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cDNA cloning of human cytosolic platelet-activating factor acetylhydrolase γ-subunit and its mRNA expression in human tissues+

Hideki Adachi[1]*, Masafumi Tsujimoto[1], Mitsuharu Hattori[2], Hiroyuki Arai[2], and Keizo Inoue[2]

[1]Suntory Institute for Biomedical Research, Mishima, Osaka 618, Japan

[2]Department of Health Chemistry, Faculty of Pharmaceutical Science, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan

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The cDNA for human cytosolic platelet-activating factor acetylhydrolase (PAF-AH) γ-subunit was isolated and the gene structure was elucidated. The complete amino acid sequence deduced from cDNA contains 231 amino acid residues. Southern blot analysis indicated the presence of a single gene. Northern blot analysis showed that 1.0 kb transcript was expressed in a tissue specific manner. Comparison of the expression pattern between adult and fetal tissues suggests that the enzyme plays an important role during the development of brain.

Platelet-activating factor acetylhydrolase (PAF-AH) catalyzes the removal of the acetyl group at *sn*-2 position of the glycerol backbone of PAF and produces biologically inactive lyso-PAF (1). PAF-AH has been shown to be present in mammalian blood (1-4), blood cells (5-7), and tissues (8-10).

Recently, we purified PAF-AH (isoform Ib) from bovine brain and found that the enzyme consisted of three subunits (*i.e.* 45-, 30- and 29-kDa subunit) (11). Subsequently, we cloned all the cDNAs for these subunits and renamed them α-, β- and γ- subunit respectively (12-14). While β- and γ- subunit retained the hydrolytic activity of PAF, α-

+*The nucleotide sequence data reported in this paper will appear in the GSDB, DDBJ, EMBL and NCBI nucleotide sequence databases with the following accession number: D63391.*

* To whom correspondence should be addressed. Fax: 81-75-962-6448.

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subunit was identified as a product of *LIS-1* gene, a causative gene of Miller-Dieker lissencephaly (MDL). MDL is a brain malformation manifested by a smooth cerebral surface and abnormal neuronal migration (15). These results suggest that the regulation of PAF concentration in brain by PAF-AH is important for the normal development of brain.

In this communication, we have cloned the cDNA for and analyzed the gene organization of γ-subunit of human PAF-AH (isoform Ib). We have also analyzed the tissue distribution of this subunit for the first time by Northern blot analysis and found that it is expressed in a tissue specific manner. Our data suggest that the enzyme plays an important role during the development of brain.

EXPERIMENTAL PROCEDURES

cDNA cloning of human PAF-AH γ-subunit - Two oligodeoxyribonucleotides, 5'-ATGTGCTGTGGCGTCTGG-3' and 5'-AGTGTGCCCGTGGTTGTT-3' from bovine PAF-AH γ-subunit cDNA sequence were used to screen human fetal liver library (12-14). After recloning into M13mp19 vector, cDNAs 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.

Cloning of PAF-AH γ-subunit gene- Human genomic DNA library (Clontech) was screened by plaque hybridization. DNA was labeled with probe-amp reagents and detected by ECL detection reagents (Amersham). DNAs of the positive clones were recloned into M13mp19 vector in both orientations and sequenced as described above.

Southern blot analysis- Human genomic DNA was digested with an excess of 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 PAF-AH γ-subunit, and washed under highly stringent conditions.

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

Expression of PAF-AH γ-subunit in E. coli- The expression vector, pUC-P_L-cI, containing the cDNA for PAF-AH γ subunit, was introduced in *E. coli* W3110 and further processed as described previously (13).

RESULTS AND DISCUSSION

Using two oligodeoxyribonucleotides derived from the cDNA sequence of bovine brain PAF-AH γ-subunit, we have screened the cDNA library prepared from human fetal liver and obtained the cDNA encoding human PAF-AH γ–subunit. The cDNA sequence and deduced amino acid sequence are shown in Fig. 1. The cDNA sequence contains an open reading frame that starts with a Met residue, which could be the initiation site of translation, and codes for 231 amino acid residues. The 5'-untranslated region of 111 nucleotides contains an in-frame stop codon preceeding a putative initiator methionine codon. Comparison of the amino acid sequences of human and bovine PAF-AH γ-subunits shows significant identity (97.8 %), indicating that this subunit is evolutionary well conserved.

