeric and monomeric Alu-repeats are shown by hatched boxes.

cross-hybridize with each other (data not presented). The EcoRI DNA fragments of these six clones were subcloned into M13 mp18 phages, and their nucleotide sequences determined as indicated in Figure 1. The combined nucleotide sequence of these PAP cDNAs contain an open reading frame. The first six amino acids Phe-Leu-Asn-Glu-Ser-Tyr, which were deduced from the cDNA sequence, were identical with residue nos. 60 to 65 from the amino-terminal sequence of the purified PAP protein (Fig. 2A). The amino acid sequences of five peptides also confirmed the cDNA-deduced sequences of residue nos. 148-153, 203-219, 240-251, 254-265 and 298-308. Therefore, the purified PAP enzyme consists of a sequence of 354 amino acids. Since these six PAP cDNAs did not possess the sequence coding for the first 59 amino acids, an EcoRI-PstI cDNA fragment of coding region (as indicated in Fig. 1) was further used as a probe to isolate full-length PAP cDNA. However, none of the additional 24 PAP cDNA clones were found to contain the coding sequence 5' to codon no. 60.

As to the 3' noncoding sequence of the PAP cDNAs (Fig. 3B), clone hP7 contained a poly (A)-tail of 51 residues added at nucleotide no. 646, and a putative polyadenylation signal of AATTAA was found at 20 nucleotides 5' to this poly (A) addition site. Clones hP26 and hP37 appeared to contain identical cDNA sequence ending at nucleotide no. 1126 without poly (A)-tail. Clone hP16 did not possess a poly (A)-tail and terminated at nucleotide no. 1442. Clone hP40 contained a poly (A)-tail of 20 residues added at nucleotide no. 1887, while clone hp38 had a poly (A)-tail of 41 residues added at nucleotide no. 1913. Both hP40 and hP38 clones contained a common

