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5'-FLANKING REGION SURROUNDING A HUMAN CYTOSOLIC PHOSPHOLIPASE A2 GENE*

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SUMMARY: The 5'-flanking region and the first four exons of a gene encoding human cytosolic phospholipase A2 (cPLA2) were isolated from a human λEMBL3 genomic library and sequenced. The 5'-flanking region is characterized by CA repeats, one of microsatellites. The analysis of the 5'-flanking region with transcription factor database suggests the existence of the transcription factor binding sites such as NF-κB, NF-IL6, AP-1, AP-2, and PEA3. These factors are well known to be induced or activated by the reagents (tumor necrosis factor α, interleukin-1, epidermal growth factor, and phorbol myristate acetate) reported as the inducers of a cPLA2 gene.

Cytosolic phospholipase A2 (cPLA2) selectively catalyzes the liberation of arachidonic acid from membrane phospholipids in response to extracellular stimulation (1-3). This reaction is a rate-limiting step for arachidonic acid cascade. Arachidonic acid and its metabolites such as eicosanoids play an important role in the various physiological and pathological processes (4-9). cPLA2 is purified from human monoblast U937 (3, 10) and its cDNA is also cloned (11, 12). The deduced amino acid sequence has no similarity to the well known secretary PLA2s, but contains a homologous structure to the C2 region of protein kinase C (11, 12).

The expression of cPLA2 gene has been reported to be induced by various reagents. Hulkower et al. (13) demonstrated that cPLA2 activity is increased 3 to 4-fold by interleukin-1β (IL-1β) in human rheumatoid synovial fibroblast. Also, in human epithelial carcinoma cell line HEp-2, tumor necrosis factor α (TNF-α) increases the activity and furthermore the effect is inhibited by glucocorticoids (14). These

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Abbreviations: cPLA2, cytosolic phospholipase A2; TNF-α, tumor necrosis factor α; IL-1, interleukin-1; M-CSF, macrophage colony-stimulating factor; EGF, epidermal growth factor; PMA, phorbol myristate acetate.

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effects require the long-term exposure of the reagents for 14 to 18 h, suggesting that the expression of a cPLA2 gene may be induced or suppressed. In fact, the protein level has been found out to be increased after 24 h incubation with IL-1α in human lung fibroblast WI-38 using Western blot analysis (15). Further, Nakamura et al. (16) recently reported a biphasic increase in cPLA2 mRNA level by macrophage colony-stimulating factor (M-CSF) in human monocytes. A monotonic increase in the mRNA level has also been reported by Maxwell et al. (17) using rat renal glomerular mesangial cells stimulated with epidermal growth factor (EGF) or phorbol myristate acetate (PMA), and by Hoech et al. (18) using human fibroblast HeLa cells stimulated with TNF-α. However, the mechanism of the gene expression remains unknown. In this report, we isolated the promoter region and the first four exons of human cPLA2 from genomic library.

MATERIALS AND METHODS

A human placenta genomic library of Sau3A1 partial digests in λphage EMBL3 SP6/T7 (Clonetech, USA) was plated at 1 x 10^5 plaques per plate. Replica filters of 2 x 10^6 plaques of the library were made using Hybond N+ membrane (Amersham, England). The filters were hybridized according to the manufacturer's recommendations with a 231 bp partial cDNA for human cPLA2 covering the nucleotide sequence from 36 to 266 (12). The cDNA was cloned using mRNA of human U937 cells by reverse transcription-mediated PCR. The probe was labeled with [α-^{32}P]dCTP (Amersham, England) by PCR (19). Isolation of λphage DNA, restriction mapping, and Southern blot analysis were performed by standard methods (20). DNA fragments were subcloned into Bluescript KS(+) vector (Stratagene, USA) and the nucleotide sequence was determined by the dideoxy nucleotide chain termination methods using a Sequenase kit (USB, USA) or a BcaBEST DNA Polymerase kit (Takara Shuzo Co.,ltd., Japan). Transcription factor binding sites were analyzed with transcription factor database (21).

