Identification of Amino Acid Determinants of the Positional Specificity of Mouse 8S-Lipoxygenase and Human 15S-Lipoxygenase-2*

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Lipoxygenases are dioxygenases containing one atom of non-heme iron in their reaction center (1). These enzymes catalyze the hydroperoxidation of polyunsaturated fatty acids, usually with high positional specificity and stereospecificity. The lipoxygenase reaction is initiated by removal of a hydrogen from a methylene group between two cis double bonds, and then molecular oxygen reacts on the opposite face of the substrate to form a hydroperoxide product with a trans-cis-conjugated diene (1). The typical substrate of mammalian lipoxygenases is arachidonic acid, which is converted to hydroperoxyeicosatetraenoic acids (HPETEs).

Phorbol ester-inducible mouse 8S-lipoxygenase (8-LOX) and its human homologue, 15S-lipoxygenase-2 (15-LOX-2), share 78% identity in amino acid sequences, yet there is no overlap in their positional specificities. In this study, we investigated the determinants of positional specificity using a random chimeragenesis approach in combination with site-directed mutagenesis. Exchange of the C-terminal one-third of the 8-LOX with the corresponding portion of 15-LOX-2 yielded a chimeric enzyme with exclusively 15S-lipoxygenase activity. The critical region was narrowed down to a cluster of five amino acids by expression of multiple cDNAs obtained by in situ chimeragenesis in Escherichia coli. Finally, a pair of amino acids, Tyr603 and His604, was identified as the positional determinant by site-directed mutagenesis. Mutation of both of these amino acids to the corresponding amino acids in 15-LOX-2 (Asp and Val, respectively) converted the positional specificity from 8S to 90% 15S without yielding any other by-products. Mutation of the corresponding residues in 15-LOX-2 to the 8-LOX sequence changed specificity to 50% oxygenation at C-8 for one amino acid substitution and 70% at C-8 for the double mutant. Based on the crystal structure of the reticulocyte 15-LOX, these two amino acids lie opposite the open coordination position of the catalytic iron in a likely site for substrate binding. The change from 8 to 15 specificity entails a switch in the head to tail binding of substrate. Enzymes that react with substrate “head first” (5-LOX and 8-LOX) have a bulky aromatic amino acid and a histidine in these positions, whereas lipoxygenases that accept substrates “tail first” (12-LOX and 15-LOX) have an aliphatic residue with a glutamine or aspartate. Thus, this positional determinant of the 8-LOX and 15-LOX-2 may have significance for other lipoxygenases.

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The abbreviations used are: H(P)ETE, hydro(pero)xyeicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid; LOX, lipoxygenase; RP-HPLC, reversed-phase high pressure liquid chromatography; PCR, polymerase chain reaction; ORF, open reading frame.

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products are quite different. The 8-LOX produces solely 8S-HPETE from arachidonic acid (12), whereas 15S-HPETE is the sole product from 15-LOX-2 (11). The hydrogen abstractions associated with these two reactions occur on different carbons (C-10 and C-13, respectively) and on different faces of the substrate. Thus, the mouse 8-LOX and human 15-LOX-2 represent new issues related to the reaction specificity of lipoxygenases. We addressed the basis of their positional specificities using chimeric enzymes and site-directed mutagenesis.

**EXPERIMENTAL PROCEDURES**

**Materials**—Complementary DNAs of mouse 8S-lipoxygenase (8-LOX) and human 15S-lipoxygenase-2 (15-LOX-2) were obtained by PCR from cDNAs prepared from the mRNAs of phorbol ester-treated mouse skin and human hair root, respectively. 8-LOX was cloned into pCR3.1 mammalian expression vector (Invitrogen, Carlsbad, CA) (12), and 15-LOX-2 was originally cloned into pcDNA3 mammalian expression vector (Invitrogen, Carlsbad, CA) (11) and then transferred to pCR3.1. Both pCR3.1 and pcDNA3 contain a T7 promoter for effective expression.

**Construction of Randomly Switched Chimera Enzymes**—Various restriction site-independent chimeras between 8-LOX and 15-LOX-2 were prepared based on the method by Moore and Blakely (17). A tandemly connected 8-LOX and 15-LOX-2 cDNA in pCR3.1 was constructed in the same way using only EcoRI except that the rest of the plasmids were dephosphorylated before ligation.