ggccgagaacagctctctcaacatgaga
-32 MetArg

A gctgcacccctctctctggccagggcagcaagcttagccttgcttctgtttctgtctttttgctggctagaccgaagtgtactagcc
 1 AtaAlaProLeuLeuLeuAlaArgAlaAlaSerLeuAlaLeuAlaSerCysPheCysPhePheCysTrpLeuAspArgSerValLeuAla
 1 LysGluLeuLysPheValThrLeuValPheArgHisGlyAspArgSerProIleAspThrPheProThrAspProIleLysGluSerSer
 31 tgccacacaggaattggccaactcaccagctgggcataggcagcagcattagaacttggagagatataagaagaagagatagaaaATTC
 31 TrpProGlnGlyPheGlyGlnLeuGlyMetGluGlnHisTyrGluLeuGlyGluTyrIleArgLysArgTyrArgLysPhe
 61 TTGAATGACTCTATAAACATGAACAGGTTTATATTTCGAAGCACAGCGTTGACCGGACTTGTAGAGTCGTATGACAAACCTGGCAGCC
 61 LeuAsnGluSerTyrLysHisGluGlnValTyrIleArgSerThrAspValAspArgThrLeuMetSerAlaMetThrAsnLeuAlaAla
 91 CTGTTTCCCCAGAGGTGTGACATCTGGAATCTACTCTGCGAGCCATCCCGGTGCACACAGTTCCTCTTCTGAAGATCAG
 91 LeuPheProProGluGlyValSerIleTrpAsnProIleLeuLeuTrpGlnProIleProPheIHisThrValProLeuSerGluAspGln
 121 TTGCTATACCTGCCCTTCAGGAATGCCCTCGTTCAGAACTTGAGAGTGAGACTTTGAAATCAGAGGAATPCCAGAAGAGCTGCAC
 121 LeuLeuTyrLeuProPheArgAsnCysProArgPheGlnGluLeuGluSerGluThrLeuLysSerGluGluPheGlnLysArgLeuHis
 151 CCTTATAAGBATTTTATAGCTACTGGGAAAACCTTCAGGATTACATGGCCAGGACCTTTTTGGAATTTGGAGTAAAGTCTACGACCT
 151 ProTyrLysAspPheIleAlaThrLeuGlyLysLeuSerGlyLeuHisGlyGlnAspLeuPheGlyIleTrpSerLysValTyrAspPro
 181 TTATATCTGAGAGTGTCCAAATTCACCTTACCCCTCTGGGCCACTGAGGACACCATGACTAAGTTGAGAGAAATGTCAGAATTTGCC
 181 LeuTyrCysGluSerValIHisAsnPheThrLeuProSerTrpAlaThrGluAspThrMetThrLysLeuArgGluLeuSerGluLeuSer
 211 CTCTGTGCCCTCTATGGAATTCACAGCAGAAAGAGAATCTAGGCTCCAAGGGGGTGTCTGGTCAATGAAATCTCAATCACATGAAG
 211 LeuLeuSerLeuTyrGlyIleHisLysGlnLysGluLysSerArgLeuGlnGlyGlyValLeuValAsnGluIleLeuAsnHisMetLys
 241 AGAGCAACTCAGATACCAAGCTACAAAACCTATCATGTATTCTGCGCATGACACTGTGACTGGCTCAGACTCAGAGTCAGAGTGTG
 241 ArgAlaThrGlnIleProSerTyrLysLysLeuIleMetTyrSerAlaHisAspThrThrValSerGlyLeuGlnMetAlaLeuAspVal
 271 TACACGGACTCCTTCTCCCTATGCTTCTGCCACTTGACGGAATTTGACTTTGAGAAGGGGAGACTTTGTGGAGATGTACTACGG
 271 TyrAsnGlyLeuLeuProProTyrAlaSerCysHisLeuThrGluLeuTyrPheGluLysGlyIleTyrPheValGluMetTyrTyrArg
 301 AATGAGACCGCAGCAGGCGTATCCCTCATGCTACCTGGCTGCAGCCAGCTGTCTCTGGAGAGGTTGTCTGAGCTGGTGGCCCT
 301 AsnGluThrGlnHisGluProTyrProLeuMetLeuProGlyCysSerProSerCysProLeuGluArgPheAlaGluLeuValGlyPro
 331 GTGATCCCTCAAGACTGTCTAGGGGGTTATGACCACAACAGCCATCAAGGTACTGAGGACAGCAGATTAAG
 331 ValIleProGlnAspTrpSerThrGluValMetThrThrAsnSerHisGlnGlyThrGluAspSerThrAsp 354

