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ORIGINAL PAPER

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Structural characterization of the catalytic domain of the human 5-lipoxygenase enzyme

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Abstract Leukotrienes are inflammatory mediators involved in several diseases. The enzyme 5-lipoxygenase initiates the synthesis of leukotrienes from arachidonic acid. Little structural information is available regarding 5-lipoxygenase. In this study, we found that the primary structure of the catalytic domain of human 5-lipoxygenase is similar to that of the rabbit 15-lipoxygenase. This similarity allowed the development of a theoretical model of the tertiary structure of the 5-lipoxygenase catalytic domain, using the resolved structure of rabbit 15-lipoxygenase as a template. This model was used in conjunction with primary and secondary structural information to investigate putative nucleotide binding sites, a MAPKAP kinase 2 phosphorylation site, and a Src homology 3 binding site on the 5-lipoxygenase protein, further. Results indicate that the putative nucleotide binding sites are spatially distinct, with one on the β-barrel domain and the other(s) on the catalytic domain. The MAPKAP kinase 2 phosphorylation site involves a four amino acid insertion in mammalian 5-lipoxygenases that significantly alters molecular structure. This target for post-translational modification is both common and unique to 5-lipoxygenases. The Src homology 3 binding site, found in all lipoxygenases, appears to lack the characteristic left-handed type II helix structure of known Src homology 3 binding sites. These results, which highlight the unique nature of the MAPKAP kinase site, underscore the utility of structural information in the analysis of protein function. Electronic supplementary material to this paper can be obtained by using the Springer LINK server located at http://dx.doi.org/10.1007/s00894-002-0076-y.

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Introduction

Leukotrienes are lipid mediators with important roles in normal host defense and inflammatory response [1, 2]. However, leukotriene overproduction contributes to a variety of diseases, including asthma [3, 4], allergic hyperresponsiveness [5], ulcerative colitis [6], psoriasis, [7] rheumatoid arthritis, [8] and ischemic reperfusion injury [9]. Understanding the regulation of overproduction of leukotrienes should help elucidate the pathways of pathogenesis for these diseases.

The enzyme 5-lipoxygenase (5-LO) plays the crucial role of catalyzing the rate-limiting first two steps in the synthesis of leukotrienes from arachidonic acid. As a result, there has been substantial interest in understanding the mode of action of this key protein. It is well established that nucleotides act as cofactors to stimulate 5-LO activity [10] and a recent study has identified potential binding sites for nucleotides on the 5-LO molecule [11]. A Src homology 3 (SH3) binding domain has been identified on 5-LO and shown to affect translocation [12]. Also, recent studies have shown that 5-LO can be phosphorylated by MAPKAP kinase 2, that phosphorylation affects activity, and that 5-LO has a site that resembles MAPKAP kinase 2 sites on other enzymes [13]. Information regarding each of these sites, the nucleotide binding site(s), the SH3 binding domain, and the MAPKAP kinase 2 phosphorylation site, is limited to the primary amino acid sequence.

For all proteins, function depends on structure. The crystal structure of 5-LO has yet to be elucidated. To date, structural information for three lipoxygenases is available, from the Research Collaboratory for Structural Bioinformatics (http://www.rcsb.org/pdb). These include rabbit reticulocyte 15-LO (1LOX [14]), the soybean LO, LOX-1 (1YGE [15] and 2SBL [16]), and the soybean LO, LOX-3 (1BYT [17] and 1LNH [18]). Comparison of

the structure of the rabbit reticulocyte 15-LO with those of the soybean LOX-1 and LOX-3 found that both mammalian and plant LOs are composed of two parts, an amino-terminal β-barrel domain and a carboxy-terminal domain composed predominantly of α-helices. Recent studies have demonstrated that the β-barrel region is a site for calcium binding [19, 20] and mediates membrane association [21]. The carboxy-terminal domain contains the iron that is essential for lipoxygenase activity and thus is the catalytic domain. Previous studies have identified residues in the catalytic domain that are essential for iron binding [22, 23], substrate positioning [14] and nuclear import [24, 25]. Again, information regarding these residues is largely limited to the primary amino acid sequence.

The absence of a 3-dimensional model for 5-LO has prevented insights into structural contributions to enzymatic function. Computer algorithms have been developed to investigate protein structure, although the results of applying these methods to 5-LO have not been published. In this study, we compared the catalytic domain of human 5-LO with those of rabbit reticulocyte 15-LO and soybean LOX-1. Preliminary analysis indicated a higher level of sequence similarity between the catalytic domains of 5-LO and rabbit 15-LO than for the corresponding β-barrel regions. As a result, our analysis focussed on the catalytic domain of 5-LO. The published structure of rabbit 15-LO was used as a template to generate a theoretical model of the catalytic domain of human 5-LO. This structural model was then used to re-evaluate previously described sites, including the nucleotide binding site(s), the MAPKAP kinase 3 phosphorylation site, and the SH3 binding domain.

Materials and methods

Materials

Amino acid sequences were obtained from Swiss-Prot from the ExPASy (Expert Protein Analysis System) proteomics server of the Swiss Institute of Bioinformatics. Primary accession numbers for proteins are: for 5-LOs, human P09917, mouse P48999, rat P12527, hamster P51399; for 15-LOs, rabbit P12530, human P16050; for 12-LOs, bovine P27479, pig P16469, rat Q02759, mouse leukocyte P39654, mouse platelet P18054; for soybeans, LOX1 P08170, LOX3 P09186.

Alignment of protein sequences was performed using CLUSTALW [26], set for accurate method, Gonnet Matrix, gap open penalty at 10, gap extension penalty at 0.1 (pairwise) and 0.2 (multiple). The Gonnet Matrix was used because it gave the highest overall alignment score; alignments obtained using either Pam or Blosum are presented in the Supplemental Material.

Modeling by homology was performed by SWISS-MODEL 3.5 (http://www.expasy.ch/swissmod/SWISS-MODEL.html). Residues 121–673 from the human 5-LO protein sequence were submitted. If submitted without a suggested template, rabbit reticulocyte 15-LO (1LOX.pdb) was chosen by the Alignmaster program. Identical models were generated when 1LOX.pdb was specified as template during sequence submission. Analysis of the generated model for the catalytic domain of 5-LO was examined by the WHATIF program version 19970813–1517 [27, 28].