The human PAF-AH γ-subunit genomic clone was isolated by means of plaque hybridization. As shown in Fig. 2, the gene contains 5

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  1  GGACGGTCCTTTGTTGCCGCGAGGGGTAGGAGTGGGCGTGGCGGAGCCAGCTCCGTTCGG    60
 61  AACACTCCCGGGCCGACCCGACTCGCTCATCCTGCAGGAGCTGCGGCGCCAAGATGAGTG   120
                                                      M  S  G      3
121  GAGAGGAGAACCCAGCCAGCAAGCCCACGCCGGTGCAGGACGTACAGGGCGACGGGCGCT   180
  4    E  E  N  P  A  S  K  P  T  P  V  Q  D  V  Q  G  D  G  R  W   23
181  GGATGTCCCTGCACCATCGGTTCGTGGCTGACAGCAAAGATAAGGAACCCGAAGTCGTCT   240
 24    M  S  L  H  H  R  F  V  A  D  S  K  D  K  E  P  E  V  V  F   43
241  TCATCGGGGACTCCTTGGTCCAGCTCATGCACCAGTGCGAGATCTGGCGCGAGCTCTTCT   300
 44    I  G  D  S  L  V  Q  L  M  H  Q  C  E  I  W  R  E  L  F  S   63
301  CTCCTCTGCATGCACTTAACTTTGGCATTGGTGGTGACGGCACACAGCATGTACTGTGGC   360
 64    P  L  H  A  L  N  F  G  I  G  G  D  G  T  Q  H  V  L  W  R   83
361  GGCTGGAGAATGGGGAGCTGGAACACATCCGGCCCAAGATTGTGGTGGTCTGGGTGGGCA   420
 84    L  E  N  G  E  L  E  H  I  R  P  K  I  V  V  V  W  V  G  T  103
421  CCAACAACCACGGACACACAGCAGAGCAGGTGACTGGTGGCATCAAGGCCATTGTGCAAC   480
104    N  N  H  G  H  T  A  E  Q  V  T  G  G  I  K  A  I  V  Q  L  123
481  TGGTGAATGAGCGACAGCCCCAGGCCCGGGTTGTGGTGCTGGGCCTGCTTCCGCGAGGCC   540
124    V  N  E  R  Q  P  Q  A  R  V  V  V  L  G  L  L  P  R  G  Q  143
541  AACATCCCAACCCACTTCGGGAGAAGAACCGACAGGTGAACGAGCTGGTACGGGCGGCAC   600
144    H  P  N  P  L  R  E  K  N  R  Q  V  N  E  L  V  R  A  A  L  163
601  TGGCTGGCCACCCTCGGGCCCACTTCCTAGATGCCGACCCTGGCTTTGTGCACTCAGATG   660
164    A  G  H  P  R  A  H  F  L  D  A  D  P  G  F  V  H  S  D  G  183
661  GCACCATCAGCCATCATGACATGTATGATTACCTGCATCTGAGCCGCCTGGGCTACACAC   720
184    T  I  S  H  H  D  M  Y  D  Y  L  H  L  S  R  L  G  Y  T  P  203
721  CTGTTTGCCGGGCTCTGCACTCCCTGCTTCTGCGTCTGCTGGCCCAAGACCAGGGCCAAG   780
204    V  C  R  A  L  H  S  L  L  L  R  L  L  A  Q  D  Q  G  Q  G  223
781  GTGCTCCCCTGCTGGAGCCCGCACCCTAAGCATCCTGCTGCCTTCCCACAACATTAAACT   840
224    A  P  L  L  E  P  A  P  *                                   231
841  CTCCTTCCTCAG                                                 852
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Fig. 1. Nucleotide and predicted amino acid sequence of the cDNA encoding human PAF-AH γ-subunit.

