The metabolic pathway called the arachidonic acid cascade produces a wide range of eicosanoids, such as prosta-
glandins, thromboxanes and leukotrienes with potent biologi-
ical activities. Recombinant DNA techniques have
made it possible to determine the nucleotide sequences of
cDNAs and/or genomic structures for the enzymes in-
volved in the pathway. Sequence comparison analyses of
the accumulated sequence data have brought great in-
sights into the structure, function and molecular evolution
of the enzymes. This paper reviews the sequence com-
parison analyses of the enzymes involved in the
arachidonic acid cascade.

Key words: Arachidonic acid cascade, Brain-type PGD syn-
thase, Cyclooxygenase, Lipoxygenases, LTA hydrolase, PGF
synthase, TXA synthase

Molecular evolution of enzymes involved in the
arachidonic acid cascade

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Introduction

Arachidonic acid is released from cell membranes
by chemical, mechanical or electrical stimuli. The
deesterified arachidonic acid is oxygenated to yield a
variety of bioactive compounds—prostaglandins
(PGs), thromboxanes (TXs), leukotrienes (LTs),
and lipoxins (LXs). These oxygenated arachidonic
acid compounds are collectively termed 'eicos-
anoids'. Most eicosanoids act as critical mediators in
inflammation, blood clotting, control of vascular
tone, renal function, reproductive systems, and cell
differentiation. In addition, neuronal and neuro-
endocrine functions of eicosanoids have been
discovered recently, and it has been indicated that
eicosanoids play more important roles in inter-
cellular signal transduction systems than those
considered previously. The metabolic pathway
where eicosanoids are formed, is called the
'arachidonic acid cascade' (see References 1, 2, 3
and 4 for reviews).

One of the prominent achievements in the
research of the arachidonic acid cascade is molecular
cloning of the cDNAs and/or genes for the key
proteins involved in the cascade.5,4 These proteins
include not only the synthases of eicosanoids, but
also a receptor for an eicosanoid5 and an activation
factor for a synthase.6 Sequence comparison
analyses of the sequence data obtained have given
clues to the structures and functions of the proteins.
We focus here on the synthases of eicosanoids, and
discuss the structure, function and molecular
evolution of these enzymes based on the sequence
comparison analyses.

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EGF-like domain: down-224 Mediators of Inflammation Vol 1992

mimically to be localized at the endoplasmic reticulum.

Cyclooxygenase has been shown immunohistochemically to be localized at the endoplasmic reticulum. Therefore, the EGF-like domain of this enzyme is considered to be a second example of the domain found in cells. However, the functional meaning of this domain in this enzyme has not yet been established which is true for most of the EGF-like domains. Cyclooxygenase contains the EGF-like domain at its N-terminal region, while thyroid peroxidase contains the EGF-like domain at its C-terminal region. Therefore, it is considered that exon shuffling had occurred independently twice, in order to introduce EGF-like domains into the members of the peroxidase family during the course of evolution.

Brain-type PGD Synthase

PGD$_2$ is a major PG produced in the brain of humans and rats, and shows a wide variety of activities in vivo such as sleep induction, hypotension, anticonvulsion, nociception, suppression of luteinizing hormone release, and modulation of the odour response. Brain-type PGD synthase catalyses the isomerization of PGH$_2$ to form PGD$_2$. The nucleotide sequences of cDNAs for the enzyme

![Diagram](image-url)
from rats\textsuperscript{28} and humans\textsuperscript{29} were determined. In addition, the genomic structure for the rat enzyme was reported.\textsuperscript{30} The deduced amino acid sequences are about 190 residues in length, including a signal sequence of 20 residues.