10 20 30 40 50 60 70 80 90 100

B 1 TGTGCACAGAGATCTCTGTAGAAGAGTAGTGCCTTCTCAGGGCAGATGATGCTTTGAGAACATACTTTGGCCATTACCCCCAGCTTGTAGGAAAA
 101 TGGGCTTGGATGATTATTTATGTTTTAGGGACCCCAACCTCAGGCAATCCATCCTTCAACCCGACCTGCCCCACTTGCATAAAAACCTTAGCT
 201 AAGTTTGTGTTTTGTTTTTCAGCGTAAATGTAAGGGGCGAGCAGTCCAAAATAAACAGAGATAAAGCTTAGGTCAAAGTTCATAGAGTCCCATGAAC
 201 ATATGACTGCCACAGGATCTTTGTTATTTAAGGATCTGAGATTTGCTTGAGCAGGATAGATAAGGCTGTCTTTAAATGCTGAAATGGAACAG
 401 ATTTCAAAAAAACCCCAACTTCCAGGTGGGAACAAGGAAGAAAGATGTGAATAGGCTGATGGGCAAAACCAATTTACCATCAGTCCAGCCCTC
 501 TCTCAAGGAGAGCAAGAAAGGAGATACAGTGGAGACATCGAAAGTTTCTCCACTGGAAAACCTGCTACTATCTGTTTTTATATTCTGTTAAAAATA
 601 TATGAGGCTACAGAACTAAAAATTAACACTCTTTGTGTCCTTGTCTGGAACATTTATGTTCTTTAAAGAAACAAAATCAAACTTTACAGAAAG
 701 ATTTGATGATGTAATACATATAGCAGCTCTTGAAGTATATATATCATAGCAAAATAGTCACTGATGAGAAACAAGCTATTTGGGCACAACACATCAGGA
 901 AAGAGAGCACCAGTGTGGAGTTTCTCCAGAAGCTCCAGTATAAGAGATGTTGACTCTAAAGTTGATTTAAGGCCAGGCATGGTGGTTTACGCCATA
 901 ATCCCAAGCATTTTGGAGCTCCGAGGTGGCAGATCACTTGAGCTCAGGAGGTCAAGATCAGCTGGGCAACATGGTGAACCTTGTCTCTACATAAAATA
 1001 CAAAAACCTTAGATGGGCATGGTGGTGTGCCTATAGTCCACTACTTGTGGGGCTAAGGCAGGAGGATCACTTGAGCCCGGAGGTCGAGGCTACAGTGA
 1101 GCCAAGAGTGCCTACTGTACTCCAGCCAGGGCAAGAGAGCAGAGCCCTGTCTCAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAA
 1201 TAAAAACAAGTTGATTAAAGAAAGGAGATAGGCTAGGCACAGTGGCTCACACCTGTAATCCTTGCAATTTTGGAAAGGCTGAGGCAGGAGGATCACTTTA
 1301 GGCCTGGTGTGTTCAAGACCAGCCTGGTCAACATAGTGAGACACTGTCTCTACCAAAAAAGGAAGGAAAGGACACATATCAAACTGAAAACAAAATAGA
 1401 AATGTAATATGTTATGTTCTAAGTGCCTCAAGTTCAAAACCTTATGGAATGTTGAGAGTGGTTCAGAAATACGTTAGGAGGACAAAAGGAAATGTTG
 1501 AGTCTTTAATGCCGATATCTTCAGAAACCTAAGCAAACTACAGGCTCGCTGAACTGCCACTCGCAAGAAATAATCATGATATAGCTTTGCCCA
 1601 TGTGCAGATCTACATGCTAGAGAACACTGTGCTCTATTACCATTATGGATAAAGATGAGATGGTTTCTAGAGATGGTTTCTACTGGCTGCCAGAATCT
 1701 AGAGCAAGCCATCCCGCTCCTGGTGGTCAGAGAATGACTGACAAGACATCGATTGATATGCTCTTTGTGTTATTCCCTCCCAAGTAATATGTTG
 1801 TCCTTGGGTCATTTCTATGCTGTAAGTCTCTTAGCAGTGAGCCAAATGTAATAAGTGAATAAGATCATTATAGGAAGTTCAAAAAGCATTGCTT
 1901 TTATAATGAACCTAAAAA

Fig. 2. The nucleotide and amino acid sequences of human PAP.

A. The amino acid sequence of PAP is numbered from the first residue of the mature enzyme, and the number of the first amino acid in each row is given on the left. The amino acid sequences obtained by direct protein sequencing are underlined. The nucleotide sequence 5' to codon no. 60 is from the previously reported sequence (20) in which a sequence of Trp-Ile-Trp-Pro-Thr-His-Pro-Ala was deduced for amino acid nos. 34 to 41. Further, Ala and Cys were reported for amino acid nos. 180 and 340, respectively (20). It may also be noted that a Gln instead of Glu at amino acid no. 2 was detected previously (10).

B. The 3' noncoding sequence of human PAP is numbered from the first nucleotide after termination codon TAG, and the number of the first nucleotide in each row is given on the left. Clones hP7, hP40 and hP38 have poly (A) addition sites (indicated by dots) at nucleotide nos. 646, 1887 and 1913, respectively. The dimer and monomer of Alu-repeats are underlined. The previously reported sequence (20) and the sequence determined in this investigation exhibit five substitutions at nucleotide nos. 156 (T for A), 157 (A for T), 168 (T for C), 385 (T for G) and 1211 (C for G); a single nucleotide (T) insertion after residue no. 256 and a double nucleotide (TT) addition after residue no. 413; eight deletions at nucleotide nos. 417, 1206, 1210, 1219, 1223, 1227, 1243 and 1842.