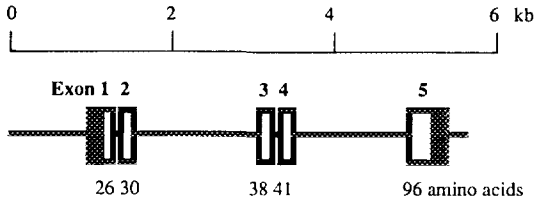

Fig. 2. Organization of the human PAF-AH γ–subunit gene.

exons interrupted by 4 introns and spans approximately 5.6 kb of DNA. The sequences of intron-exon junctions confirm to the concensus sequence of eukaryotic splice junctions (not shown).

Figure 3 shows the result of Southern blot hybridization of human genomic DNA with the complete PAF-AH γ-subunit cDNA. Since we employed highly stringent condition, cDNAs for β– and γ– subunit were

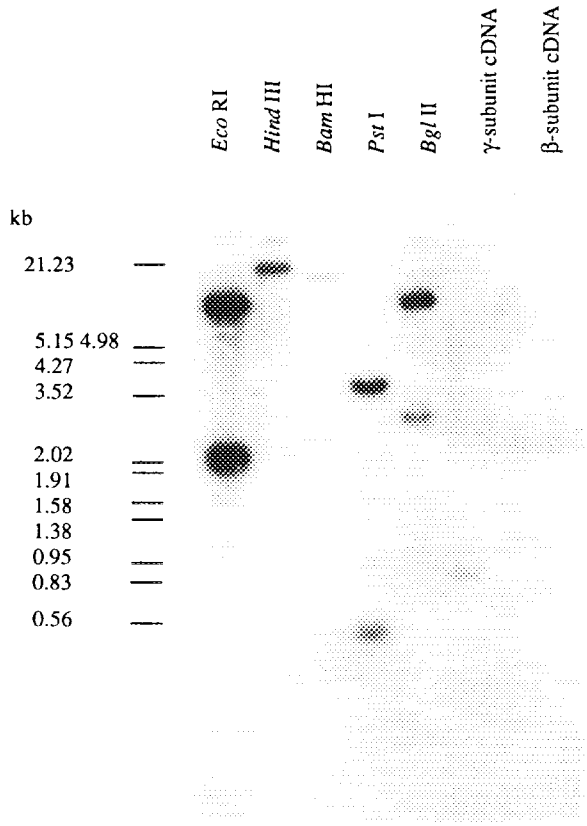

Fig. 3. Southern blot analysis of the human gene for PAF-AH γ-subunit. Human genomic DNA was digested with various restriction endonucleases and probed with ^{32}P-labeled human cDNA for the enzyme.

distinguishable from each other. Under such conditions, both *Hind* III and *Bam* HI digestion gave a single DNA fragment of >21 kb, suggesting the presence of a single gene. The existence of multiple DNA fragments in *Eco* RI, *Pst* I and *Bgl* II digests reflects the presence of restriction sites in the introns that are present in the genomic DNA.

Expression of mRNA for PAF-AH γ–subunit was examined by Northern blot analysis employing complete cDNA as a probe. As shown in Fig. 4A, a single transcript of about 1.0 kb long was detected in several adult tissues such as brain, skeletal muscle, kidney, spleen, and colon. On the other hand, little mRNA was present in heart, lung, and liver, indicating the tissue specific expression of the transcript. Figure 4B shows the result of fetal tissues. Fetal brain expressed the largest amount of the transcript among tissues tested. In other fetal tissues such as lung, liver and kidney, we could detect the transcript but the amount of which was significantly less than that of brain.