This enzyme shows weak (10–33\% identity) but significant sequence homology to the members of the lipocalin family in their entire regions.\textsuperscript{29,31} The lipocalin family consists of a wide variety of small secretory proteins (160–200 amino acids in length), which includes \(\beta\)-lactoglobulin, retinol-binding protein, bilin-binding protein, \(\alpha_1\)-acid glycoprotein, odorant-binding protein and \(\alpha_2\)-urinary globulin.\textsuperscript{32,33} These proteins are bound to small lipophilic molecules and are involved in the transport of those molecules. The amino acid sequences of the members are different from one another, but six conserved regions are identified, which characterize the lipocalins (alignment positions 51, 54–56, 109, 150–152, 181 and 220 in Fig. 2(a)).\textsuperscript{32} All these

![Alignment of rat brain-type PGD synthase and seven lipocalins whose genomic structures are known. Redrawn from the alignment in Ref. 30. Asterisks indicate stop codons. The positions of the exon/intron junction are indicated by the numbers on the sequences. '0' between two residues indicates splicing between codons. '1' and '2' on a residue indicate splicing between the first and second positions of a codon, and that between the second and third positions of a codon, respectively. Closed circles, open circles, and '-' have the same meanings as those in Fig. 1(a). (1) Rat brain-type PGD synthase, (2) Mouse \(\alpha_2\)-urinary globulin, (3) Oxine \(\beta\)-lactoglobulin, (4) Human placental protein 14 (A35570), (5) Human \(\alpha_1\)-microglobulin, (6) Human \(\alpha_2\)-acid glycoprotein, (7) Rat retinol-binding protein (VART), and (8) Human apolipoprotein D (A26968). The codes in the parentheses indicate accession numbers in the NBRF data base as references.](image)

![Model tertiary structure of PGD synthase. Redrawn from the model in Ref. 29.](image)

![Unrooted phylogenetic tree of 26 lipocalins including brain-type PGD synthases derived from humans and rats. Redrawn from the alignment in Ref. 30. Lipocalins whose tertiary structures are known are boxed. The abbreviations are as follows: RPDS, rat brain-type PGD synthase; HPDS, human brain-type PGD synthases; 24p3, mouse 24p3 protein (S07397); CB, human complement component C8\(\beta\) chain (CHUG); \(\alpha_2\)UG, rat \(\alpha_2\)-urinary globulin; \(\alpha_2\)P20, chicken quiescence-specific polypeptide 20K (A30230); CGL, chicken \(\beta\)-lactoglobulin; PP14, human placental protein 14 (A31242); OBP, rat odorant-binding protein (A28713); APHR, hamster aphrodisin (A31243); PRB, rat probasin (A32602); PZBP, bovine pyrazine-binding protein (S06843); \(\alpha_1\)GP, human \(\alpha_1\)-acid glycoprotein (OMHU1); \(\alpha_1\)MG, human \(\alpha_1\)-microglobulin (HCHU); OBPII, rat odorant-binding protein II; VEGP, rat von Ebner's gland protein (S08161); BGP, frog, Rana pipiens, Bowman's gland protein (OVFGP); 18.5K, rat androgen-dependent epididymal 18.5K protein (SORTAD); RBP, rat retinol-binding protein (VART); PURN, chicken retinol-binding protein (A26969); MBP, tobacco hornworm, Manduca sexta, bilin-binding protein (CUW10); PBBP, butterfly, Pieris brassica, bilin-binding protein (S00819); ALPD, human apolipoprotein D (A26968); CNA2, lobster, Homarus gammarus, crusteaynin A\textsubscript{2}; CNC, lobster, Homarus gammarus, crusteaynin C\textsubscript{1}. The codes in the parentheses indicate the accession numbers of the proteins in the NBRF database as references.](image)
conserved regions are also found in the PGD synthases.\textsuperscript{29} In addition to the similarity in sequence and size, the positions of exon/intron junctions and the phases of the splicing of this enzyme are similar to those of the lipocalins (see Fig. 2(a)).\textsuperscript{30}

The fact that tertiary structures of proteins are more conserved than their primary structures has been amply documented.\textsuperscript{34-36} In fact, three lipocalins, \(\beta\)-lactoglobulin,\textsuperscript{37,38} retinol-binding protein\textsuperscript{39,40} and bilin-binding protein,\textsuperscript{41,42} share a common folding pattern called \(\beta\)-barrel structure in spite of the high sequence divergence among them (13–22\% identity). The hydrophobic ligands are bound in the barrel structure. The sequence homology between PGD synthases and lipocalins suggests that the enzyme also has a \(\beta\)-barrel structure\textsuperscript{29,31} (Fig. 2(b)). The enzyme is known to bind to several small lipophilic molecules including PGH\(_2\), the substrate for this enzyme.\textsuperscript{29} These molecules may be bound in the barrel structure as with other lipocalins.