Results

Primary structure

As noted above, LO proteins are known to be composed of an N-terminal β-barrel domain and a C-terminal catalytic domain [14, 15, 16, 18, 29]. In mammals, the β-barrel region consists of ~110–120 amino acids and there is a random coil of ~10 amino acids between the β-barrel and catalytic domains. For this reason, D121 was arbitrarily chosen as the beginning of the catalytic domain for human 5-LO. This sequence from 5-LO was aligned with the full length sequences of rabbit 15-LO and soy LOX-1 using CLUSTALW. [26] By this approach, D121 from 5-LO is appropriately aligned with G118 from 15-LO, a residue between the last sheet of the β-barrel domain and the first helix of the catalytic domain (Fig. 1a). However, this approach aligns 5-LO D121 with G235 from soy LOX-1, which omits ~85 residues from the start of the LOX-1 catalytic domain. As a second approach to alignment, CLUSTALW was used to align the known catalytic domains of 15-LO and LOX-1 with amino acids 121–673 of 5-LO. This approach aligned 5-LO D121 with soy LOX-1 E163, a residue appropriately just beyond the last sheet of the soy β-barrel region. This alignment resulted in 3 gaps of 19–32 amino acids in the first 100 bases of both 5-LO and 15-LO, indicating substantial differences in the catalytic domains of the mammalian LOXs and the plant LOX (Fig. 1a). CLUSTALW comparison of just the mammalian LOXs (5-LO and 15-LO) gave a much higher sequence similarity, with 40% identity, 63% positive and 0.36% gapped (Fig. 1b). A more thorough CLUSTALW comparison of catalytic domain sequences from human, mouse, rat and hamster 5-LO with 15-LO from rabbit and human as well as 12-LO from human, bovine, pig, rat and mouse platelet, leukocyte and epidermal indicated that most of the gaps were common and unique to the 5-LOs (data not shown). That is, in all of the 5-LOs, there are identical insertions at or near residues 200, 265, 300, 465 and 580 and a gap at or near residue 328.

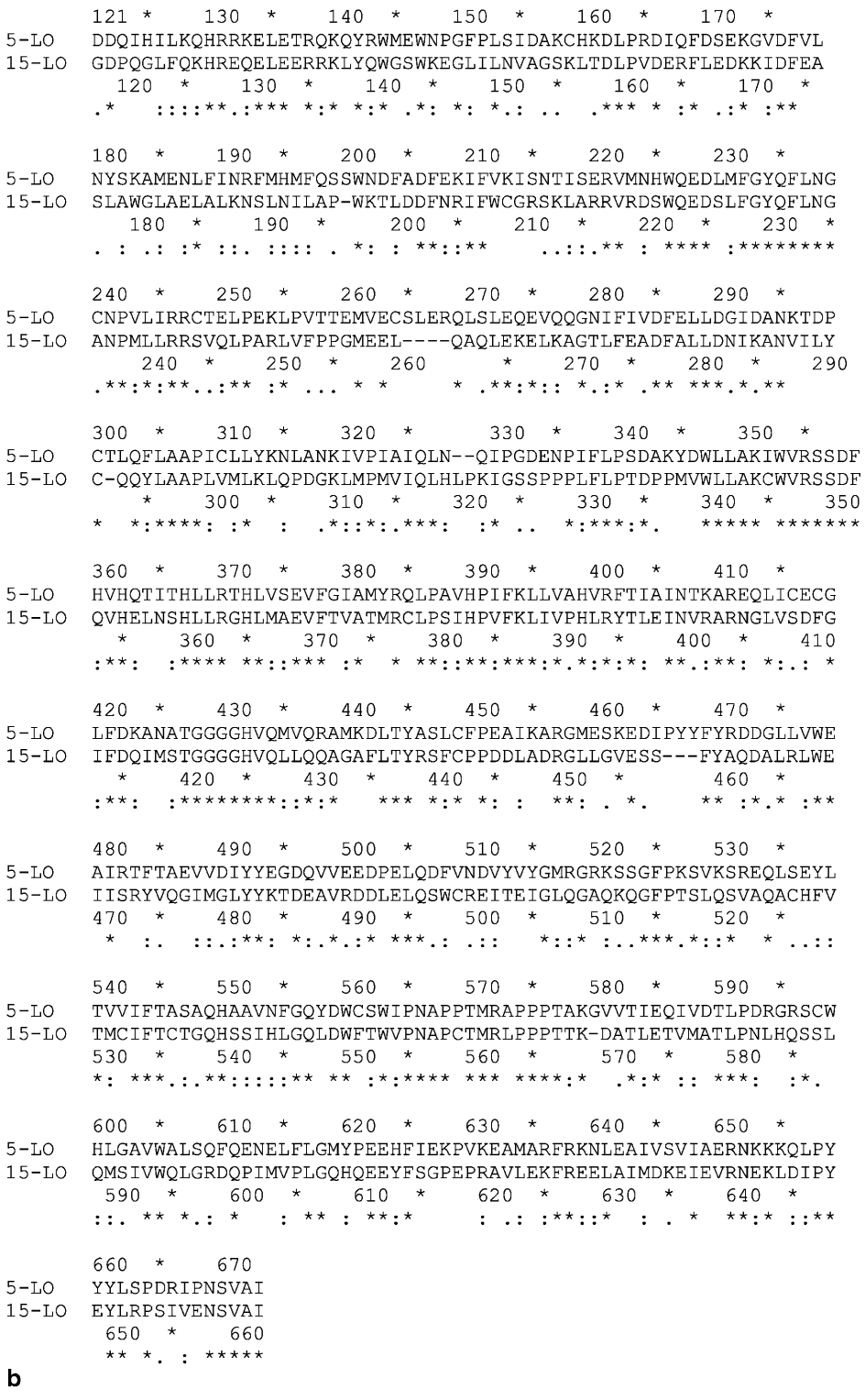
Theoretical model

The high similarity between the catalytic domains of human 5-LO and rabbit 15-LO indicated that the 15-LO might be a good template for modeling the catalytic domain of 5-LO by homology. SwissModel 3.5 [30, 31, 32] was used to generate the model, using the resolved structure for 15-LO (1LOX.pdb, [14] Fig. 2a) as a template. As expected, the modeled catalytic domain of 5-LO (Fig. 2b) superficially resembled the catalytic domain of 15-LO. The predicted protein was analyzed using the WHATIF program version 19970813–1517 [27, 28] and the results are summarized in Table 1. Z-scores presented by WHATIF are the number of standard deviations that the score deviates from the expected value; positive structure z-scores are better than average, while RMS