**Construction of Restriction Site-switched Chimera Enzymes**—Various restriction site-independent chimeras between 8-LOX and 15-LOX-2 were prepared by mixed ligation between the N-terminal chimeras, I and II (Fig. 2A), were prepared by mixed ligation between the C-terminal region cut by double digestion of HindIII and XcmI and the rest of the plasmids. N-terminal chimeras, III and IV (Fig. 2A), were prepared in the same way using only EcoRV except that the rest of the plasmids were dephosphorylated before ligation.

**RESULTS**

**Construction of Randomly Switched Chimera Enzymes**—A tandemly connected 8-LOX and 15-LOX-2 cDNA in pCR3.1 was constructed in two steps in order to get some proper restriction sites between the two ORFs (Fig. 3A). First, 15-LOX-2 cDNA cut out from pCR3.1 using Xhel and XbaI was cloned into pSE280 (Invitrogen) pre-cut with XbaI. A cloned fragment containing the 15-LOX-2 cDNA in an inverse direction compared with the T7 promoter of pSE280 was selected and digested with NotI to obtain a 15-LOX-2-XbaI fragment containing an XbaI site in the 5’ side of 15-LOX-2. This fragment was then transferred into a pcRIII/L-8-LOX linearized by cutting with NotI. A clone containing tandemly connected 8-LOX and 15-LOX-2 cDNAs was selected, linearized by cutting the linker region between 8-LOX and 15-LOX-2 cDNAs with HpaI and KpnI, and purified from agarose gel using QIAEX II resin (Qiagen, Chatsworth, CA). Using 100 μg of the purified linear plasmid, TOP10°F-compent cells (Invitrogen) were transformed by heat shock at 42°C. After selection of the correct size clones by gel electrophoresis, the switching point in each chimera cDNA was narrowed down by examining the restriction cut profiles and finally determined by sequencing. This first trial of chimera preparation ultimately yielded 3 useful chimeras, V, VI, and XII (Fig. 4).

**Construction of Restriction Site-switched Chimera Enzymes**—The C-terminal region, a new tandem clone containing a full-length of 8-LOX cDNA and C-terminal 426 bases of 15-LOX-2 was constructed (Fig. 3B). The C-terminal portion of the parent 15-LOX-2 cDNA in pcRIII.1 was cut out with SmaI and EcoRV and was cloned into pBl-Stratagene, La Jolla, CA). The target portion was cut out again with ApaI/XbaI and transferred into pSE280 (Invitrogen), and then cut out again with ApaI and NotI and cloned into the downstream of the 8-LOX ORF in pCR3.1. The resulting chimera cDNA composed of a full-length of 8-LOX ORF and C-terminal 426 bases of 15-LOX-2 was linearized by cutting with NotI and XbaI and used to transform TOP10°F-compent cells. This chimerization resulted in 5 useful chimeras.
Site-directed Mutagenesis—Site-directed mutagenesis in the Ser 600-
His604 region in 8-LOX and Tyr 602-Asp603 in 15-LOX-2 was performed
by PCR-based overlap extension mutagenesis using mutated synthetic
oligonucleotides. The mismatching primers used are shown in Table I.
Four kinds of mutants of successive two amino acids and then three
ekinds of mutants of one amino acid were prepared from 8-LOX, and
three kinds of mutants were prepared from 15-LOX-2. The specific
upstream primers for the primary PCR of 8-LOX and 15-LOX-2 anneal
to SFVSEIV region (5'-GAGCTTTGTCTCTGAAATAGTCAG 3') and
GFSELIQR region (5'-GGCTTCTCTGAGTTGATACAGG 3'), respec-
tively. The pCR3.1 reverse primer was used as the specific downstream
primer for both enzymes. The resulting mutated fragments were di-
gested with EcoRV and then exchanged with the corresponding region
of the wild type cDNA.

Expression and Lipoxigenase Activity Assay—All the cDNAs were
expressed in HeLa cells using VTF-7, a recombinant vaccinia virus
containing the T7 RNA polymerase gene (18). Cells plated at 1 × 10^6
cells/35-mm well 48 h earlier were transfected with 1 μg of plasmid
dNA and 3 μg of Lipofectin and harvested after 12 h. The harvested
cells were sonicated on ice, and the resulting homogenate was used for
LOX activity assay and Western analysis.

The cell homogenates were incubated with 100 μM [1-14C]arachidonic
acid for 45 min at room temperature. The products were extracted by
the method of Bligh and Dyer (19), and the extracts were analyzed
directly by RP-HPLC first and then by normal phase HPLC after
reduction using triphenylphosphine. For stereochemical analysis, the
reduced hydroperoxide products were purified by normal phase HPLC,
methylated with diazomethane, re-purified by normal phase HPLC,
and then applied to chiral HPLC as described previously (11, 12).