However, this enzyme has two distinctive characteristics compared to other lipocalins.\textsuperscript{29,31} One is that this enzyme has an enzymatic activity, whereas the other lipocalins are involved in the transport of small lipophilic molecules. Since the enzyme requires sulphhydryl compounds for the reaction and is inactivated by treatment with sulphhydryl modifers, Cys residues are considered to be involved in the catalytic site of the enzyme. The sequence comparison between the enzymes from humans and rats revealed three conserved Cys residues. Two of them (alignment positions 109 and 220 in Fig. 2(a)) are also conserved in the other lipocalins, and are involved in the disulphide bridge formation. The remaining Cys residue is specifically conserved in the PGD synthase (alignment site 82 in Fig. 2(a)), which may play an important role in its enzymatic activity. The Cys residue of alignment site 82 is present in the predicted \(\beta\)-barrel structure favourable for interaction with its substrate (see Fig. 2(b)).

The other distinctive characteristic is that this enzyme is a membrane associated protein, whereas the other lipocalins, except for an isoform of probasin (which reportedly translocates to the nucleus\textsuperscript{45}), are secretory proteins. It is an important and interesting problem not only for molecular evolution but also for protein engineering to establish the mechanism for the acquisition of the two characteristics.

Figure 2 (c) shows a phylogenetic tree of the 26 lipocalins including three PGD synthases.\textsuperscript{30} The tree shows that PGD synthase is relatively close to the p23 oncogene product and the complement component C8 \(\gamma\) chain, although the similarities of the enzyme to these two lipocalins are low (23–33\% identity).

**PGF Synthase**

PGF\(_{2\alpha}\) shows activities of bronchoconstriction, vasoconstriction, luteolysis, and acetylcholine release. On the other hand, 11-\(\epsilon\)-PGF\(_{2\alpha}\), the isomer of PGF\(_{2\alpha}\), causes bronchoconstriction, vasoconstriction, Na\(^+\) excretion, urinary excretion, and antiaggregation. The former is converted from PGH\(_2\) and the latter from PGD\(_2\) by the same enzyme, PGF synthase. Although both activities of this enzyme require NADPH, the active site for the PGF\(_{2\alpha}\) formation is different from that for 11-\(\epsilon\)-PGF\(_{2\alpha}\) formation.\textsuperscript{44,45} The cDNAs encoding this enzyme were isolated from bovine lung\textsuperscript{46} and liver.\textsuperscript{47} Both cDNAs encode proteins of 323 amino acids which are closely related to each other (99\% identity).