a

Fig. 1 Alignment of the catalytic domains of human 5-LO, soybean LOX-1 and rabbit reticulocyte 15-LO. Catalytic domains were aligned using CLUSTALW. **a)** Alignment of domains from all three proteins. **b)** Alignment of 5-LO with 15-LO. Upper and lower numbers indicate residue numbers from 5-LO and 15-LO, respectively.

Fig. 1b



b

z-scores should be close to 1. All scores were rated as acceptable by WHATIF except for the structure score for 2nd generation packing quality. The score of –3.79 was interpreted as indicating that the protein is probably threaded correctly, but either poorly refined, or it is just a protein with an unusual (but correct) structure. To evaluate the significance of the problems highlighted by the WHATIF program, the sequence of the catalytic domain of rabbit reticulocyte 15-LO was also submitted for modeling. As shown in Table 1, the scores from the corresponding WhatCheck analysis for modeling of the 15-LO sequence, using its own structure as a template, were only moderately better than those obtained for 5-LO.

The structural elements for 5-LO, as predicted by SwissModel, are summarized in Fig. 3 and Table 2.

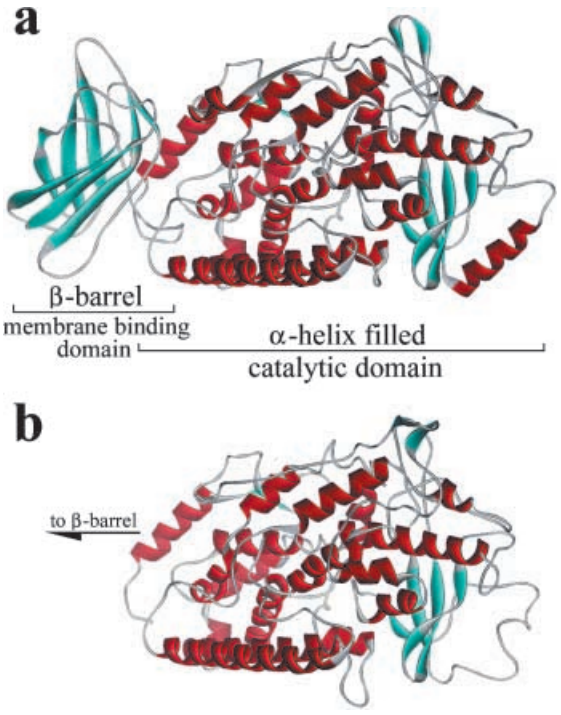


Fig. 2 Comparison of the theoretical model for the catalytic domain of 5-LO with the resolved structure of 15-LO. **a)** Entire 15-LO molecule, from 1LOX.pdb. **b)** Theoretical model of the catalytic domain of human 5-LO.

Table 1 Summary of protein structural analyses for catalytic domains from human 5-LO and 15-LO, as performed by WHATIF. The evaluation of the significance of each score, given in parentheses, was provided by WHATIF.

	5-LO cat dom	15-LO cat dom
Structure Z-scores:		
1st generation packing quality	–1.835 (OK)	–1.383 (OK)
2nd generation packing quality	–3.790 (poor)	–2.674 (OK)
Ramachandran plot appearance	–1.366 (OK)	–0.829 (OK)
chi-1/chi-2 rotamer normality	0.211 (OK)	0.400 (OK)
Backbone conformation	–1.905 (OK)	–1.268 (OK)
RMS Z-scores:		
Bond lengths	0.764 (normal)	2.447 (loose)
Bond angles	1.186 (normal)	1.174 (normal)
Omega angle restraints	1.065 (OK)	0.753 (OK)
Side chain planarity	1.943 (OK)	3.311 (loose)
Improper dihedral distribution	1.387 (OK)	1.625 (loose)
Inside/Outside distribution	1.094 (normal)	1.074 (normal)

Again as expected, helices and sheets in the 5-LO model matched many of the structures of the soybean LOX (Fig. 3a) and most of those of 15-LO (Fig. 3b). Notably, helices of 5 or fewer aa were not recognized as helices by SwissModel. Major structural elements included 16 α-helices. Two helices, labeled 7a and b, were separated by two residues in the model but, in soy LOX3 (1LNH.pdb), form a single helix running through the center of the domain. A cluster of β-sheets, labeled 4–6, was located distal from the β-barrel region (Fig. 4a). When rotated horizontally a quarter turn, several helices

Table 2 Summary of major secondary structural elements from 15-LO (LOX1.pdb) and the theoretical model for the catalytic domain of 5-LO. Elements are named by first residue letter and sequence number followed by last residue letter and number of residues in the structure.

15-LO	structure	5-LO	Structure Number (5-LO)
Q125L14	helix	K128Q14	1
K213S9	helix	T217H9	2
D225N10	helix	D229N10	3
L273D5	sheet	I281D5	4
L300L6	sheet	I309K6	5
L311Q7	sheet	I320P3 X A324Q3	6A, 6B
P337L21	helix	K344L26	7A
H365C14	helix	H372Q14	7B
P384L10	helix	P391V10	8
T396N10	helix	T403A8	9
G424F12	helix	G431M10	10
P443D7	helix	F450A7	11
F459L23	helix	Y470V20	12
D486R5	helix	D496E5	13
L493T11	helix	P503Y11	14
V522T14	helix	R532A15	15
G538W13	helix	A548N7	16
L573T7	helix	E585T6	17
L583Q13	helix	R594Q16	18
P617K27	helix	K628K26	19

(3, 7a, 7b, 11, 12, 17, 19) were found to run parallel to one another, projecting toward the β-barrel domain (Fig. 4b). When further rotated downward approximately 45°, several other helices (1, 9, 14, 16, 18) were found to run parallel to one another (Fig. 4c).