Western Analysis—The expression level of each enzyme was esti-
mated by Western analysis. The cell homogenate containing 20 μg
of protein was separated by SDS-polyacrylamide gel electrophoresis and
then transferred to a nylon membrane (Amersham Pharmacia Biotech).
The enzymes were stained as described previously (12) using an anti-
sera raised against 15-LOX-2, which cross-reacts with 8-LOX but not
with 15-LOX-1, and an ECL detection kit (Amersham Pharmacia Bio-
tech), according to the manufacturers specifications.

FIG. 3. Construction of in situ random chimeras. A, chimera construction
using the full-length ORF of 8-LOX and 15-LOX-2. B, chimera construction using
the full-length ORF of 8-LOX and the C-
terminal region of 15-LOX-2.

FIG. 4. Structure of the in situ random chimeras. The C-terminal regions
of 8-LOX and 15-LOX-2 containing EcoRV
and Smal sites are shown. Amino acid
differences are marked with asterisks.
The black dots indicate the switching
point of each chimera.
RESULTS

Construction and Expression of Chimeras Using Common Restriction Sites—The mouse 8-LOX cDNA and the human 15-LOX-2 cDNA contain XcmI and EcoRV sites at equivalent positions (at 730 and 1380 base pairs, respectively, in the murine sequence). These restriction sites separate the cDNAs into three almost equally sized parts: N-terminal, middle, and C-terminal regions. Four chimeric cDNAs were prepared using these restriction sites (Fig. 2A). Chimeras I and II have the N-terminal regions exchanged at the XcmI site, whereas chimeras III and IV have the C-terminal regions exchanged at the EcoRV site. Following transfection into HeLa cells, all chimeras were expressed at the same level as the original wild type 8-LOX and 15-LOX-2 (Fig. 2B). Thus, substitution of their N- or C-terminal regions does not affect the enzyme expression probably due to the high sequential homology between the original enzymes.

When expressed in HeLa cells, the wild type 8-LOX and 15-LOX-2 converted arachidonic acid exclusively to 8-S-HETE (Fig. 2C) and 15-HETE (Fig. 2D), respectively. These data indicate that the C-terminal region of the mouse 8-LOX contains structural elements that determine its 8-S specificity.

In Situ Random Chimeragenesis—In order to narrow down the positional determinants in the C-terminal region of the 8-LOX, a variety of chimeras were produced by in situ chimeragenesis in Escherichia coli (17). In the first trial the bacteria were transformed with a plasmid containing the full-length 8-LOX and 15-LOX-2 cDNAs. We identified constructs that contained part of the C-terminal region of 15-LOX-2 cDNA exchanged with the corresponding region in the wild type 8-LOX sequence. Three useful chimeras were obtained (Fig. 3A): chimera V, switched at Trp 482; chimera VI at His 554; and chimera VII at Leu 610 (Fig. 4). After expression in HeLa cells, chimeras V and VI exhibited purely 15-LOX activity, similar to have the C-terminal regions exchanged at the EcoRV site. Following transfection into HeLa cells, all chimeras were expressed at the same level as the original wild type 8-LOX and 15-LOX-2 (Fig. 2B). Thus, substitution of their N- or C-terminal regions does not affect the enzyme expression probably due to the high sequential homology between the original enzymes.

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Table I

| Name  | Sequence              |
|-------|-----------------------|
| Wild type | CCAGCAGTTAATTCCCAAGTTAACATTGCTTC |
| SS/AT | CCAGCAGTTAATTCCGAAGTTAACATTCT |
| SS/TC | GCGTTAATTCCGCAAGTTAACATTCT |
| SY/CD | GTTAATTCCGCAAGTTAACATTCT |
| YH/DV | AAAGTTACGAAGTTAACATTCT |
| S602C | GTTAATTCCGCAAGTTAACATTCT |
| Y603D | AATTTCCGCAAGTTAACATTCT |
| H604V | TCAGTTAATTCCGAAGTTAACATTCT |

Fig. 5. Analysis of the arachidonic acid metabolites of double amino acid mutants of 8- and 15-lipoxygenase. The amino acids Ser602, Tyr603, and His604 inclusive in 8-LOX were changed to those in 15-LOX-2 by two amino acid mutations at a time. Each mutant enzyme was expressed in vaccinia virus-infected HeLa cells, and the arachidonic acid (AA) metabolites were analyzed by HPLC.