RESULTS AND DISCUSSION

In order to isolate the promoter region of a cPLA2 gene, a human genomic library was screened using a cDNA probe covering 103 bp noncoding region and 128 bp coding region. The screening resulted in a genomic clone covering 15 kbp, two overlapping clones covering 27 kbp, and two overlapping clones covering 15 kbp (Fig. 1). A restriction map was established and DNA fragments hybridizing to cDNA probe were sequenced. The boundary between exon and intron was determined by comparing the genomic nucleotide sequences with that of human cPLA2 cDNA (11, 12) and consequently first four exons were identified. Exon 1 contained at least 63 bp of noncoding region. Exon 2 contained both 69 bp of noncoding and 33 bp of coding region. Exons 3 and 3' contained 82 bp and 33 bp of coding regions, respectively. The nucleotide sequences at splicing junctions agreed with a consensus sequence for splicing donor acceptor site (GT/AG rule) (22). The genomic clone λPLI-3 extended about 7 kbp downstream of exon 1 and λPLI-5 extended about 18 kbp upstream of exon 2. However, their restriction maps showed no overlap, nor they hybridized to each other. This indicates that the first intron may be longer than 25 kbp. Also, we

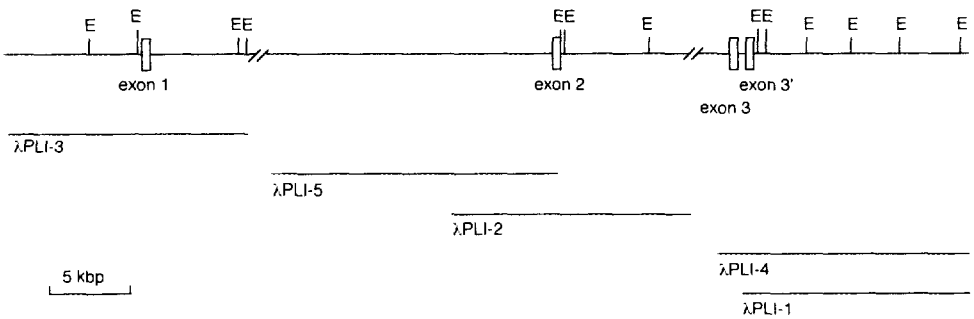

Figure 1. *Map of genomic clones containing human cytosolic phospholipase A2 sequences.* The genomic gene structure is shown with exons represented as boxes and the locations of the isolated phage clones are shown below the gene. Restriction sites for EcoRI are indicated by E. The nucleotide sequence data reported in this paper will appear in the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence databases with the following accession numbers: D38178 for the promoter region, the first exon, and the first intron, D38176 for exon 2, and D38177 for exons 3 and 3'.

could find out no overlap between the downstream of exon 2 and the upstream of exon 3, indicating that the second intron may be longer than 8 kbp. Therefore, these data suggest that a human cPLA2 gene may be consisted of a huge genomic DNA covering longer than 50 kbp.

The 110 bp nucleotide sequence of exon 1 and the upstream was aligned with that of rat reported by Tay et al. (23). The sequence of human showed a high homology of 76 % to that of rat (Fig. 2). Especially, the sequence around the transcription initiation site reported in rat was highly conserved in that of human. Therefore, the transcription might initiate at the same position as that of rat. The search of DNA binding element revealed TATA-box consensus sequence situated at -397, -559, -975, -1679, -1741, -2867, -2882, and -3516, and CCAAT-box like element at -1850. The transcription strictly initiates at about 25 bp or about 75 bp from TATA-box or CCAAT box, respectively (24, 25). All positions of searched TATA-box and CCAAT-box like elements did not agree with the rule and therefore the transcription of cPLA2 gene is unlikely to initiate at these typical promoter sites. Also, we could not find other known initiator elements (26, 27).

The 4178 bp nucleotide sequence containing the 5'-flanking region, the first exon, and the first intron was determined. Fig. 3 shows the first exon, the first intron,

```
HUMAN   CTTTAATTCC ACCTTAAAAC ATCTGCAAAA GCGCAAGGAG ACCAGCCCAC ATTTTAGCCC
        ::: :::::: ::::::: : ::: :: :: ::: ::::::::: : :::::::
RAT     CTTGAATTCC ACCTTAA--C ATC--CA--- ---CA--GAG ACCAGCCCAT TTCTTAGCCC

HUMAN   CTCCTACT-- CAGGATAAGA CTTTCTCTAA GTCCGGAGCT GAAAAAGGAT CC
        ::::::: : ::: :::: ::::::: :: ::: ::: :: : :: :: : ::
RAT     CTCCTACCAG CGGGAGAAGA CTTTCTCAAA GTC-GGAACT GTGAAGGGCT CC
```

Figure 2. *Alignment of nucleotide sequences surrounding the transcription initiation sites in human and rat.* The rat nucleotide sequences are reported by Tay et al. (23). The alignment of the two sequences was carried out using "Gene Works" (IntelliGenetics, Inc., USA). The sequences for first exons are underlined and colons mark the nucleotide identities.

