Vol. 171, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 31, 1990 Pages 216-221

Molecular Evolution and Zinc Ion Binding Motif of Leukotriene A_4 Hydrolase *

Hiroyuki Toh[1], Michiko Minami[2] and Takao Shimizu[2]

[1]*Protein Engineering Research Institute, 6-2-3 Furuedai, Suita, Osaka 565 Japan*

[2]*Department of Physiological Chemistry and Nutrition, Faculty of Medicine, University of Tokyo Tokyo 113 Japan*

Received July 9, 1990

Summary : Leukotriene A_4 (LTA_4) hydrolase belongs to the aminopeptidase N family. In order to investigate the molecular evolution and physiological significance of LTA_4 hydrolase, the enzymes belonging to the family were aligned and a phylogenetic tree was constructed. From the alignment, it was found that three residues involved in zinc binding and one residue of the active sites of aminopeptidases N were conserved in LTA_4 hydrolase. In agreement with theobservation, LTA_4hydrolase is a zinc protein as determined by atomic absorption spectroscopy.

Leukotrienes (LTs) constitute a class of eicosanoids which have various physiological activities and pathological functions (1,2). All kinds of LTs are synthesized via LTA_4, which is synthesized from arachidonic acid by the action of 5-lipoxygenase (3,4).

LTA_4 hydrolase is an enzyme which hydrolyzes an epoxide moiety of LTA_4 to yield LTB_4. LTB_4 is a potent chemotactic compound of granulocytes and closely related to the immune system (1, 2,4,5). LTA_4 hydrolase is a cytosolic enzyme with a molecular weight of 68,000 - 70,000 (6-8). cDNAs of LTA_4 hydrolase was cloned from human spleen and placental libraries, and the primary structure was determined (9,10). Furthermore, the cDNA of the enzyme was expressed with a full enzyme activity in *Escherischia coli* (11).

A weak homology was reported between LTA_4 hydrolase and aminopeptidases (12). In order to investigate evolution and function of LTA_4 hydrolase, the amino acid sequence of LTA_4 hydrolase deduced from the cDNA sequence was aligned with those of aminopep-

* This work was supported in part by a grant-in-aid from the Ministry of Education, Science and Culture of Japan.

[1] Corresponding author.

0006-291X/90 $1.50

tidases. According to the alignment, a phylogenetic tree was constructed. Furthermore, it was found that the purified preparation of LTA_4 hydrolase contains a zinc ion.

Materials and Methods

LTA_4 hydrolase was subjected to the computational analysis with the method of sequence comparison of protein primary structures. For the pairwise alignment, a program for weak homology detection was used (13). The sequence data were taken from a protein primary structure data base, PRF (rel.89.9) (14). A multiple alignment was constructed, according to the pairwise alignment made by the homology detection program. The significance of the detected sequence homology was checked by statistical test with 100 randomized sequence pairs (15, 16). According to the alignment, a phylogenetic tree was constructed by the neighbor-joining method (17).

The references used in this study were as follows ; ref.9 and ref.10 for human LTA_4 hydrolase, ref.18 for human aminopeptidase N, ref.19 for rabbit aminopeptidase N, ref.12 for rat aminopeptidase M, ref.20 for *E.coli* aminopeptidase N, ref.21 for *Bacillus stearothermophilus* thermolysin, ref.22 for *Bacillus thermoproteolyticus* thermolysin, ref.23 for *Bacillus subtilis* thermolysin, ref.24 for *Bacillus amyloliquefaciens* thermolysin, ref.25 for *Bacillus cereus* neutral proteinase, ref.26 for *Pseudomanas aeroguinosa* elastase, ref.27 for rabbit endopeptidase, ref.28 for rat endopeptidase, ref.29 for rabbit collagenase, ref.30 for human collagenase, human stromelysin and rat stromelysin, ref.31 for human collagenase IV, ref.32 for rat transin 2, ref.33 for human stromelysin 2, and ref.34 for rabbit proactivator.

LTA_4 hydrolase was obtained as described previously (11). The zinc content of LTA_4 hydrolase was determined with a Hitachi atomic absorption spectrometer model Z-6100.