A cluster of four antiparallel β-sheets was also observed (Fig. 5). This cluster was found on both mammalian and plant LOs. The overall structure of this cluster on 5-LO was essentially identical to that on 15-LO (Fig. 5a, b). However, significant helical structure was evident on an intervening loop of the plant LO, LOX 1 (Fig. 5c). The cluster was distal to the β-barrel domain (Fig. 2).

Potential nucleotide binding sites

Affinity labeling has been used to identify potential binding sites for nucleotides on 5-LO [11]. In those studies, one probe was found to bind at a mole ratio of 1.4, whereas a second probe bound at 0.94, suggesting 1 or 2 binding sites. Two fragments of 5-LO (K73-K83 and F193-K209) were frequently found to bind the probes; W75 and W201 were modified, suggesting direct interactions. A third, less common, 5-LO fragment, N315-Q326, was also found. A common motif for nucleotide binding is the P-loop, although not all nucleotide-binding proteins contain this P-loop [33]. None of the fragments fits the consensus P-loop sequence ([AG]-x(4)-G-K-[ST]). Similarly, they were not found to be similar to any of the other nucleotide-, ATP-, or GTP-binding sequence signatures (motifs listed at http://hits.isb-sib.

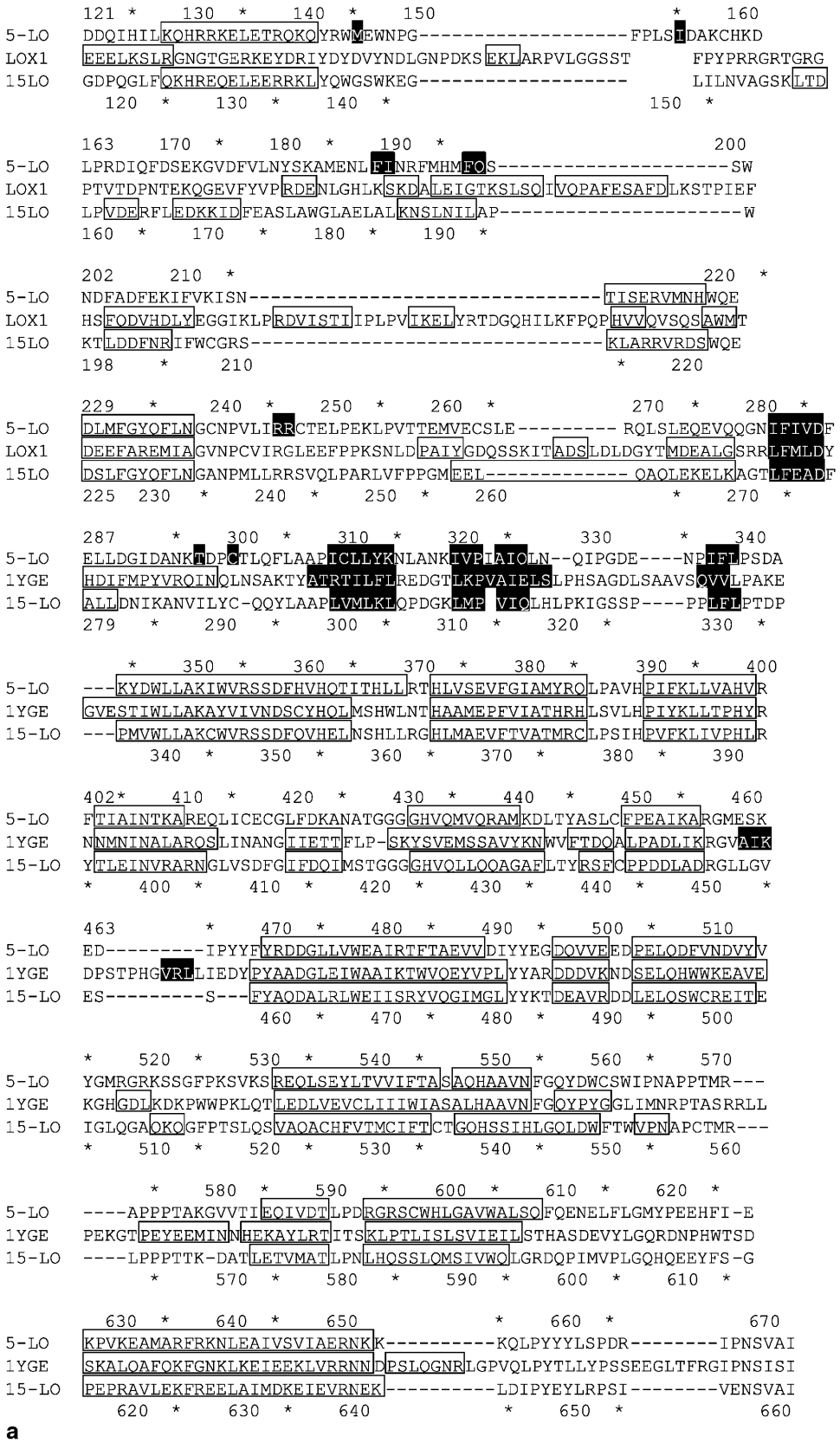
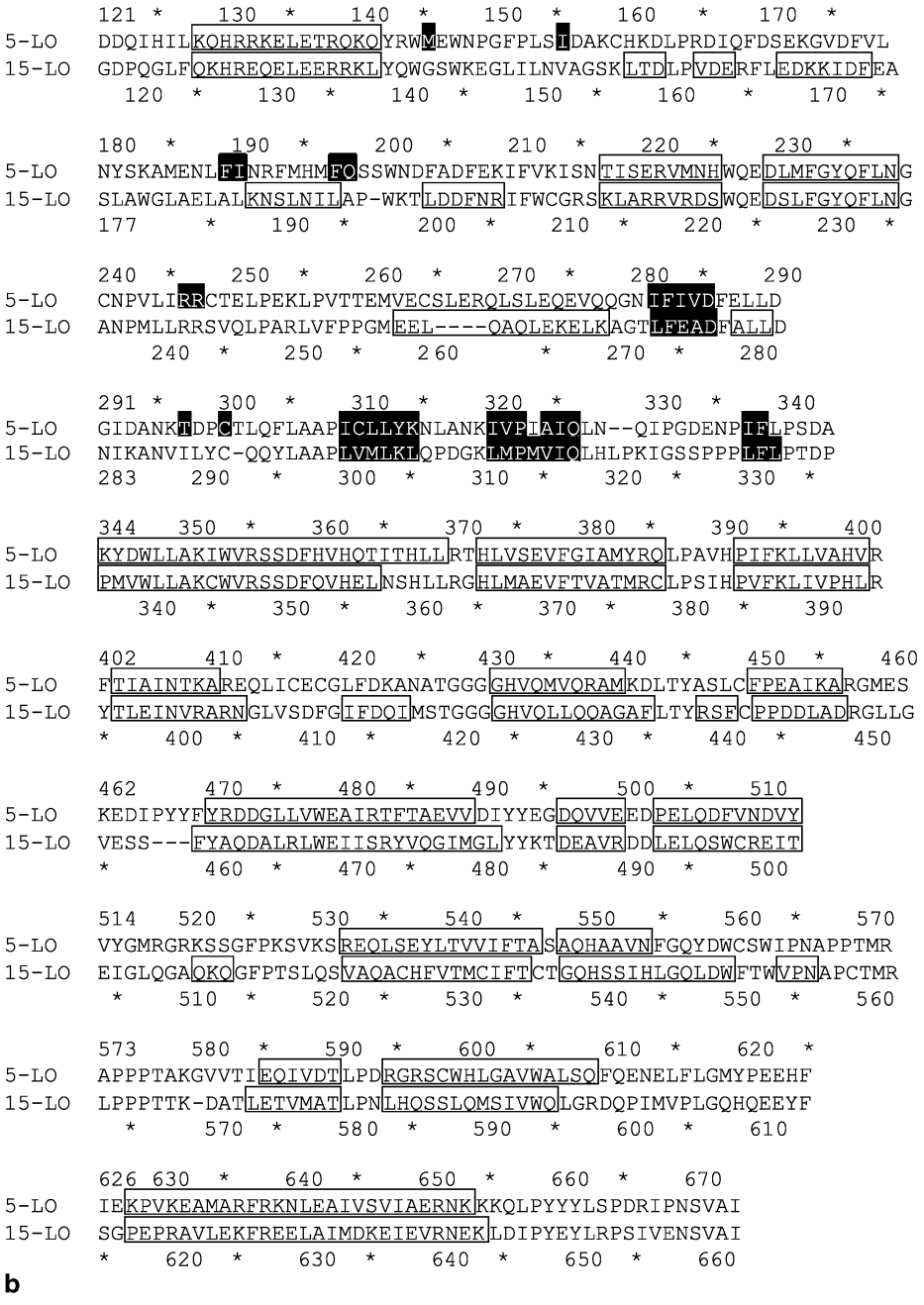


