BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **233,** 10–13 (1997)
ARTICLE NO. RC976383

Differential Tissue Distribution of the β- and γ-Subunits of Human Cytosolic Platelet-Activating Factor Acetylhydrolase (Isoform I)[1]

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Received February 13, 1997

The cDNA for human platelet-activating factor acetylhydrolase (PAF-AH) β-subunit was cloned. The complete amino acid sequence deduced from cDNA contains 229 amino acids and is completely identical with that of the bovine subunit. Moreover, the sequence of the human β-subunit protein shows 62.4% identity with the human γ-subunit at the amino acid level. Southern blot analysis suggested the presence of multiple genes and we indeed found another closely related pseudo gene. Northern blot analysis showed that the 4.0 kb transcript was expressed in all tissues tested, suggesting the ubiquitous distribution of the subunit protein. Differential distribution of β- and γ-subunits might suggest that the oligomeric structure of PAF-AH is different from tissue to tissue.

Platelet-activating factor acetylhydrolase (PAF-AH), which inactivates PAF by removing the acetyl group at the *sn*-2 position of glycerol backbone, is widely distributed in plasma and tissues (1-5). Recently, PAF acetylhydrolases have been purified from several sources and determined primary structures by cDNA cloning (6-15). In bovine brain, at least three isoforms designated isoforms Ia, Ib and II, were detected as cytoplasmic proteins. While isoform II is monomeric (12), isoform Ib is a heterotrimeric enzyme composed of 45 (α-), 30 (β-), and 29 (γ-) kDa subunits and isoform Ia is composed of β - and γ -subunits (8). It was shown that β- and γ- subunits retained the hydrolytic activity of PAF (10,11) and α-subunit was identified as a product of *LIS-1* gene, a causative gene of Miller-Dieker lissencephaly (MDL) which is a brain malformation manifested by a smooth cerebral surface and abnormal neuronal migration (9,16). These results suggest that PAF-AH (isoform Ib) plays an important role in the brain development. However, the biological significance of other two catalytic subunits are largely unknown.

In this communication, we have cloned the cDNA for the β-subunit of human PAF-AH (isoform I) as a first step to elucidate the physiological function of the subunit. We have examined the tissue distribution of the subunit by Northern blot analysis and found that in contrast with γ-subunit which was expressed in a tissue specific manner, β-subunit was expressed all tissues and cell lines tested, suggesting its ubiquitous distribution. Our results suggest that each subunit of the enzyme is evolutionarily well conserved and oligomeric structure of the enzyme is different from tissue to tissue.

EXPERIMENTAL PROCEDURES

cDNA cloning of the human PAF-AH β-subunit. Two oligodeoxyribonucleotides, CCTGTTCGTGGGGGACTC and CCCAGACAACGATGACCT from bovine PAF-AH β-subunit cDNA sequence were used to screen human fetal liver library (9-11). After recloning into M13mp19 vector, cDNA of positive clones were deleted using double-stranded nested deletion kit (Pharmacia). DNA sequencing was carried out using Taq Dyedeoxy Terminal Cycle Sequencing kit and Applied Biosystems 377A fluorescence DNA sequencer.

Southern blot analysis. Human genomic DNA was digested with restriction endonucleases and size-fractionated by electrophoresis on 1.0 % agarose gels. The fragments were denatured, transferred to a nylon membrane, hybridized with ^{32}P-labeled human cDNA for β-subunit, and washed under highly stringent conditions.

Northern blot analysis. The human multiple tissue northern (MTN) blots were obtained from Clontech. The filter-bound RNA was hybridized with ^{32}P-labeled cDNA for PAF-AH β-subunit following the instruction manual.

[1] The nucleotide sequence data reported in this paper will appear in the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence databases under Accession No. D63390.

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0006-291X/97 $25.00

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        10         20         30         40         50         60
CGCGCCGGAGCGGGACCGACGGGACCGAGCGAGCGACCGACGCGCCACCCGCCGACGCCT
        70         80         90        100        110        120
CAGCCGCTTGGGGCCCGCACGGACCCTCTACTTCAGTGTAGAATGAGCCAAGGAGACTCA
                                          M  S  Q  G  D  S

       130        140        150        160        170        180
AACCCAGCAGCTATTCCGCATGCAGCAGAAGATATTCAAGGAGATGACCGATGGATGTCT
 N  P  A  A  I  P  H  A  A  E  D  I  Q  G  D  D  R  W  M  S

       190        200        210        220        230        240
CAGCACAACAGATTTGTTTTGGACTGTAAAGACAAAGAGCCTGATGTACTGTTCGTGGGA
 Q  H  N  R  F  V  L  D  C  K  D  K  E  P  D  V  L  F  V  G