Results and Discussion

From computer assisted sequence comparison, it was suggested that LTA_4 hydrolase is a member of aminopeptidase N family, although the function of LTA_4 hydrolase is quite different from those of the aminopeptidases (12). In order to investigate the molecular evolution of these enzymes and their enzymatic functions, we compared the amino acid sequences of these enzymes. As a result, a region of LTA_4 hydrolase of 611 residues long (from 106 to 326 residue) was aligned with that of human intestine aminopeptidase N of 967 residues long (from 180 to 418 residue), that of rabbit intestine aminopeptidase N of 791 residues long, (from 1 to 239 residue), that of rat kidney aminopeptidase M of 964 residues long (from 180 to 417 residue) and that of *E.coli* aminopeptidase N of 870 residues long (from 75 to 327 residue). The N-terminal sequence of the the rabbit aminopeptidase N has not been determined yet. Fig. 1 shows the alignment of the homologous region. The C-terminal half of the alignment are more conservative than the N-terminal half. The Cys residue which is considered to be involved in the LTA_4 hydrolase activity is present in the aligned region (position 119 in the alignment) (9, 10). In order to check the significance of the detected homology, the alignment was subjected to the statistical test and the normalized alignment scores were calculated. The normalized score for the pair of LTA_4 hydrolase and human aminopeptidase N is 9.45 S.D., that of LTA_4 hydrolase and rabbit aminopeptidase N is 8.29 S.D., and that of LTA_4 hydrolase and rat aminopeptidase M is 7.70 S.D., although that of LTA_4 hydrolase and *E.coli* aminopeptidase N is 2.86 S.D. To determine further, the alignment of the C-terminal consevative region (position 181 - 264 of the alignment) of the pair of LTA_4 hydrolase and *E.coli* aminopeptidase N

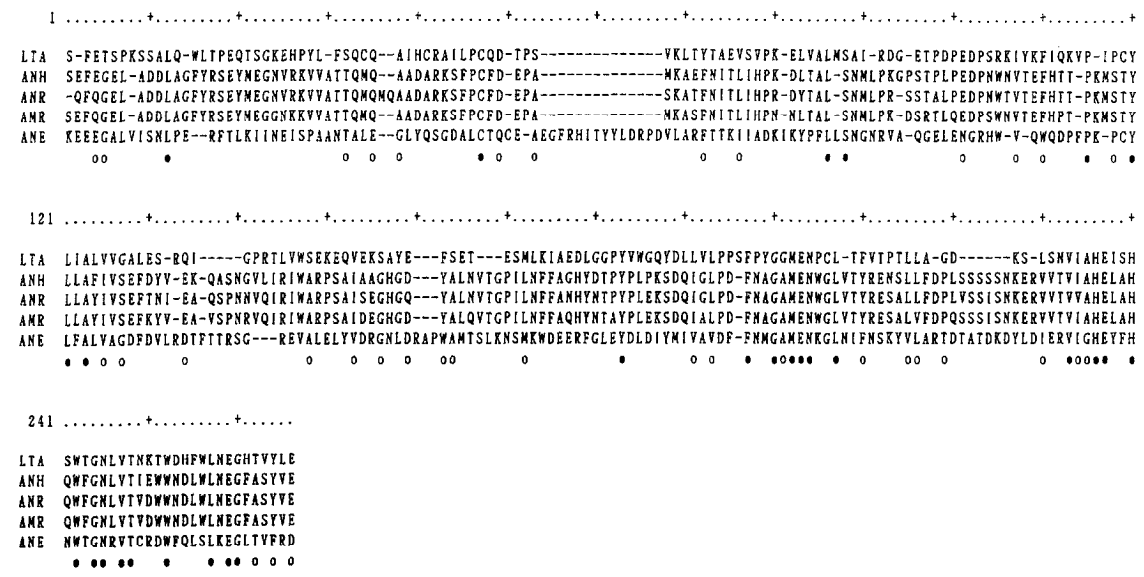

Fig. 1 Alignment of LTA_4 hydrolase and aminopeptidase N derived from human and *E.coli*. Open circle indicates the site occupied by chemically similar residues and closed circle indicates the site occupied by identical residues. Abbreviated name of each enzyme is written on the left side of the aligned sequence. The abbreviations are as follows, LTA : human LTA_4 hydrolase, ANH ; human aminopeptidase N, ANR ; rabbit aminopeptidase N, AMR ; rat aminopeptidase M, ANE ; *E.coli* aminopeptidase N.

was subjected to the statistical test. The normalized alignment score of this region LTA_4 hydrolase and *E.coli* aminopeptidase N is 5.66 S.D. These results of the calculation suggest that the detected sequence homology is statistically significant, that is, LTA_4 hydrolase is evolutionarily related to the aminopeptidase N, at least, at the homologous region.