Fig. 3 Comparison of the secondary structural elements predicted by the theoretical model for 5-LO with those from soy LOX1 and rabbit 15-LO. **a)** 5-LO, LOX1 and 15-LO. **b)** 5-LO vs. 15-LO. *Boxed areas* indicate α-helices; *shaded areas* indicate β-sheet structure.

Fig. 3b
Legend see page 107



ch/). However, the third fragment was found to be similar to the guanine-nucleotide dissociation stimulator motif (PS00741), which has a consensus sequence of [LM]-x(2)-[LIVMFYW]-L-x(2)-P-[LIVM]-x(2)-[LIVM]-x-[KRS]-x(2)-L-x-[LIVM]-x-[DEQ]-[LIVM]-x(3)-[ST].

Without information on tertiary structure, it was unclear whether these fragments cooperated in nucleotide binding or represented distinct sites. As indicated in Table 3, the majority of the first fragment, and in particular W75, aligned with the random coil region between two β-sheets on the β-barrel domain. The W75 residue was found to be unique to the 5-LOs but replaced with R on rat 5-LO. Again, in the second fragment, the aromatic residue W201 was found on a random coil between two structural elements, α-helices in this case. This residue was found to be conserved across all mammalian LOXs. The third fragment was completely conserved in all 5-LOs but was poorly conserved across other LOXs. In the predicted structure, the second and third fragments appeared to be distinct from one another, as well as distant from the first fragment (Fig. 6a). The second fragment, F193-K209, lost the helical structure found in 15-LO. Instead, it was a random coil between helices 2, 16 and 18. The third fragment spanned a loop between two of the β-sheets in a cluster of sheets that were spatially distant from the first two fragments.