A–D, RP-HPLC profiles of the arachidonic acid metabolites of the S602C, Y603D, and H604V mutants, respectively. The SS/TC, SY/CD, and YH/DV mutants were analyzed by HPLC.
chimera III, and without any other detectable products including 8-HETE (data not shown). The activity of chimera XII was identical to the original 8-LOX (data not shown). This first set of chimeras prepared in situ showed that the positional determinants of the 8-LOX were located between the switching points of chimera VI (switched at His554) and XII (switched at Leu610) that the downstream region beyond Leu610 is not involved.

To obtain a greater number of chimeras switched in the region between His604 and Leu610, the second set of chimeras was prepared using the full-length 8-LOX cDNA in series with only the C-terminal region. From analysis of the various C-terminal chimeras, a cluster of 5 amino acids was identified (SSSYH, that was used to elute the unreacted arachidonic acid.)

A

B

C

Retention time (min)

0 15 30 45

0 8 15

0 15 30

FIG. 7. Analysis of the arachidonic acid metabolites of 15-LOX-2 mutants. Asp602Y and Val603H in 15-LOX-2 were substituted with the corresponding amino acids in 8-LOX, Tyr603 and His604, respectively, both singly and as a pair. The resulting one amino acid mutants, D602Y and V603H, and the double mutant, DV/YH, were expressed in HeLa cells, and their LOX activity was determined using [1-14C]arachidonic acid (AA). The resulting metabolites were analyzed by RP-HPLC with a solvent of MeOH/H2O/acetic acid (80:20:0.01) at a flow rate of 1.1 ml/min. A, D603Y; B, V604H; C, DV/YH; D, wild type of 15-LOX-2. (In C, the small radioactive peak at about 25 min is not 5-HETE; it is an artifactual peak related to the abrupt switch of solvent to 100% MeOH that was used to elute the unreacted arachidonic acid.)

D

E

F

not shown) and the SS/TC mutant showed unchanged regio-specificity from wild type 8-LOX (Fig. 5, A and B). On the other hand, the SY/CD mutant produced mainly 8-HETE and a significant amount of 15-HETE product (Fig. 5C). The YH/DV mutant produced mainly 15-HETE and a small amount of 8-HETE (Fig. 5D). Chiral HPLC analyses showed that both the 8- and 15-HETE produced by the SS/TC mutant and the 15-HETE by the YH/DV mutant were S isomers (Fig. 5, E and F). These data suggested that the positional determinants of the 8-LOX reside within the three amino acids, Ser602, Tyr603, and His604.

To define further the positional determinants, Ser602, Tyr603, and His604 in 8-LOX were substituted with the corresponding amino acids in 15-LOX-2 (Cys, Asp, and Val, respectively) by site-directed mutagenesis using the primers shown in Table I. After expression in HeLa cells, the S602C mutant produced only 8-HETE, indicating that this mutation has no effect on regio-specificity (Fig. 6A). The Y603D mutant still produced mainly 8S-HETE, whereas there is also a significant amount of 15-HETE product (8S-HETE:15-HETE = 11:1) (Fig. 6B). The H604V mutant gave significant amounts of 8- and 15-HETEs (38:62) (Fig. 6C), and furthermore, both products were S-enantiomers (Fig. 6D). Thus, His604 appears to be a key amino acid determinant of positional specificity, and the neighboring Tyr603 seems to support it. Change at both positions is required to switch the 8-LOX to an enzyme with predominantly 15-lipoxygenase activity.

In order to examine whether the Tyr-His pair can also function as an 8-prefering determinant in 15-LOX-2, the corresponding amino acids in 15-LOX-2 were mutated to Tyr or His. Switching of Asp602 to Tyr did not affect the positional specificity of the wild type 15-LOX-2 (Fig. 7A), whereas the Val603→His mutant gave almost equal amounts of 15- and 8-HETEs (38:62) (Fig. 6C), and furthermore, both products were S-enantiomers (Fig. 6D). Thus, His604 appears to be a key amino acid determinant of positional specificity, and the neighboring Tyr603 seems to support it. Change at both positions is required to switch the 8-LOX to an enzyme with predominantly 15-lipoxygenase activity.