	Z* Z		Z
leukotriene A_4 hydrolase	VIAHEISHSW	(14 residues)	LNEGH
aminopeptidase N human	VIAHELAHQW	(14 residues)	LNEGF
aminopeptidase N rabbit	VVAHELAHQW	(14 residues)	LNEGF
aminopeptidase M rat	VIAHELAHQW	(14 residues)	LNEGF
aminopeptidase N E.coli	VIGHEYFHNW	(14 residues)	LKEGL
thermolysin B.stearothermophilus	VVAHELTHAV	(15 residues)	INEAI
thermolysin B.thermoproteolyticus	VVAHELTHAV	(15 residues)	INEAI
thermolysin B.subtilis	VTAHEMTHGV	(15 residues)	LNESF
thermolysin B.amyloliquefaciens	VTAHEMTHGV	(15 residues)	LNESF
neutral proteinase B.cereus	VIGHELTHAV	(15 residues)	LNEAI
elastase P.aeruginosa	VAAHEVSHGF	(15 residues)	MNEAF
endopeptidase rabbit	VIGHEITHGF		
endopeptidase rat	VIGHEITHGF		
collagenase rabbit	VAAHELGHSL		
collagenase human	VAAHELGHSL		
collagenase IV human	VAAHEFGHAM		
transin 2 rat	VAAHELGHSL		
stromelysin human	VAAHEIGHSL		
stromelysin 2 human	VAAHELGHSL		
stromelysin rat	VAAHELGHSL		
proactivator rabbit	VAAHELGHSL		

Fig. 2 Alignment of the motif region. 'Z' indicates the site, which is consdered to act as ligand of zinc ion. '*' indicates the site, which is considered to be active site for peptidase activity. References of the sequence data are listed under 'Materials and Methods'. Numbers in the parenthesis indicate the lengths of the regions connecting two motifs.

In the aminopeptidase N, a highly conservative region (position 231 - 258 of the alignment) is considered to constitute a functional protease domain (15,16), because the region contains a sequence motif, VXXHEXXH, which is a ubiquitous sequence motif of zinc-metalloproteinases (see Fig.2). There are four classes of zinc-metalloproteinases, aminopeptidase N, thermolysin, endopeptidase, and collagenase, all contain the sequence motif (12,18-34). Among these proteinases, thermolysin has been characterized extensively. Two invariant His residues in the motif act as ligands of zinc ion, and an invariant Glu residue is one of the active sites for hydrolysis of peptides (35) (see Fig.2). As shown in Fig.1 and Fig.2, the sequence motif is also found in the LTA_4 hydrolase. In the thermolysin, it is known that a zinc ion binds two His residues in the sequence motif and a Glu residue in the downstream of the motif. Corresponding Glu residue is conserved in aminopeptidases N (12,18-20) and LTA_4 hydrolase, although corresponding residue has not been identified in the other two classes of proteinase, endopeptidase and collagenase (see Fig.1 and Fig.2). These observation suggests that a zinc ion may be present in the LTA_4 hydrolase.

By atomic absorption spectroscopy, the purified LTA_4 hydrolase contains a zinc ion. Furthermore, the recombinant LTA_4 hydrolase has a peptidase activity. More detailed experimental procedures and quantitative data will be published elsewhere. Further studies are on going to elucidate whether or not the peptidase activity of LTA_4 hydrolase is due to the inherited structural homology of the enzyme. It is still possible that a small amount of peptidase(s) is contaminated in the purified enzyme.

The immunohistochemical study of LTA_4 hydrolase has demonstrated that the enzyme is rich in the epithelial cells of small intestine (36). The peptidase activity of LTA_4 hydrolase may explain, at least partly, the physiological significance of the enzyme in the gastrointestinal tract. Thus, the results of sequence analysis described above are consistent with the experimental investigations.