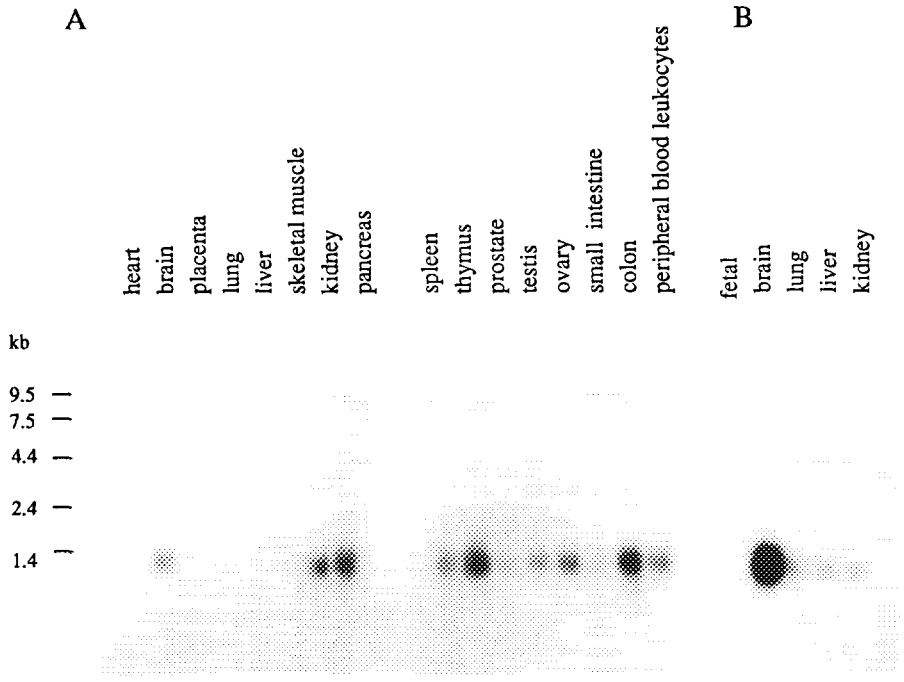

Fig. 4. Northern blot analysis of poly(A)$^+$ RNA from various human adult A) and fetal B) tissues.

The obtained cDNA was transfected into *E. coli*, and the enzyme activity of the 100,000 x g supernatant of the cell sonicates was measured. While no activity was detected in non-transfected control cells, the supernatant of the transfected cells (designated as clone 29-1) contained a significant level of PAF acetylhydrolase activity (Fig. 5). This result indicates that as is bovine homologue, trimer formation is not essential for the catalytic activity of human PAF-AH γ–subunit .

In the current study, we have cloned cDNA encoding human PAF-AH γ-subunit and analyzed its gene structure. The human enzyme γ-subunit consists of 231 amino acid residues. Striking identity was observed between human and bovine subunits and of 231 amino acid residues, only 5 were different from each other. The region around the active serine residue (*i.e.* Ser^{47}) including PAF receptor homologous sequence is also conserved nearly completely (only exception is Gly^{77} in human and Ser^{77} in bovine) (13, 16, 17). In our previous work, we observed that α-subunit of the enzyme also showed a striking homology between human and bovine and of 410 amino acid residues, only 3 were different from each other (13, 15). These results indicate that each subunit of the enzyme is evolutionary well conserved.

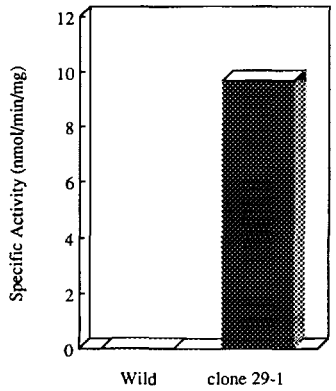

Fig. 5. PAF acetylhydrolase activities of the recombinant PAF-AH γ-subunit polypeptides expressed in *E.coli*. Cells were transfected with (clone 29-1) or without (wild) the cDNA for the enzyme. PAF acetylhydrolase activity was measured as described previously (13).

We have also analyzed the tissue distribution of PAF-AH γ–subunit by Northern blot analysis and found that mRNA for the subunit was expressed in a tissue specific manner. It is not unexpected that fetal brain is the major tissue which expresses the mRNA since another subunit of the enzyme (*i.e.* α-subunit) is a product of MDL gene which affect the development of brain (15). These results suggest that the enzyme plays an important role in the development of brain. It is conceivable that PAF acetylhydrolase activity mediated by the protein/protein interaction between α-subunit and its unknown target molecule is essential for the normal development of brain (18). It is noteworthy here that differential tissue distribution between β- and γ-subunit was observed (data not shown), suggesting that oligomeric structure of PAF-AH might be different from tissue to tissue. Biological significance of the differential distribution of these two catalytic subunits should be elucidated in the future studies.