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TCTAGAAATT CAGGGATTTC TTTGGAAAGA GAAATTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT GAGACGGAGT CTCGCTCTGT -1251

GGCCCAGGCT GGAGTGCAAT GGCGCAACTT GGCTCACTGC AAGCTCTGCC TCCCGGGTTC ACGCCATTCT CCTGCCTCAG CCTCCCAAGT AGCTGGGACT -1151
ACAGGCGCCC GCCACCACAC CCGGCTAATT TTTTGTATTT TTAGTAGAGA CGGGGGTTTC ACCGTGTTAG CCAGGATAGT CTCGATCTCC TGACCTCGTG -1051

ATCCGCCCGC CTTGGCCTCC CAAAGTGCTG GGATTACAGG CGTGAGCCAC CACGCCCAGC CCGAAAAATG TTTATTATAA ATCTATCCTT CCTAAAAAAG -951

ACATTACTGG CTTTTTTTTT TTCAAAGAGA GATTTATAAT ATGCCAGGTT GAATTTAGGG GAAAAAAATA AAAACAAATA AGAGTTGAAT GTTGGGCTAC -851

AGAAAGCAGA TATTCCAGAA GATTTAGTGA AATTATATTC TTTGAAAATA TTGTAAGAGA GTTAGGAGAG TGAGTTCAGT ATTTAATTAA AGGCAAAAAC -751

AAGATCCTGT GTTTGAAAAA ACAAGATATA CTAAATGAAC ATGTCAATCT TTAAAGACAT GAATTGATTC TCATGGAATT TGGGTACATT TAAACAATGC -651

ACCTTCTTCA TTACAGTGCT ATAAGGTATT TGGATTTACA TTGCAAATTT GGAATTCAAC CTGATTTCAT TTTCTTCCCT AAATCTCAAC ATATATATTA -551

CATTATTTGG TATGCCTTTA ATCTTAAATT TACAAAGAAA TTATTATCAA CACAGGTGTT CTGAGTCTGG AGTGGAAATA TTTAAACAGA TTTGATTTTT -451
AAAGTGGTCT GATACTCATT TTTTAAAATG TACATTTTAT ATTTGGGGTT TGTTATAAAC TTTGCCTTTT TAAATAATGC AAATATGCTC TAGGTTATTC -351