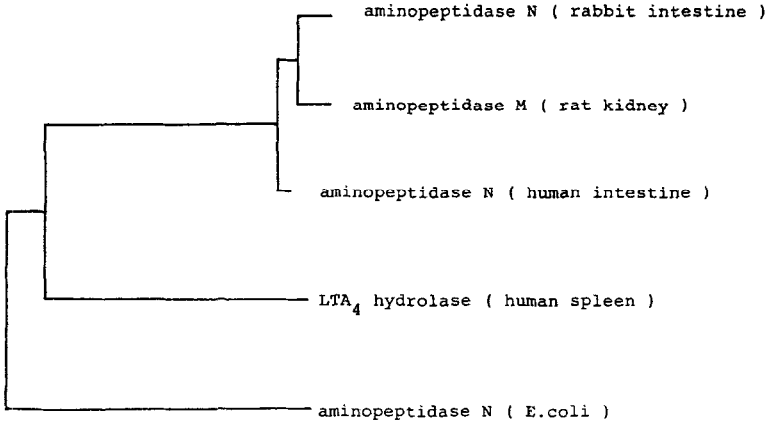

Fig. 3 Phylogenetic tree of aminopeptidase N family and LTA_4 hydrolase.

A phylogenetic tree was constructed, according to the alignment (see Fig.3). The tree shows the early divergence of LTA_4 hydrolase from aminopeptidase family, although the age has not been determined. Last year, a novel evolutionary strategy was proposed from the investigations of crystallins, which is called gene sharing (37). Gene sharing means that a gene acquires a novel function without duplication and keeps its original function. The sequence homology, together with the recent experimental investigation, suggests that LTA_4 hydrolase have two different enzymatic activities, LTA_4 hydrolase activity and peptidase activity. LTA_4 hydrolase may have evolved from aminopeptidase N by gene sharing, or vice versa.

Acknowledgment. We thank Dr.Yoshihiro Urade for stimulating discussion and advice.

References

1 Samuelsson,B., Dahlén,S.-E., Lindgren, J.Å.., Rouzer,C.A. and Serhan,C.N. (1987) *Science*, **237**, 1171-1176.
2 Shimizu,T. and Wolfe,L.S. (1990) *J. Neurochem.* **55**, 1-15.
3 Samuelsson,B. and Funk,C.D. (1989) *J.Biol.Chem.* **264**, 19469-19472.
4 Shimizu,T. (1988) *Int.J.Biochem.* **20**, 661-666.
5 Needleman,P., Turk,J., Jakscih,B.A., Morrison,A.R. and Lefkowith,J.B. (1986) *Annu. Rev. Biochem.* **55**, 69-102.
6 Rådmark,O., Shimizu,T., and Jörnvall,H. and Samuelsson,B. (1984) *J. Biol. Chem.* **259**, 12339-12345.
7 Evans,J.F., Dupuis,P. and Ford-Hutchinson,A.W. (1985) *Biochim. Biophys. Acta* **840**, 43-50.
8 Bito,H., Ohishi,N., Miki,I., Minami,M., Tanabe,T., Shimizu,T. and Seyama,Y. (1989) *J.Biochem.* **105**, 261-264.
9 Funk,C.D., Rådmark,O., Fu,J.Y., Matsumoto,T., Jörnvall,H., Shimizu,T. and Samuelsson,B. (1987) *Proc. Natl. Acad. Sci. USA* **84**,6677-6681.
10 Minami,M., Ohno,S., Kawasaki,H., Rådmark,O., Samuelsson,B., Jörnvall,H., Shimizu,T., Seyama,Y. and Suzuki,K. (1987) *J. Biol. Chem.* **262**, 13873-13876.
11 Minami,M., Minami,Y., Emori,Y., Kawasaki,H., Ohno,S., Suzuki,K., Ohishi,N., Shimizu,T. and Seyama,Y. (1988) *FEBS Lett.* **229**, 279-282.
12 Malfroy,B., Kado-Fong,H., Gros,C., Giros,B., Schwartz,J.-C. and Hellmis,R. (1989) *Biochem.Biophys.Res.Commun.* **161**, 236-241.
13 Nishikawa K., Nakashima H., Kanehisa M. and Ooi T. (1987) *Protein Sequences and Data Analysis* **1**, 107-116.
14 Seto,Y., Ihara,S., Kohtsuki,S., Ooi,T. and Sakakibara,S. (1988) In: Lesk AM (ed) *Computational Molecular Biology* pp27-37 Oxford University Press, Oxford.
15 Scwartz,R.M. and Dayhoff,M.O. (1978) *Matrices for Detecting Distant Relationships* in *Atlas of Protein Sequence and Structure* Vol.5 Suppl.3 M.O.Dayhoff Ed., pp353-358. The National Biomedical Research Foundation.
16 Toh,H., Hayashida,H. and Miyata,T. (1983) *Nature* **305**, 827-829.
17 Saitou,N. and Nei,M. (1987) *Mol.Biol.Evol.* **4**, 406-425.
18 Olsen,J., Cowell,G.M., Kønigshøfer,E., Danielsen,E.M., Møller,J., Laustsen,L., Hansen,O.C., Welinder,K.G., Engberg,J., Hunziker,W., Spiess,M., Sjöström,H. and Norén,O. (1988) *FEBS Lett.* **238**, 307-314.
19 Norén,O., Dabelsteen,E., Høyer,P.E., Olsen,J., Sjöström,H., Hansen,G.H. (1989) *FEBS Lett.* **259**, 107-112.