       250        260        270        280        290        300
GACTCCATGGTGCAGTTAATGCAGCAATATGAGATATGGCGAGAGCTTTTTTCCCCACTT
 D  S  M  V  Q  L  M  Q  Q  Y  E  I  W  R  E  L  F  S  P  L

       310        320        330        340        350        360
CATGCACTGAATTTTGGAATTGGGGGAGATACAACAAGACATGTTTTGTGGAGACTAAAG
 H  A  L  N  F  G  I  G  G  D  T  T  R  H  V  L  W  R  L  K

       370        380        390        400        410        420
AATGGAGAACTGGAGAATATTAAGCCTAAGGTCATTGTTGTCTGGGTAGGAACAAATAAC
 N  G  E  L  E  N  I  K  P  K  V  I  V  V  W  V  G  T  N  N

       430        440        450        460        470        480
CACGAAAATACAGCAGAAGAAGTAGCAGGTGGGATCGAGGCCATTGTACAACTTATCAAC
 H  E  N  T  A  E  E  V  A  G  G  I  E  A  I  V  Q  L  I  N

       490        500        510        520        530        540
ACAAGGCAGCCACAGGCCAAAATCATTGTATTGGGTTTGTTACCTCGAGGTGAGAAACCC
 T  R  Q  P  Q  A  K  I  I  V  L  G  L  L  P  R  G  E  K  P

       550        560        570        580        590        600
AATCCTTTGAGGCAAAAGAACGCCAAGGTGAACCAACTCCTCAAGGTTTCGCTGCCGAAG
 N  P  L  R  Q  K  N  A  K  V  N  Q  L  L  K  V  S  L  P  K

       610        620        630        640        650        660
CTTGCCAACGTGCAGCTCCTGGATACCGACGGGGGTTTTGTGCACTCGGACGGTGCCATC
 L  A  N  V  Q  L  L  D  T  D  G  G  F  V  H  S  D  G  A  I

       670        680        690        700        710        720
TCCTGCCACGACATGTTTGATTTTCTGCATCTGACAGGAGGGGGCTATGCAAAGATCTGC
 S  C  H  D  M  F  D  F  L  H  L  T  G  G  G  Y  A  K  I  C

       730        740        750        760        770        780
AAACCCCTGCATGAACTGATCATGCAGTTGTTGGAGGAAACACCTGAGGAGAAACAAACC
 K  P  L  H  E  L  I  M  Q  L  L  E  E  T  P  E  E  K  Q  T

       790        800        810        820        830        840
ACCATTGCCTGACTGGCTCTTATCAGTGTTAATAGCATCTCAGCTTCCTCAGATCAGTTC
 T  I  A  *

       850        860        870        880        890        900
TATCACTGGCACTACAGAATCCTTCTCTTTCTTAAGGCACTTTGCATTGTAGAATGTTCC
       910        920        930        940        950        960
TGGATGTTCATATCTAGTGTTTGAAGGGGAGGAGGGATTTAAACTGGTCCTGTACATAGA
       970        980        990       1000       1010       1020
AGGTTTGTTTGACAGAGGAGAAAAATTAGCCAAGGAAGATTGTTGTTTAAATTCATTTGA
      1030       1040       1050       1060       1070       1080
AACCAGAAGGGGACTTTTTAGTTGTATGTGTAACACATTCATTGAATTATTATCACTGTT
      1090       1100       1110       1120       1130       1140
TTCTTGGGACAACATCAAGCCTAAATACTGAACAATATGAAGATTCTTTTCTTGGCCTTT
      1150       1160       1170       1180       1190       1200
CTGTGGATTATGTCATATATAATAATTATCAGAATCATTCTACTTGGCTTTTTCC
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FIG. 1. Nucleotide and predicted amino acid sequences of the cDNA encoding human PAF-AH β-subunit.

Expression of PAF-AH proteins. The expression vector, pUC-P_L-cI, containing the cDNA or gene for desired protein, was introduced in *E.coli* W3110 and further processed as described previously (10).

RESULTS AND DISCUSSION

Two oligodeoxyribonucleotides derived from cDNA sequence of bovine PAF-AH β-subunit were synthesized and used for the screening of the cDNA library prepared from human fetal liver to obtain the human homologue. The cDNA sequence and the deduced amino acid sequence of human PAF-AH β-subunit is shown in Fig. 1. The cDNA sequence contains an open reading frame with a Met residue, which could be the initiation site of translation, and codes for 229 amino acid residues. Strikingly no difference was observed between human and bovine amino acid sequences of PAF-AH β-subunit whereas 94.2 % homology was observed in the nucleotide sequences of the coding region of these two species, indicating that the subunit is evolutionarily well conserved. The homology between human β- and γ-subunits is 62.4 % at the amino acid level, suggesting that the genes encoding these two proteins develop from a common ancestral gene.