ACTGTCTTTT CAATTTTGTT CTCCTTTGGA AAAAAGGAGG AAGTAGTTAC TACTTAAGGA CAAGTAGCAA TTTCAGACGC GCGCACGCAC ACACACACAC -251

ACACACACAC ACATCACACA CACAGAAATC CACAACAGCA CTCATGGAAT TTAGGACTGA TTAATTTACA TATTATTTAA GCATATTTTC TGACTTCAAA -151

CTCCTGGTTC TAATAACTAA GCATTTACAT TTACAATATT AGCTTCTATG AGAAGAGAGC GTTCTCCCTC TTCCCCTTTA ATTCCACCTT AAAACATCTG -51

CAAAAGCGCA AGGAGACCAG CCCACATTTT AGCCCCTCCT ACTCAGGATA AGACTTTCTC TAAGTCCGGA GCTGAAAAAG GATCCTGACT GAAAGCTAGA 50

GGCATTGAGG AGCCTGAAGA TTCTCAGGTA ACTCTGGGAA CTGTAAGCAA TATATTTATT CTTCCTCTGC TCCACTTCTC TCTCCCACCC TCCTTAGCTT 150

TTACTTGGTA GTTTACTTGG AAGGAAGAAA GGACGGAGGG AACCTCGCTG CTTAAAGTCA GCATGTCCTC TGCTTTGAAG TGACACAGAT AGGACTCATT 250

CTCAACCACG AGTTGCTCTG ATCTAATGAC TTGAGTCAGT AGGTCTGTGA TCAGAGTAGC ATCAACGCGT AGTGCGTATA GCGTCACGGA TG 342
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Figure 3. *Nucleotide sequence of the 5' region of human cytoplasmic phospholipase A2 gene.* The sequence is numbered from the putative transcription initiation site. The underline and the dotted underline represent the first exon. The underlined exon was identified by comparing the genomic sequence with that of cDNA (11,12). The dotted-underlined exon was extended by comparing human genomic sequence with that of rat as shown in Fig. 2. Putative transcription controlling sequences and CA repeats are shown by double underline. Further 5'-flanking nucleotide sequence is available through the accession number D38178 in the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence databases.

and 1.35 kbp sequence of the upstream. The 5'-flanking region is characterized by CA repeats, one of microsatellites, at -263 and T repeated sequence as long as 46 bp around -1366. The putative transcriptional regulatory elements were searched by using transcription factor database (21). Two potential SP1 binding sites are situated at -1706 and -2582. A modified NF-κB element is found at -1099. Also, five NF-IL6 elements are located at -1330, -1714, -2962, and -2975, and -3030. These factors are well known to be induced or activated by IL-1 (28) which induces a cPLA2 gene in human rheumatoid fibroblast (13) and human lung fibroblast WI-38 (15). Recently, we found the gene induction by IL-1β in rat C6 glioma cells (29) in which NF-κB is reported to be rapidly activated by IL-1β (30). Although aspirin and sodium salicylate, anti-inflammation drugs, is well known to inhibit cyclooxygenase in prostaglandin production, Kopp and Ghosh (31) reported that these drugs inhibit the activation of NF-κB by preventing the degradation of IκB, an inhibitory protein of NF-κB. These data suggest that the activation of NF-κB may play an important role in the induction of cPLA2 gene by IL-1. NF-κB is activated not only by IL-1, but also by TNF and PMA which have been reported as the inducer in human epithelial carcinoma cell lines HEp-2 (14), HeLa cells (18), and rat renal glomerular mesangial cells (17). In addition to classical AP-1 element at 282 and -2096, allowing for one base-pair mismatch, three modified AP-1 elements are noted at -193, -2922, and -3580. Further, six AP-2 elements are located at -996, -2344, -2350, -2355, -2357, and -2751. Five PEA3 elements are situated at 172, -313, -1775, -1938, and -3057. These factors are known

to be induced by PMA or EGF which induce cPLA2 gene in rat renal glomerular mesangial cells (17). Although glucocorticoids inhibit the induction by TNF-α in HEp-2 cells (14) and HeLa cells (18) and that by IL-1α in WI-38 cells (15), a glucocorticoid responsive element element is found at 267 within the first intron. In addition, we could find out two OCT elements at -374 and -3122 and two C/EBP elements at -1714 and -2962. Thus, the putative transcription factors are closely related to the inducers of cPLA2 gene. Further, the promoter sequence is characterized by three clusters of the putative transcription control sites. First is one NF-κB, one NF-IL6, and one AP-2 element around -1000. Second is one SP-1, one NF-IL6, one AP-1, four AP-2, two PEA3, and one C/EBP element around -2000. Third is three NF-IL6, one AP-1, one AP-2, one PEA3, one OCT, and one C/EBP element around -3000.

The sequence of exon 3' was identical to that of the latter half of exon 3. Further, the sequences of the following introns exhibited a high homology of 92 %, allowing for 14 bp mismatch of 176 bp, although the intron between exons 3 and 3' has only 195 bp long. In contrast, the upstream regions showed no homology. Exon 3' may be originated from the repeat of exon 3 and the following intron by a recombination. This leads to a possibility of the regulation of cPLA2 gene expression by an alternative splicing mechanism although the subtype has remained unknown. The detailed transcriptional regulation is currently investigated.