20 Bally,M., Foglino,M., Bruschi,M., Murgier,M. and Lazdunski,A. (1986) *Eur.J.Biochem.* **155**, 565-569.
21 Takagi,M., Imanaka,T. and Aiba,S. (1985) *J.Bacteriol.* **163**, 824-831.
22 Titani,K., Hermodson,M.A., Ericsson,L.H., Walsh,K.A. and Neurath,H. (1972) *Nature New Biol.* **238**, 35-37.
23 Yang, M.Y., Ferrari,E. and Henner,D.J. (1984) *J.Bacteriol. 160*, 15-21.
24 Vasantha,N., Thompson,L.D., Rhodes,C., Banner,C., Nagle,J. and Filpula,D. (1984) *J.Bacteriol.* **159**, 811-819.
25 Sidler,W., Niederer,E., Suter,F. and Zuber,H. (1986) *Biol.Chem.Hoppe-Seyler* **367**, 643-657.
26 Fukushima,J., Yamamoto,S., Morihara,K., Atsumi,Y., Takeuchi,H., Kawamoto,S. and Okuda,K. (1989) *J.Bacteriol.* **171**, 1698-1704.
27 Devault,A., Lazure,C., Nault,C., Le Moual,H., Seidah,N.G., Chretien,M., Kahn,P., Powell,J., Mallet,J., Beaumont,A., Roques,B.P., Crine,P. and Boileau,G. (1987) *EMBO J.* **6**, 1317-1322.
28 Malfroy,B., Schofield,P.R., Kuang,W.J., Seeburg,P.H., Mason,A.J. and Henzel,W.J. (1987) *Biochem.Biophys.Res.Commun.* **144**, 59-66.
29 Fini,M.E., Plucinska,I.M., Mayer,A.S., Gross,R.H. and Brinckerhoff,C.E. (1987) *Biochemistry* **26**, 6156-6165.
30 Whitham,S.E., Murphy,G., Angel,P., Rahmsdorf,H.J., Smith,B.J., Lyons,A., Harris,T.J.R., Reynolds,J.J., Herrlich,P. and Docherty,A.J.P. (1986) *Biochem.J.* **240**, 913-916.
31 Collier,I.E., Wilhelm,S.M., Eisen,A.Z., Marmer,B.L., Grant,G.A., Seltzer,J.L., Kronberger,A., He,C., Bauer,E.A. and Goldberg,G.I. (1988) *J.Biol.Chem.* **263**, 6579-6587.
32 Breathnach,R., Matrisian,L.M., Gesnel,M.C., Staub,A. and Leroy,P. (1987) *Nucleic Acids Res.* **15**, 1139-1151.
33 Muller,D., Quantin,B., Gesnel,M.C., Millon-Collard,R., Abecassis,J. and Breathnach,R. (1988) *Biochem.J.* **253**, 187-192.
34 Fini,M.E., Karmilowicz,M.J., Ruby,P.L., Beeman,A.M., Borges,K.A. and Brinckerhoff,C.E. (1987) *Arthritis Rheum.* **30**, 1254-1264.
35 Matthews,B.W., Jansonius,J.N., Colman,P.M., Schoenborn,B.P. and Dupourque,D. (1972) *Nature New Biol.* **238**, 37-41.
36 Ohishi,N., Minami,M., Kobayashi,J., Seyama,Y., Hata,J., Yotsumoto,H., Takaku,F. and Shimizu,T. (1990) *J.Biol.Chem.* **265**, 7520-7525.
37 Piatigorsky,J. and Wistow,G.J. (1989) *Cell* **57**, 197-199.