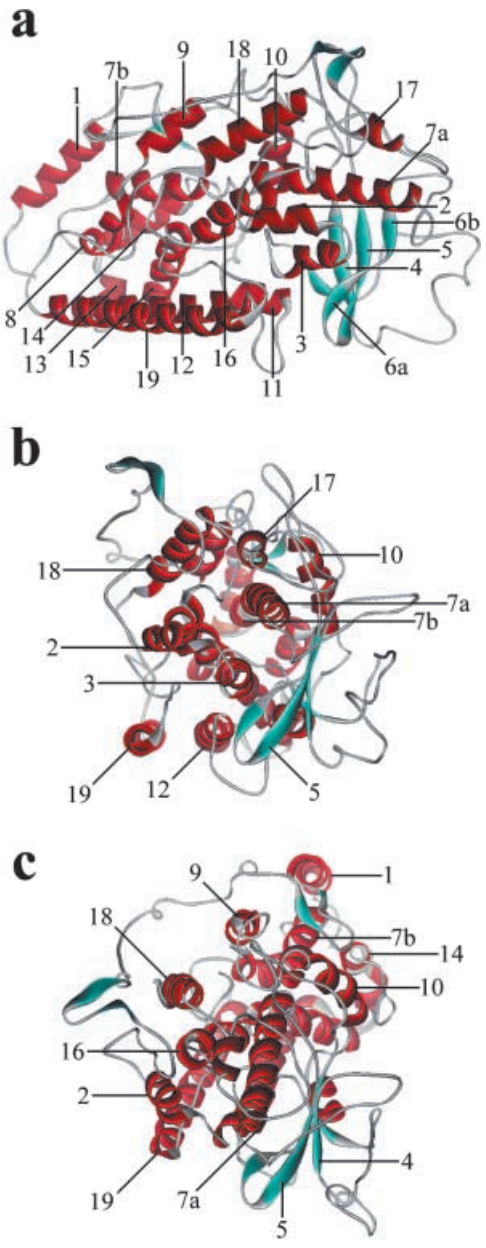


Fig. 4 Organization of the catalytic domain of human 5-LO. **a)** Localization of major structural elements, numbered sequentially as in Table 2. **b)** Visualization of structural elements running parallel to the central 7a and 7b helices, viewed on end. **c)** Helices running parallel to one another and approximately 45° from the central 7a and 7b helices.

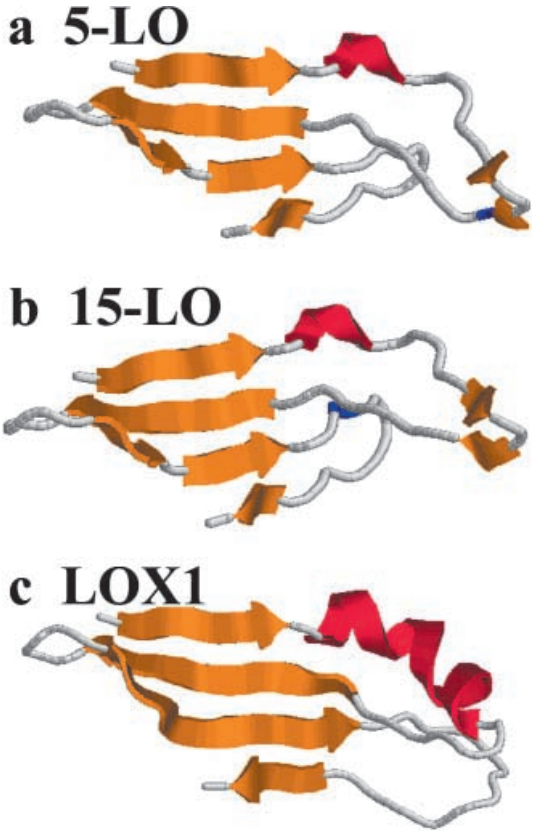


Fig. 5 Comparison of β-sheet portions of catalytic domains of **a)** 5-LO, **b)** 15-LO and **c)** LOX1. Sheets run sequentially and antiparallel in all LOX proteins. Loops between sheets are very similar in the mammalian LOXs.

The MAPKAP kinase 2 phosphorylation site

The MAPKAP kinase 2 phosphorylation motif of 5-LO was previously identified based on similarity to sequences in two other proteins, heat shock protein 27 and lymphocyte-specific protein 1 [13]. The proteins share the motif LxRxxS, with phosphorylation on the terminal S. However, this motif, conserved across the 5-LOs, was not examined in other LOs. Using CLUSTALW, this motif was found to be common as well as unique to all 5-LOs, resulting from an insertion of four amino acids into the highly conserved LO sequence (Table 4). This insertion occurs in the middle of a sequence that forms an α-helix in rabbit 15-LO. The predicted structure in 5-LO is not an α-helix, as in 15-LO. Instead, the key hydrophobic L266 residue projects inward and the putative phosphorylation target, S271, is presented outward (Fig. 6b). The basic R268 is positioned between two acidic residues, E263 and E275, a positioning that might be expected to stabilize this structure and maintain S271 in an outward orientation.

The SH3 binding domain

The putative SH3 binding domain identified [12] and characterized further [34] by Fitzpatrick and colleagues was examined for structural characteristics. Essential primary structural features of SH3 binding domains includes a PXXP motif, with each P preceded by an aliphatic residue (A, I, L or V), and an additional non-P, typically R, forming part of the binding core that contacts the SH3 domain [35, 36]. All of these features were found to surround the PNAP core of 5-LO (Table 5). The primary sequence was extremely well conserved in the 5-LOs. The key elements (core PNAP