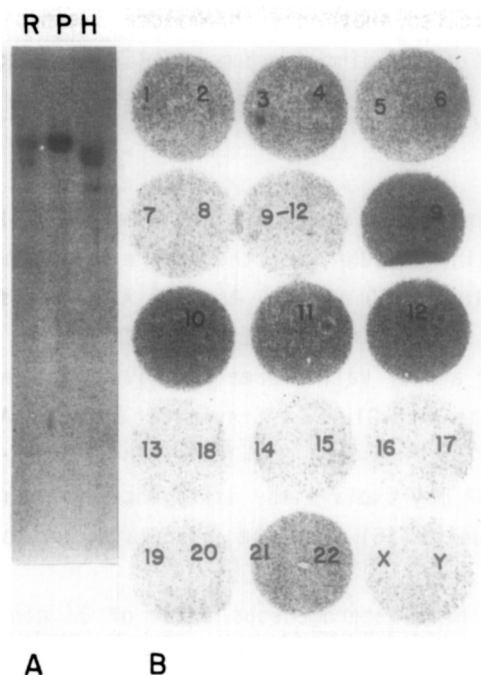


Fig. 3. Analyses of human genomic blot and chromosome spot-blots using human PAP cDNA probe.

A. The total human genomic DNA was cleaved with restriction endonucleases EcoRI (R), PstI (P) or HindIII (H), and electrophoresed on 0.75% agarose gel. The DNA fragments were transferred to nitrocellulose filter and hybridized with 32 P-labeled coding probe of PAP cDNA as indicated in Fig. 1. The estimated sizes of major DNA fragments are: 4.6Kb for EcoRI and PstI, and 3.8Kb for HindIII.

B. The human chromosomes of each type were flow-sorted directly onto nitrocellulose discs as previously described (16). The spot-blots were hybridized with the same coding probe used in genomic blot analysis.

polyadenylation signal of AATAAA located at 18 and 44 nucleotides, respectively, upstream to their poly (A) addition sites. Thus, clones hP26, hP37, hP16, hP40 and hP38 appeared to have originated from mRNAs in which the 3' noncoding sequences contain a dimer and a monomer of Alu-repetitive sequences. The dimeric Alu-sequence including 12 repeats of TAAA is flanked by direct repeats of AAAGTTGATT (nucleotide nos. 861-1216), while the monomeric Alu-sequence is surrounded by direct repeats of AAGGAAG (nucleotide nos. 1222-1370).

Gene mapping:

The genomic blot analysis of human total DNA using PAP coding probe showed a single hybridization band in PstI cut DNA (Fig. 3A). The detection of a strong band and a weak band in the DNAs of either EcoRI or HindIII cleavage suggested the presence of an intron within the cDNA region used as a probe, since this cDNA sequence contains neither an internal EcoRI nor a HindIII site. The results of chromosome spot-blot hybridization with the same

PAP coding probe indicated that only chromosome 3 exhibits a positive signal (Fig. 3B). Thus, the gene coding for human PAP is located on chromosome 3.

Discussion

The complete amino acid sequence of purified human PAP was determined by sequencing both protein and cDNA, and the mature enzyme contains a sequence of 354 amino acids, including 10 Ala, 15 Arg, 10 Asn, 16 Asp, 5 Cys, 17 Gln, 29 Glu, 19 Gly, 13 His, 14 Ile, 44 Leu, 19 Lys, 10 Met, 15 Phe, 25 Pro, 27 Ser, 24 Thr, 6 Trp, 19 Tyr and 17 Val. Three putative N-linked glycosylation sites of PAP were found at Asn-Glu-Ser (residues 62-64), Asn-Phe-Thr (residues 188-190) and Asn-Glu-Thr (residues 301-303). The glycosylation at these three potential sites of PAP may explain the difference between the estimated 50 kDa of glycosylated subunits (6) and the calculated Mr of 40,939 for mature polypeptide chains.

Human PAP mRNAs have heterogeneous sizes of 3' noncoding region. Clone hP7 contain only 646 nucleotides of the 3' noncoding sequence; while clones hP40 and hP38 have 3' noncoding regions of 1887 and 1913 nucleotides, respectively. A dimer and a monomer of Alu-repeats are present in the longer 3' noncoding sequences, and these two Alu-repeats exhibit approximately 80% homology with the consensus Alu-sequence (18). It is of interest that an Alu-repeat is also present in the 3' noncoding region of the cDNA encoding for human placental alkaline phosphatase (19). However, the biological significance of the Alu-repeats in the 3' noncoding region of PAP mRNAs remains to be determined.