DISCUSSION

Initially, the presence of common restriction sites in the mouse 8-LOX and human 15-LOX-2 permitted ready exchange of their N and C termini. Two chimeras containing the C-terminal region of 15-LOX-2 exhibited significant 15S-lipoxygenase activity with no other products formed, suggesting that the C-terminal region of the 8-LOX contains positional determinants that distinguish the 8S and 15S specificities. To narrow this down, a variety of chimeric enzymes was prepared by the random mutagenesis method of Moore and Blakely (17). The two cDNAs to be interchanged are cloned into a single plasmid is transfected into E. coli. Because of the mismatching ends, the bacteria are unable to effect repair by re-ligation. Instead, the repair systems align the two related sequences and reconstitute chimeric sequences with a homologous region of one sequence exchanged with the other. Repair of the linearized construct gives many different recombinants, mainly consisting of circularized plasmids containing a single chimeric species of cDNA. As a refinement of this approach, we placed the full-length 8-LOX cDNA in series with only the C-terminal part of 15-LOX-2, thus effectively focusing the switching onto the C-terminal region. From analysis of the various C-terminal chimeras, a cluster of 5 amino acids was identified (SSSYH,
positions 600–604 of the mouse 8-LOX) that conferred 15-lipoxygenase activity on the murine 8-LOX. Further site-directed mutagenesis showed that His604 is the major positional determinant as its mutation to valine led to formation of almost comparable amounts of 15- and 8-products, both of which were pure S-isomers (Fig. 6D). The nearly complete conversion of positional specificity observed in the YH/DV double mutant indicated that Tyr603 augments the 8 specificity favored by His604 (Fig. 5D). The reverse substitutions in the human 15-LOX-2 produced the opposite effects, with the double mutant having primarily 8-lipoxygenase activity (Fig. 7C). Three serines in this region do not affect the positional specificity.

Our approach to identification of the positional determinants was independent of structural considerations. The results immediately beg the question, where do these critical amino acids lie in the enzyme three-dimensional structure? Although three-dimensional data are not available on the mouse 8-LOX or human 15-LOX-2, the primary structures of all the mammalian lipoxygenases show an unambiguous alignment in the region encompassing Tyr603 and His604 (Fig. 8). Consequently, the positions of the corresponding residues in the x-ray structure of the rabbit reticulocyte 15-LOX should predict the approximate location of these residues (Fig. 9). The reticulocyte 15-LOX residues (Leu589 and Gln590) lie in the catalytic domain, on helix 21, on the opposite side of the iron from the \( \beta \)-barrel domain (5).

The non-heme iron of the catalytic domain is surrounded by five amino acids lying approximately at the corners of an octahedron, with the sixth position open, or in some structures occupied by a water molecule (3). This open side of the iron also corresponds to the area where an inhibitor was bound in the x-ray crystal structure of the reticulocyte 15-LOX enzyme (5). The Leu589/Gln590 in the reticulocyte 15-LOX structure stand back about 15–20 Å from this open face of the iron (Fig. 9). This is somewhat further away from the iron than usually anticipated for substrate binding. Nonetheless, the linear length of arachidonic acid, \( \approx 24 \) Å, is easily sufficient to reach this area. Assuming that the residues Tyr603/His604 of themurine 8-LOX lie in very approximately the same position as the Leu-Gln of the reticulocyte LOX structure, we can infer that they may have direct contact with the substrate and/or they may alter the positions of the helices that help form the putative substrate binding pocket in this region.

When the amino acid alignments are extended to include plant lipoxygenases, there are lower overall similarities to the mouse 8-LOX and 15-LOX-2. Nonetheless, alignments utilizing either the clustal or Jotun-Hein algorithms of the DNASTar program identify Ile746/Ser747 of the soybean l-1 isozyme as the residues corresponding to Tyr603/His604 of the mouse 8-LOX (Fig. 8). These soybean amino acids lie in helix 21 (2) in app-
proximately the equivalent positions identified in the reticu-
lo-LOX structure.