Figure 2 shows the Southern blot analysis of human genomic DNA employing the complete PAF-AH β-subunit cDNA as a probe. As mentioned previously (17), cDNA for β-subunit did not hybridize with that for γ-subunit, since we employed highly stringent condition. In contrast with the analysis of γ-subunit gene which showed the presence of a single gene, the results of the β-subunit analysis was rather complicated and multiple bands were detected in all the endonuclease digests tested, suggesting the presence of other closely related gene(s). Therefore we examined the plaque hybridization assay and isolated a gene showing 80.0 % identity with β-subunit. However, stop codon was inserted after Tyr^{175} , suggesting that it might be a pseudo gene or even if the gene could be translated into protein, the product might have no enzymatic activity. In fact, several attempts to express the gene in *E. coli* or COS-1 cells and obtain the active enzyme were unsuccessful.

Expression of mRNA for PAF-AH β-subunit was examined by Northern blot analysis employing complete cDNA as a probe. As shown in Fig. 3, a single transcript of about 4.0 kb long was detected in all the adult and fetal tissues tested. We also examined the expression in several human cell lines derived from various tissues and found that all cell lines tested expressed the tran-

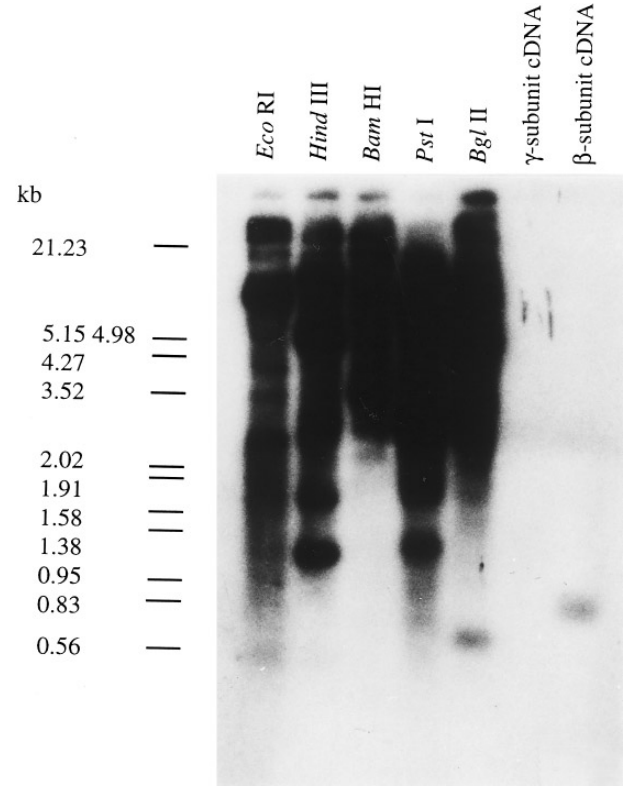

FIG. 2. Southern blot analysis of the human gene for PAF-AH β-subunit. Human genomic DNA was digested with various restriction endonucleases and probed with ^{32}P-labeled human cDNA for the subunit.