PGF synthase shows high sequence similarity to the members of the aldo-keto reductase family (36–74\% identity).\textsuperscript{46} In the presence of NADPH, PGF synthase catalyses the reduction of not only PGD\(_2\) and PGH\(_2\), but also several carbonyl compounds including 9,10-phenanthrenequinone, a substrate of the aldo-keto reductases.\textsuperscript{48} On the other hand, human liver aldehyde reductase, whose sequence shows 40\% identity to that of the PGF synthase, catalyses the reduction of PGH\(_2\), although it does not catalyse the reduction of PGD\(_2\).\textsuperscript{49} The aldo-keto reductase family consists of a wide variety of reductases, such as human liver chlordechone reductase, Corynebacterium diketogluconic acid reductase, human liver aldehyde reductase, and aldose reductases from various mammals. Figure 3(a) is a multiple alignment of the family including the PGF synthases, and Fig. 3(b) shows an unrooted phylogenetic tree of the family. As shown in the figure, this enzyme shows the highest sequence similarity to chlordechone reductase within the members of the family (74\% identity). It is interesting that frog \(\rho\)-crystallin occupies a position relatively close to PGF synthase and chlordechone reductase in the tree.\textsuperscript{50,51} The amino acid sequence of frog \(\rho\)-crystallin shows 59\% identity against that of PGF synthase or chlordechone reductase. Recently, it was revealed that the \(\rho\)-crystallin has an NADPH-binding activity and shows a weak enzymatic activity to convert PGH\(_2\) to PGF\(_{2\alpha}\) (5\% of the activity of PGF synthase), although it does not show the activity to convert PGD\(_2\) to 11-\(\epsilon\)-PGF\(_{2\alpha}\).\textsuperscript{51}

There are several crystallins apart from the frog \(\rho\)-crystallin, which have been identified from various animals and show high sequence similarity to the other functionally important enzymes. For example, \(\epsilon\)-crystallin from avian and crocodilian lenses shows high sequence similarity to the lactate dehydrogenase B4. Furthermore, the \(\epsilon\)-crystallin shows an enzymatic activity comparable to the
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![Alignment of aldo-keto reductase family including PGF synthase. Redrawn from the alignment in Ref. 51. Open circles, closed circles, and '-' have the same meanings as those in Fig. 1(a). (1) Bovine lung PGF synthase, (2) Human chlordecone reductase (A34263), (3) Frog Rana temporaria, α-crystallin, (4) Corynebacterium diketogluconic acid reductase, (5) Leishmania major P100/11E gene product (A32950), (6) Yeast nuclear gene GCY product, (7) Barley aldose reductase-like protein (S15024), (8) Human aldehyde reductase (A37990), aldoses derived from (10) Bovine (A35452), (11) Human (S06591), and (12) Rabbit (A34406), and (13) Rat. Δ4-3-ketosteroid 5β-reductase (S15835). The codes in the parentheses indicate the accession numbers of the proteins in the NBRF database as references.

(b) Unrooted phylogenetic tree of the aldo-keto reductase family. The nodes in a circle indicate proteins which belong to a group designated near the circle.

| Accession Number | Protein Name |
|------------------|-------------|
| A34263           | Human chlordecone reductase |
| A32950           | Leishmania major P100/11E gene product |
| A35452           | Human aldehyde reductase |
| A37990           | Human aldehyde reductase |
| S15024           | Human aldehyde reductase |
| S06591           | Human aldehyde reductase |
| A34406           | Rabbit aldehyde reductase |
| A35835           | Rat Δ4-3-ketosteroid 5β-reductase |
| A35482           | Human aldehyde reductase |

Recent observations suggest another evolutionary mechanism, that is, a gene can acquire new functions without loss of the original function or gene duplication. This new mechanism is called 'gene sharing'. The sequence homology between PGF synthase and frog α-crystallin gives an example of gene sharing.

Recently, the crystal structure of pig aldose reductase was determined. The enzyme exhibits a single domain protein with an eight-stranded parallel α/β barrel, or a so-called triose phosphate isomerase (TIM)-barrel structure. The crystallographic analysis revealed that 2'-monophosphoadenosine-5'-diphospho-ribose, which competitively inhibits the binding of NADPH, binds to a cleft located at the C-terminal region of the TIM-barrel.
structure. This observation suggests that PGF synthase also has a TIM-barrel structure and NADPH is bound to the C-terminal region of this enzyme.

**TXA Synthase**

TXA$_2$ is a potent inducer of platelet aggregation and a constrictor of smooth muscles. It is known that this eicosanoid is involved in several diseases such as thrombosis, atherosclerosis and asthma. TXA synthase catalyses the conversion of PGH$_2$ to TXA$_2$. TXA synthase and PGI synthase were purified to homogeneity, and were found to be the cytochrome P-450 enzymes. Recently, cDNA of this enzyme was identified from human platelet and lung cDNA libraries, and the complete sequence of the cDNA was reported. The cDNA encodes an amino acid sequence of 533 amino acids.