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REFERENCES

1. Leslie C.C., Voelker D.R., Channon J.Y., Wall M.M. and Zelarney P.T. (1988) *Biochim. Biophys. Acta*, **963**, 476-492.
2. Diez E. and Mong S. (1990) *J. Biol. Chem.*, **265**, 14654-14661.
3. Clark J.D., Milona N. and Knopf J.L. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 7708-7712.
4. Blackburn E.H. (1991) *Annu. Rev. Biochem.*, **61**, 113-129.
5. Sammuelson B., Goldyne M., Branstrom E., Hamberg M., Hammarstrom S. and Malmsten C. (1978) *Annu. Rev. Biochem.*, **47**, 997-1029.
6. Wolfe L.S. and Coceani F. (1979) *Annu. Rev. Physiol.*, **41**, 669-684.
7. Hayaishi O. (1989) *J. Biol. Chem.*, **263**, 14593-14596.
8. Watanabe Y., Mori K., Imamura K., Takagi S.-F. and Hayaishi O. (1986) *Brain Res.*, **378**, 216-222.
9. Bazan N.G. (1976) *in "Function and Metabolism of Phospholipids in Central and Peripheral Nervous System"*, pp. 317-335, Plenum Press, New York.
10. Kramer R.M., Roberts E.F., Manetta J. and Putnam J.E. (1991) *J. Biol. Chem.*, **266**, 5268-5272.
11. Clark J.D., Lin L.-L., Kriz R.W., Ramesha C.S., Sultzman L.A., Lin A.Y., Milona N. and Knopf J.L. (1991) *Cell*, **65**, 1043-1051.
12. Sharp J.D., White D.L., Chiou G., Goodson T., Gamboa G.C., McClure D., Burgett S., Hoskins J., Skatrud P.L., Sportsman J.R., Becker G.W., Kang

L.H., Roberts E.F. and Kramer R.M. (1991) *J. Biol. Chem.*, **266**, 14850-14853.
13. Hulkower K.I., Hope W.C., Chen T., Anderson C.M., Coffey J.W. and Morgan D.W. (1992) *Biochem. Biophys. Res. Commun.*, **184**, 712-718.
14. Goppelt-Struebe M. and Rehfeldt W. (1992) *Biochim. Biophys. Acta*, **1127**, 163-167.
15. Lin L.-L., Lin A.Y. and DeWitt D.L. (1992) *J. Biol. Chem.*, **267**, 23451-23454.
16. Nakamura T., Lin L.-L., Kharbanda S., Knopf J. and Kufe D. (1992) *EMBO J.*, **11**, 4917-4922.
17. Maxwell A.P., Goldberg H.J., Tay A. H.-N., Li Z.-G., Arbus G.S. and Skorecki K.L. (1993) *Biochem. J.*, **295**, 763-766.
18. Hoeck W.G., Ramesha C.S., Chang D.J., Fan N. and Heller R.A. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 4475-4479.
19. Schwalter D.B. and Sommer S.S. (1989) *Anal. Biochem.*, **177**, 90-94.
20. Sambrook J., Fritsch E.F. and Maniatis T. (1989) "*Molecular Cloning: A LABORATORY MANUAL (2nd ed.)*", Cold Spring Harbor Laboratory Press, New York.
21. Ghosh D. (1990) *Nucleic Acids Res.*, **18**, 1749-1756.
22. Csank C., Taylor F.M. and Martindale D.W. (1990) *Nucleic Acids Res.*, **18**, 5133-5141.
23. Tay A., Maxwell P., Li Z.-G., Goldberg H. and Skorecki K. (1994) *Biochim. Biophys. Acta*, **1217**, 345-347.
24. Xu L.C., Thali M. and Schaffner W. (1991) *Nucleic Acids Res.*, **19**, 6699-6704.
25. Bucher P. and Trifonov E.N. (1986) *Nucleic Acids Res.*, **14**, 10009-10026.
26. Smale S.T. and Baltimore D. (1989) *Cell*, **57**, 103-113.
27. Roy A.L., Meisterernst M., Pognonec P. and Roeder R.G. (1991) *Nature*, **354**, 245-248.
28. Faisst S. and Meyer S. (1992) *Nucleic Acids Res.*, **20**, 3-26.
29. Ozaki M., Morii H., Qvist R. and Watanabe Y. (1994) *Biochem. Biophys. Res. Commun.*, in press.
30. Moynagh P.N., Williams D.C. and O'Neill L.A.J. (1993) *Biochem. J.*, **294**, 343-347.
31. Kopp E. and Ghosh S. (1994) *Science*, **265**, 956-959.