The change from 8S to 15S oxygenation would appear to
tau a major alteration in specificity, yet it can be accounted
for by a relatively simple change in binding of the substrate.
For all lipoygenases, the same stereochemical relationship
pertains between the initial hydrogen abstraction and the re-
action with molecular oxygen, namely that hydrogen removal
from the CH₂ group between two cis double bonds occurs on one
face of the substrate and oxygen reacts on the opposite face
(20). This antarafacial “rule” applies to all lipoygenases tested
(at least 10 different enzymes, including the mouse 8-LOX
(21)), and it should apply to each of the selective oxygenations
of the 8-LOX, the 15-LOX-2, and the chimeras. Fig. 10 illus-
trates how a reorientation of the substrate can allow 8S or 15S
oxygenation through stereochemically equivalent hydrogen ab-
stractions and reactions with molecular oxygen. This change in
binding provides a simple and straightforward mechanism that
can account for the apparently major shift in positional speci-
city. There is ample precedent for this model in the lipoxyge-
nase literature (e.g. Refs. 22 and 23). The appropriateness of
the model is further supported by the fact that the single amino
acid mutants produce both 8S- and 15S-hydroperoxides, and
only these products (Fig. 6, C and D). There were no products at
intermediary positions or products of opposite stereoconfigura-
tion. This all but eliminates the possibility of a frameshift type
of binding change (5) in which the substrate sinks deeper into
the active site pocket as oxygenation specificity changes be-
tween C-15 and C-8.

A potential role of the individual amino acids in substrate
binding is speculative. The basic side chain of His₆⁰⁴ could be a
strong acceptor for the polar carboxyl group of the substrate
fatty acid, whereas Tyr₆⁰³ could support the substrate binding
through hydrogen bonding and/or π-π interaction with the C
terminus. Mutation of Tyr₆⁰³ and His₆⁰⁴ to Asp and Val, re-
spectively, changes the character of the polarity and introduces
a negative charge. This charge reversal might encourage a
major repositioning of the substrate carboxyl group and hence
promote the type of change proposed in Fig. 10. It is notable,
however, that the experiments with single amino acid sub-
tstitutions include the expression of the Tyr-Val (neutral) and
Asp-His (acid-base) mutants; both of these enzymes produced
mixtures of 8S- and 15S-hydroperoxides. It might be concluded,
therefore, that in the context of the similar active sites of the
8-LOX and 15-LOX, substitutions in these key positions alter
the contacts between amino acids on adjacent helices, the con-
sequence being a change in the available space for substrate
binding. This indirect effect may determine the shift in
specificity.

As noted in the Introduction, the molecular mechanisms
determining the positional specificity in lipoygenases have
been approached before mainly using enzymes that exhibit
frameshift differences in specificity. Several 12S-LOX and 15S-
LOX-1 have received the most attention. Sequence compari-
sions led to the identification of residues of differing size that
might influence the available space in the substrate binding
pocket (6). The evidence suggests that making more space is
associated with oxygenation further along the carbon chain
and, assuming a tail-first projection of the substrate into the
active site, produces a change from 15S to 12S specificity.
These previously identified positional determinants do not seem
to be applicable directly to 8-LOX and 15-LOX-2. Either the
previously identified residues are not conserved in 8-LOX
and 15-LOX-2 (this applies to Refs. 6 and 8) or they are con-
served but are present in both enzymes (7).

More recently, an amino acid was identified from modeling
and sequence comparisons that could change a plant 13S-LOX
to 9S-LOX specificity (24). This change is more akin to the
positional specificity issues with the enzymes we have been
studying. In both instances the change in LOX specificity must
involve a turning around of the substrate in the active site. The
residue identified in the plant enzyme is His⁶⁰⁴ of the cucumber
lipid body 13-LOX. (Owing to the longer N-terminal sequence of
plant lipoygenases, this is roughly equivalent to position 420
in the mammalian enzymes.) Due to the weak percent identi-
ties of plant and mammalian lipoygenases in this region,
location of the equivalent residues in the 8-LOX and 15-LOX-2
is problematic; the alignments point to residues within a few
amino acids of the mammalian consensus Gly-Gly-Gly-Gly mo-
tif that is not found in the plant enzymes.

From sequence alignments, there appears to be a strong
possibility that the residues we identified in the mouse 8-LOX
will have an influence on the specificity of other mammalian
lipoygenases. This applies especially to the mammalian
5-LOX enzymes, as they conserve the histidine equivalent to
His⁶⁰⁴ of the mouse 8-LOX (Fig. 8). A potential role of this
5-LOX histidine in positional specificity has been speculated
upon before on the basis of sequence alignment and modeling
studies (10). In addition, the conserved tryptophan just before
the histidine in the 5-LOX primary structures has a related
character to Tyr⁶⁰³ of the 8-LOX. The likeness between the
5-LOX and 8-LOX is all the more compelling because both C-5
and C-8 oxygenations involve a similar mode of substrate bind-
ing (10, 23), differing only in a frameshift along the carbon
chain. Mutation studies of these amino acids of mammalian
5-LOX would be interesting.

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