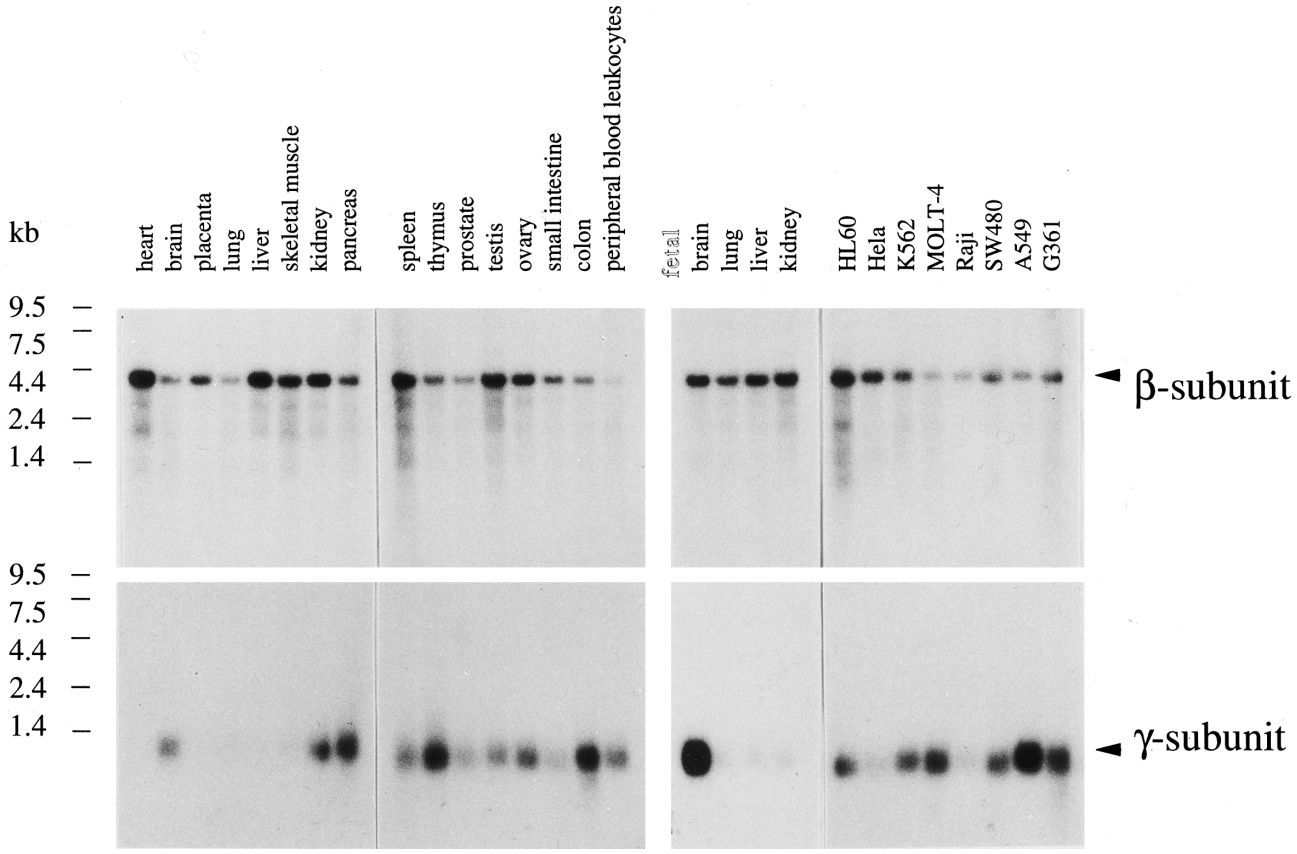

FIG. 3. Northern blot analysis of poly(A)$^+$ RNA from various human tissues probed with ^{32}P-labeled human cDNA for the subunit. For comparison, results employing γ-subunit probe are also shown (17).

script, indicating that the expression pattern of β-subunit is quite different from that of γ-subunit (17). While β-subunit is expressed ubiquitously, γ-subunit is rather in a tissue specific manner. Therefore in some tissues such as liver and placenta, it is possible that only β-subunit is expressed and it may act as an active enzyme without heterodimer formation with γ-subunit.

The cDNA for β- and γ-subunits and the putative pseudo β-gene were transfected into *E. coli*, and the enzyme activities of the 100,000 $\times$ g supernatants of the cell sonicates were measured. While the supernatants of β- and γ-subunit transfected cells contained significant levels of PAF acetylhydrolase activity, no activity was detected in the supernatant of pseudo β-gene transfected cell (data not shown). These results indicated that both subunits were enzymatically active presumably through the homodimmer formation (11) and suggested that the product of pseudo β-gene was inactive even if it was translated.

In this communication, we have cloned cDNA encoding human PAF-AH β-subunit. The subunit is enzymatically active, consists of 229 amino acids and is completely identical with bovine homologue at amino acid level. In our previous works, we observed that both α- and γ- subunits showed striking homology between human and bovine (10,16,17). Taken together, these results suggest that all subunit of the enzyme is evolutionarily well conserved and plays an important role in the maintenance of homeostasis through degradation of PAF which is a potent phospholipid mediator and implicated in several pathological processes (18,19).

Studies on the tissue distribution of β-subunit by Northern blot analysis indicated the ubiquitous expression of the subunit. Since γ-subunit was expressed in a tissue specific manner (17), the expression patterns of β- and γ-subunits were quite different from each other. Because α-subunit of the enzyme which has no enzyme activity is shown to affect brain development (8,16), it seems reasonable that fetal brain is the major tissue which express the γ-subunit. On the other hand, at present, it is difficult to speculate the biological significance of the ubiquitous distribution of β-subunit. However, it should be safe to mention that oligomeric structure of PAF-AH might be different from tissue to tissue. We have shown that while the β- and γ-subunits formed a heterocomplex in the native enzyme, both re-

combinant subunits existed as a enzymatically active homodimer (8,11). Studies on the biological significance of the differential distribution of these two catalytic subunits are now in progress.

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