Cytochrome P-450s are ubiquitous in nature and are found in animals, plants, fungi and bacteria. P-450s comprise multigene families in living organisms, and evolutionary relationships among them have been investigated extensively. TXA synthase (and PGI synthase) is involved in the cyclooxygenase pathway. Additionally, P-450s are involved in the cytochrome P-450 pathway to yield various epoxy-eicosatrienoic acids from arachidonic acid by their monooxygenase activities. On the other hand, PG $\omega$-hydroxylase was cloned from rabbit lung, and is involved in $\omega$-oxidation, a step in metabolic inactivation of PGs. PG $\omega$-hydroxylase also belongs to the cytochrome P-450 family.

Figure 4(a) shows the phylogenetic relationships between 50 members of the P-450 family including TXA synthase and PG $\omega$-hydroxylase. PG $\omega$-hydroxylase belongs to the P-450 IV family. Contrary to that, TXA synthase occupies a unique position in the tree, but it is relatively close to the P-450 III family, as noted previously. Figure 4(b) shows the alignment of the TXA synthase and cytochrome P-450 III. The Glu (alignment site 406), Arg (alignment site 409), Phe (alignment site 479), Cys (alignment site 486) residues are invariant in the 50 sequences used for construction of the phylogenetic tree.

The tertiary structure of P-450 CI (or P-450cam) has been determined. Although the sequence identity of P-450 CI to other eukaryotic P-450s is very weak, the other P-450s are considered also to have a similar folding pattern to that of P-450 CI, and modellings of the tertiary structures of other P-450s according to the sequence homology were tried. The sequence identity between P-450 CI and TXA synthase is also low, but it is considered that TXA synthase also has a tertiary structure like that of P-450 CI.

**5-12- and 15-Lipoxygenases**

We have discussed the structure, function and molecular evolution of the enzymes involved in the cyclooxygenase pathway. Now we will discuss the enzymes involved in the lipoxygenase pathway, the other constituent of the arachidonic acid cascade. The lipoxygenase pathway is further divided into three pathways, each of which is initiated by the oxygenation of arachidonic acid by 5-, 12- and 15-lipoxygenases, respectively. The numbers attached to the names of the enzymes indicate the carbon positions of the arachidonic acid, which are subjected to the oxygenation catalysed by the corresponding enzymes. The nucleotide sequences of the cDNAs for the three types of the lipoxygenases (5-lipoxygenases derived from humans and rats; 12-lipoxygenases derived from humans and pigs; and 15-lipoxygenases derived from humans and rabbits) have been determined. Furthermore, the genomic structures of human 5-lipoxygenase and rabbit 15-lipoxygenases were determined. The deduced amino acid sequences are about 700 residues in length, and the primary structures of 5-, 12- and 15-lipoxygenases are homologous to each other in their entire regions. Plants also contain several lipoxygenases of about 900 amino acid residues in length. The animal and plant lipoxygenases show sequence similarity at their C-terminal regions of about 500 amino acid residues. However, their N-terminal regions are different not only in amino acid sequences but also in the lengths.

Sequence comparison of animal and plant lipoxygenases suggests that their primary structures contain a cluster of invariant His residues (Fig. 5(a)). It is known that lipoxygenases contain a non-heme iron co-factor, and the invariant His residues were proposed to act as ligands for the iron co-factor. On the other hand, animal 12- and 15-lipoxygenases were reported to contain a zinc finger-like motif, which was also proposed to act as a ligand for the iron co-factor. However, the motif is absent in animal 5-lipoxygenases and plant lipoxygenases (Fig. 5(b)).