Table 3 Alignment of mammalian LOX sequences corresponding to the putative nucleotide binding fragments from 5-LO. Alignments performed by CLUSTALW. Residues involved in an α-helix in r15-LO are boxed; residues forming a β-sheet are shaded. The specific fragments sequenced as nucleotide binding in human 5-LO are highlighted in bold.

```
Bov 12LO          LLFVKLRKRHLLSDDAWFCNWISVQG
Pig 12LO          LLFVKLRKRHLLQDDAWFCNWISVQG
Rat 12LO          LLFVRVQKWHYLTDDAWFCNWISVKG
Mou 12LO leuk     LLFVRVQKWHYLKEDAWFCNWISVKG
Mou 12LO plat     LQFVKLHKQHTVVDDAWFCNLITVQG
Hum 12LO          LQFVRLRKHHWLVDDAWFCDRITVQG
Hum 15LO          LLFVKLRKRHLLKDDAWFCNWISVQG
Rab 15LO          LLFVRLRKKHFLKEDAWFCNWISVQA
Ham 5-LO          IQLVRIEKRKYWLHDDWYLKYITLKT
Rat 5-LO          IYLVKIEKRKYRLHDDWYLKYITLKT
Mou 5-LO          IYLVKIEKRKYWLHDDWYLKYITLKT
Hum 5-LO (64)     IQLVRIEKRKYWLNDDWYLKYITLKT
                  : :*::.* :   .* *: . *:::

Bov 12LO          KDSLNILT-CWKSLDDFNR
Pig 12LO          KDSLNVLM-SWNSLDSFNR
Rat 12LO          NFPINTVT-CWKSLDDFNC
Mou 12LO leuk     NFPKNTVT-CWKSLDDFNY
Mou 12LO plat     KRVYTLLR-SWNHLEDFDQ
Hum 12LO          KRVYTLLS-SWNCLEDFDQ
Hum 15LO          KDSLNVLT-CWKDLDDFNR
Rab 15LO (188)    KNSLNILA-PWKTLDDFNR
Ham 5-LO          NRFMHMFQSSWNDFADFEK
Rat 5-LO          NRFMHMFQSSWHDFADFEK
Mou 5-LO          NRFMHMFQSSWHDFADFEK
Hum 5-LO (191)    NRFMHMFQSSWNDFADFEK
                  :     .   *: : .*:

Bov 12LO          QYVAAPLVMLKLQPDGKLLPMAIQLQ
Pig 12LO          QYLAVPLVMLKLQPDGKLLPMVIQLQ
Rat 12LO          QYLAAPLVMLKLMPDGQLLPIAIQLE
Mou 12LO leuk     QYLAAPLVMLKLQPDGQLLPIAIQLE
Mou 12LO plat     QYLAAPLVMLRMDPGGKLLPMAIQIQ
Hum 12LO          QYLAAPLVMLKMEPNGKLQPMVIQIQ
Hum 15LO          QHLAAPLVMLKLQPDGKLLPMVIQLQ
Rab 15LO (294)    QYLAAPLVMLKLQPDGKLMPMVIQLH
Ham 5-LO          QFLAAPICLLYKNLANKIVPIAIQLN
Rat 5-LO          QFLAAPICLLYKNLANKIVPIAIQLN
Mou 5-LO          QFLAAPICLLYKNLANKIVPIAIQLN
Hum 5-LO (303)    QFLAAPICLLYKNLANKIVPIAIQLN
                  *.:*.*: :*     .:: *:.**:.
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Table 4 The 5-LO MAPKAP phosphorylation site: comparison with primary sequences from various mammalian lipoxygenases. Alignments performed by CLUSTALW. Residues forming an α-helix in r15-LO are boxed; residues forming a β-sheet are shaded.

```
Bov 12LO          GMGELQAE----LEKELQQGTLFEADFS
Pig 12LO          GMEELQAQ----LEKELQGGTLFEADFS
Rat 12LO          GMEKLQAQ----LNKELQKGTLFEADFF
Mou 12LO leuk     GMEKLQAQ----LDEELKKGTLFEADFF
Mou 12LO plat     GMEELQAQ----LEKELKNGSLFEADFI
Hum 12LO          GMEELQAQ----LEKELQNGSLFEADFI
Hum 15LO          GMEELQAQ----LEKELEGGTLFEADFS
Rab 15LO (257)    GMEELQAQ----LEKELKAGTLFEADFA
Ham 5-LO          EMVECSLERHLSLEQEVQEGNIFIVDYE
Rat 5-LO          EMVECSLERQLSLEQEVQEGNIFIVDYE
Mou 5-LO          EMVECSLERQLSLEQEVQEGNIFIVDYE
Hum 5-LO (260)    EMVECSLERQLSLEQEVQQGNIFIVDFE
                   * : . :    *::*:: *.:* .*:
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Table 5 The 5-LO SH3 binding domain: comparison with primary sequences from several mammalian lipoxygenases. Alignments performed by CLUSTALW. Residues involved in helical structures on r15-LO are boxed.

```
Bov 12LO          WYSWVPNAPCTMRLPPPTTK
Pig 12LO          WYTWVPNAPCTMRLPPPTTK
Rat 12LO          WFYWVPNAPCTMRLPPPTTK
Mou 12LO leuk     WFYWVPNAPCTMRLPPPKTK
Mou 12LO plat     WYGWVPNAPCTMRMPPPTSK
Hum 12LO          WYAWVPNAPCTMRMPPPTTK
Hum 15LO          WYSWVPNAPCTMRLPPPTTK
Rab 15LO (552)    WFTWVPNAPCTMRLPPPTTK
Ham 5-LO          WCSWIPNAPPTMRAPPATAK
Rat 5-LO          WCSWIPNAPPTMRAPPPTAK
Mou 5-LO          WCSWIPNAPPTMRAPPPTAK
Hum 5-LO (560)    WCSWIPNAPPTMRAPPPTAK
                  *  *:**** *** **..:*
Consensus         W--W-PNAP-TMR-PP---K
```

sequence, aliphatic residues preceding prolines, neighboring R) were present in mammalian 12-LOs and 15-LOs, as well. A single P (569) was consistently replaced with C in those LOs. Secondary structure characteristics of SH3 binding domains involve the formation of a left-handed poly-P type II helix [37, 38]. The resolved structure of 15-LO did not contain this structure (Fig. 6c). The predicted structure of 5LO, similarly did not contain this structure (Fig. 6d). The affinity of an SH3 binding peptide for its SH3 domain can be enhanced by contextual elements [39]. The predicted structure of 5LO placed the PNAP core sequence close to helices 7a, 17 and 18 (Fig. 6d).

Discussion

In proteins, structure is critical to function. This study presents the first model of the structure of the catalytic domain of human 5-LO. Confidence in the general strength of the model is supported by overall similarity in primary and secondary structural characteristics to rabbit 15-LO, regional similarity to soybean LOX1, and a generally strong WHATIF analysis of the theoretical model. The development of a model for 5-LO represents a significant step toward understanding functional aspects of the 5-LO protein. Without a model, it has been impossible to evaluate the significance of recent findings, such as the putative nucleotide binding fragments.