This investigation found that none of the isolated 30 PAP cDNA clones contain the coding sequence 5' to codon no. 60, while several PAP cDNAs truncated at the same AAATTC site of codon no. 59-60. This result may indicate that EcoRI restriction endonuclease cleaves the EcoRI-like sequence, but EcoRI methylase did not protect this sequence during the cDNA cloning (9). Thus, a different procedure without using EcoRI restriction endonuclease must be utilized to establish the cDNA library in order to obtain full-length PAP cDNA.

While this work was in progress, human PAP cDNA was independently cloned (20). A comparison of protein sequence revealed 10 amino acid differences at residue nos. 34 to 41, 180 and 340. The differences between the sequence of Gly-Phe-Gly-Gln-Leu-Thr-Gln-Leu obtained by direct protein sequencing in this investigation and the predicted sequence of Trp-Ile-Trp-Pro-Thr-His-Pro-Ala can simply be due to an insertion of T at codon no. 34 and deletion of G at codon no. 41 in the reported cDNA sequence (20). It should also be noted that the amino acid sequence of residue nos. 34 to 39 obtained by direct protein sequencing is identical to the corresponding sequence of human LAP (21) as

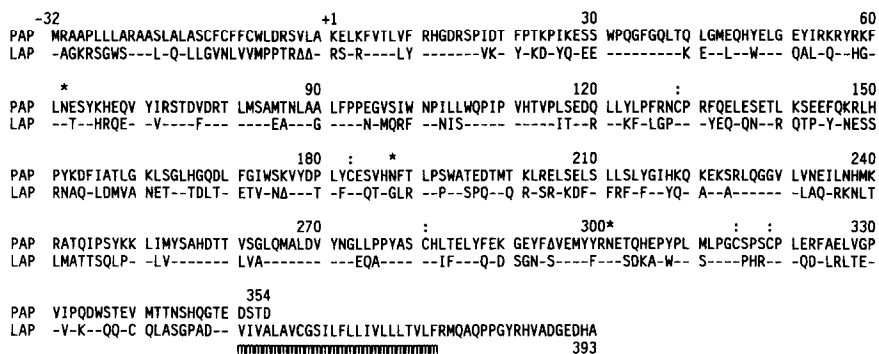


Fig. 4. Comparison of amino acid sequences of human PAP and LAP.

The amino acids of LAP (21) different from PAP are given; while those identical residues are indicated by hyphen. The open triangle means the addition/deletion in PAP and LAP sequences. The conserved Cys residues are shown by dots, while the potential N-linked glycosylation sites are labeled by star. The transmembrane peptide of LAP is denoted by m.

indicated in Figure 4. The differences at amino acid nos. 180 (Pro vs Ala) and 340 (Val vs Cys) may represent genetic polymorphisms in human population. At the 3' noncoding region of 1913 nucleotides (Fig. 2B) there are 5 nucleotide substitutions and 11 additions/deletions between the reported sequence (20) and the sequence determined in this investigation.

The gene coding for PAP was mapped onto human chromosome 3, and this gene is designated as Acp-3. The Acp-1 gene coding for red blood cell acid phosphatase is located on chromosome 2; the Acp-2 gene coding for lysosomal acid phosphatase (LAP) is on the short arm of chromosome 11 (21-23). Recently, human LAP was reported to contain 393 amino acids, including a transmembrane sequence (21). There is 50% (177/351) homology between the corresponding amino acid sequences of human PAP and LAP (Fig. 4). All five Cys residues as well as the first and third potential N-linked glycosylation sites of PAP sequence were conserved in the LAP sequence. Human PAP and LAP precursor proteins contain hydrophobic signal peptides of 32 and 30 amino acids, respectively (20-21). Both PAP and LAP enzymes are sensitive to inhibition by L-tartrate (6,21). These structural and functional similarities suggest that the genes coding for human prostatic and lysosomal acid phosphatases belong to a multi-gene family originated from an ancestral gene during the course of evolution.

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