Figure 5(c) shows a phylogenetic tree of the lipoxygenases. Two hypothetical ancestral lipoxygenases are shown in the tree (lipoxygenase-X and lipoxygenase-Y). Animal and plant lipoxygenases diverged at node 'a'. Then, 5-lipoxygenase and lipoxygenase-X diverged by gene duplication at node 'b'. Next, human 12-lipoxygenase and lipoxygenase-X functionally diverged at node 'c' by gene duplication of lipoxygenase-X. After that, pig 12-lipoxygenase and 15-lipoxygenase diverged at node 'd' by gene duplication of lipoxygenase-Y. Hence, the tree suggests that enzymes with 12-lipoxygenase activity were created independently.
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LG

LTA Hydrolase

Leukotriene (LT) A₄ is synthesized from arachidonic acid by the activity of 5-lipoxygenase, and all kinds of LTs are synthesized via LTA₄. LTA
hydrolase is an enzyme which catalyses the hydrolysis of an epoxide moiety of LTA₄ to yield LTB₄. LTB₄ is a potent chemotactic compound for granulocytes and is closely related to the immune system.8-82 The nucleotide sequences of cDNAs for this enzyme derived from human spleen, lung and placenta,83,84 and mouse spleen85 have been determined, all of which encode proteins 611 amino acid residues in length. The two human enzymes are identical to each other and the amino acid identity between human and mouse enzymes is 93%.

This enzyme shows weak but significant sequence homology to the members of the aminopeptidase N family (Fig. 6(a)),86,87 although the functions of LTA hydrolase is quite different from that of aminopeptidases. Figure 6(b) shows the phylogenetic relationships within the aminopeptidase family including LTA hydrolases.87 One of the highly conserved regions between them corresponds with a ubiquitous sequence motif found in a wide variety of zinc metalloenzymes.87,88 The motif sequence, VXXHHEXXH (alignment sites 349–356), contains two invariant His residues, which act as ligands for the zinc ion in the proteases. In addition, it is known that an invariant Glu residue in the motif is one of the active sites of the peptidases. These residues are also conserved in the LTA hydrolase.

After the determination of the sequence homology, it was revealed by atomic absorption spectrometry that LTA hydrolase contains one zinc ion per enzyme molecule.89,90 Furthermore, LTA hydrolase exhibits peptidase activity toward the synthetic substrates alanine-4-nitroanilide and leucine-4-nitroanilide.85,91 The zinc ion was shown to be involved in both LTA hydrolase activity and peptidase activity of the enzyme, that is, the apoenzyme of LTA hydrolase is virtually inactive for both activities but can be reactivated to show both activities by the addition of stoichiometric amounts of zinc or cobalt ion. The LTA hydrolase undergoes suicide type inactivation, and the inactivated LTA hydrolase does not show peptidase activity. The experimental results, in addition to the

![Diagram of phylogenetic tree of lipoxygenase family](image-url)
sequence homology, suggest that the catalytic mechanism of LTA hydrolase is similar to those of zinc metallopeptidases.

It has been a riddle that LTA hydrolase activity is found in a wide variety of tissues and cells, some of which do not express 5-lipoxygenase activity and, therefore, are unable to synthesize LTA, the substrate for this enzyme. The finding of peptidase activity of this enzyme offers an interpretation that this enzyme may exert different functions in different cells and tissues. Further discussion should be suspended until peptidase activity of this enzyme is found in vivo. However, it should be noted that LTA hydrolase is a new type of enzyme which can utilize two different cellular compounds, lipids and peptides, as substrates.

**Conclusion**

We have discussed the structure, function and molecular evolution of the enzymes involved in eicosanoid synthesis. Each enzyme discussed here
involved in the arachidonic acid cascade, but also the evolution of the cascade itself. Further, experimental and theoretical approaches will reveal not only the evolution of the enzymes involved in the arachidonic acid cascade, but also the evolution of the cascade itself.

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Note added in proof:

Recently, the crystal structure of myeloperoxidase was determined (Zeng, Fenna R. F. J Mol Biol 1992; 226: 185-207), which may be useful in investigations of the structure and function of cyclooxygenase.

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