Since PAF is a potent phospholipid mediator and implicated in several pathological processes (19, 20), decrease in PAF activity *via* degradation by PAF-AH might be important to maintain the homeostasis. Availability of cDNA and recombinant human PAF AH γ–subunit protein will make it possible to characterize this new enzyme with respect to its physiological and pathological functions in detail.

REFERENCES

1. Blank, M. L., Lee, T-C., Fitzgerald, V., and Snyder, F. (1981) *J. Biol. Chem.* **256**, 175-178
2. Farr, R. S., Cox, C. P., Wardlow, M. L., and Jorgensen, R. (1980) *Clin. Immunol. Immunopathol.* **15**, 318-330
3. Stafforini, D. M., McIntyre, T. M., Carter, M. E., and Prescott, S. M. (1987) *J. Biol. Chem.* **262**, 4215-4222
4. Stafforini, D. M., Prescott, S. M., and McIntyre, T.M. (1987) *J. Biol. Chem.* **262**, 4223-4230
5. Stafforini, D.M., Prescott, S. M., Zimmerman, G. A., and McIntyre, T. M. (1991) *Lipids*, **26**, 979-985
6. Stafforini, D. M., Rollins, E. N., Prescott, S. M., and McIntyre, T. M. (1993) *J. Biol. Chem.* **268**, 3857-3865
7. Korth, R., Bidault, J., Palmantier, R., Benveniste, J., and Ninio, E. (1993) *Lipids*, **28**, 193-199

8. Lee, T-C., Malone, B., Wasserman, S. I., Fitzgerald, V., and Snyder, F. (1982) *Biochem. Biophys. Res. Commun.* **105**, 1303-1308
9. Yanosita, R., Kudo, I., Ikizawa, K., Chang, H. W., Kobayashi, S., Ohno, M., Nojima, S., and Inoue, K. (1988) *J. Biochem.* **103**, 815-819
10. Nijssen, J. G., Roosenbloom, C. F. P., and van den Bosch, H. (1986) *Biochim. Biophys. Acta* **876**, 611-618
11. Hattori, M., Arai, H., and Inoue, K. (1993) *J. Biol. Chem.* **268**, 18748-18753
12. Hattori, M., Adachi, H., Tsujimoto, M., Arai, H., and Inoue, K. (1994) *Nature*, **370**, 216-218
13. Hattori, M., Adachi, H., Tsujimoto, M., Arai, H., and Inoue, K. (1994) *J. Biol. Chem.* **269**, 23150-23155
14. Hattori, M., Adachi, H., Aoki, J., Tsujimoto, M., Arai, H., and Inoue, K. (1995) *submitted for publication*
15. Reiner, O., Carrozzo, R., Shen, Y., Wehnert, M., Faustinella, F., Dobyns, W. B., Caskey, C. T., and Ledbetter, D. H. (1993) *Nature*, **364**, 717-721
16. Honda, Z., Nakamura, M., Miki, I., Minami, M., Watanabe, T., Seyama, Y., Okado, H., Toh, H., Ito, K., Miyamoto, T., and Shimizu, T. (1991) *Nature*, **349**, 342-346
17. Nakamura, M., Honda, Z., Izumi, T., Sakanaka, C., Mutoh, H., Minami, M., Bito, H., Seyama, Y., Matsumoto, T., Noma, M., and Shimizu, T. (1991) *J. Biol. Chem.* **266**, 20400-20405
18. Wang, D-S., Shaw, R., Hattori, M., Arai, H., Inoue, K., and Shaw, G. (1995) *Biochem. Biophys. Res. Commun.* **209**, 622-629
19. Snyder, F. (1985) *Med. Res. Rev.* **5**, 107-140
20. Hanahan, D. J. (1986) *Annu. Rev. Biochem.* **55**, 483-509