The development of a theoretical model has helped to highlight what appears to be a “lipoxygenase” tertiary motif. Previous studies have noted that both plant and animal LOs have the two domains, the β-barrel domain and the catalytic domain. Also, two LO motifs in primary structure, both for iron binding, have been described. These are LOX iron-binding signature 1 (PS00711): H-[EQ]-x(3)-H-x-[LM]-[NEQHRC]-[GSTA]-H-[LIVMSTAC](2)-x-E; and LOX iron-binding signature 2 (PS00081): [LIVMAC]-H-P-[LIVM]-x-[KRQ]-[LIVMF](2)-x-[AP]-H. Together, these features have led to the recognition of a lipoxygenase family of proteins (pfam

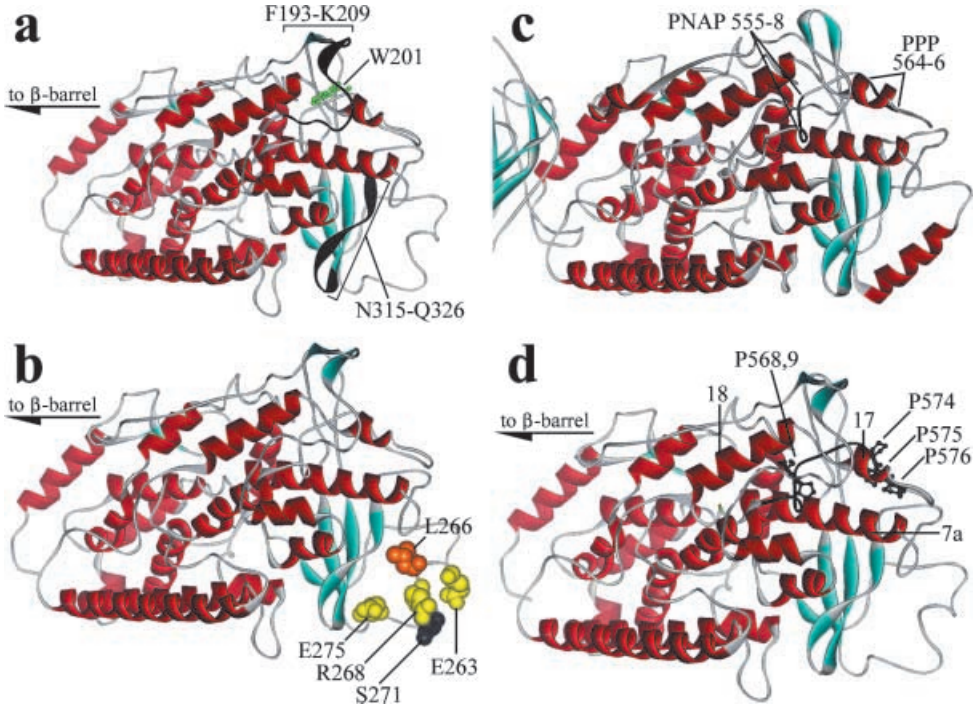

Fig. 6 Localization of functional elements of the catalytic domain. **a)** The putative nucleotide binding fragments on the catalytic domain of 5-LO. **b)** The MAPKAP 2 phosphorylation site. **c)** Region, on rabbit 15-LO, corresponding to the SH3-binding domain of 5-LO. **d)** SH3-binding domain on the catalytic domain of 5-LO.

PF00305 or INTERPRO entry IPR000907). The description of this family may be extended to incorporate tertiary structure. The tertiary motif, as outlined in Results, involves 5 α-helices running parallel to a central helix or pair of continuing sub-helices (in this study, labeled 7a and b). These helices are essentially perpendicular to the β-barrel domain. Both of the iron-binding signatures are located on the central helix or helix pair. In addition to this group of helices, there is a second group of helices which runs parallel to one another and at an angle of approximately 45° to the first group. Finally, there is also a small cluster of β-sheets on the opposite end of the catalytic domain from the β-barrel domain. These structures are common to both the plant and animal LOs and thus appear to provide a general overall structure.

The analysis presented provides some insight regarding potential sites for nucleotide binding. Each fragment is highly conserved across the different 5-LOs and shows low homology with other mammalian LOXs. Also, the three fragments appear to be spatially separated, indicating that they do not cooperate in nucleotide binding. A common motif for nucleotide binding is the P-loop, although not all nucleotide-binding proteins contain this P-loop [33]. None of the fragments fits the consensus P-loop sequence ([AG]-x(4)-G-K-[ST]). However, the third fragment is similar to the guanine-nucleotide dissociation stimulator motif (PS00741). Further work will need to determine if any or all of these fragments actually bind nucleotides *in vivo* and how nucleotide binding might be important for 5-LO function.

The finding that the RQLS sequence is unique to the 5-LOs stands in stark contrast to the high degree of similarity between the LOs. This indicates that the function of this site will be unique to the 5-LOs. The predicted structure through this region of the 5-LOs, resulting in the presentation of Ser271 to the cytoplasm, suggests that this residue is highly accessible for phosphorylation. These details strongly suggest that this site will be important in the function of the 5-LOs.

Regarding the SH3 binding domain, the high degree of sequence similarity across the different mammalian LOs suggests that this domain does not have a function that is unique to the 5-LOs. On the other hand, the single change from 569Pro in the 5-LOs to Cys in other mammalian LOXs may be a critical difference. Also, the 15-LO structure and the 5-LO model indicate that this domain does not have the characteristic left-handed poly-Pro type II helix secondary structure. Finally, neighboring structures may reduce accessibility. The studies of Fitzpatrick and colleagues [12, 34] have demonstrated that the SH3 binding domain of 5-LO can interact with proteins containing SH3 domains. It is possible that the Pro for Cys replacement, found in the 5-LOs, serves to position the SH3 binding domain closer to the surface of the molecule. Alternatively, other events, such as cofactor binding, might rearrange the neighboring structures and in this way make the binding domain more accessible.

In summary, we have developed a theoretical model for the structure of the catalytic domain. This model allows inspection of specific residues and domains in the context of the three dimensional molecule. Such an examination has indicated that the MAPKAP kinase phosphorylation site of the 5-LOs represents a site that is unique to the 5-LOs and appears to be structurally consistent with functional significance. In contrast, the putative nucleotide binding fragments appear to be distinct sites which lack structural similarity to other nucleotide

binding domains and thus require further examination. The theoretical model should be similarly useful in other analyses of 5-LO structure and function.

Supplementary material

1 Sequence alignments obtained using different matrices with CLUSTALW is available in the supplementary material.
2 The source of most images is available as a pdf-file in the supplementary material.

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