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Recognition of polyunsaturated acyl chains by enzymes acting on membrane lipids ☆

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ABSTRACT

Polyunsaturated acyl chains play an important role in human biology. These lipids cannot be synthesized *de novo* and they are selectively distributed to certain organs and are found predominantly only in certain lipid classes. Their selective distribution is a consequence of the specificity of the binding of these lipids by certain proteins. Lipoxygenases are a group of well studied enzymes that specifically oxidize polyunsaturated fatty acids. We propose that certain features of the interaction of lipoxygenases with polyunsaturated acyl chains are also found in other unrelated proteins that act on lipids with these moieties. The features common to several of the enzymes that specifically interact with polyunsaturated acyl chains include the fact that the polyunsaturated chain is drawn out of the membrane to bind to a hydrophobic channel within the protein and that a similar pattern of required amino acids residues comprises part of the binding site for the polyunsaturated chain. This article is part of a Special Issue entitled: Protein Folding in Membranes.

1. Introduction

1.1. Roles of lipid headgroups and acyl chains in the interactions of proteins with membranes

The interaction of proteins with membranes will often involve both non-specific interactions as well as binding to specific groups. Examples of non-specific interactions can include electrostatic, hydrophobic, van der Waals and H-bonding interactions. In contrast, specific interactions depend on a combination of several of these types of non-covalent interactions localized within a folded domain, resulting in a higher affinity binding. Examples have been given indicating that the binding of the coagulation factor X [1] or the binding of annexin V with Ca^{2+} [2] is dependent only on non-specific electrostatic interactions with the membrane. In contrast, other proteins have folded domains that bind to specific lipid molecules in a membrane. There are many examples of specific interactions with lipids among proteins involved in signal transduction [3].

The role of lipid acyl chains in binding to proteins, is generally non-specific. Unlike the headgroup, the acyl chains have a limited degree of specific structural features and this region of the membrane has a high degree of disorder in ordinary liquid crystalline bilayers. Furthermore, the principal types of non-covalent binding forces that can occur with these hydrocarbon chains are limited, involving principally hydrophobic and van der Waals interactions. In addition to direct binding interactions with the protein, the acyl chains can also contribute to determining the degree of non-specific interactions through their role in determining the membrane lateral pressure profile that will contribute to the partitioning of molecules into the membrane [4]. The acyl chains will contribute to determining the thickness of the bilayer that has to match the thickness of protein segments that traverse the membrane as predicted by the mattress model [5]. Nevertheless there are examples indicating specificity of binding to particular acyl chains. For example, covalent modification of proteins by acylation on an amino terminal Gly occurs always with the myristic acid moiety and not any other acyl chain, while covalent modification by acylation of the sulphydryl group of proteins is with palmitoyl moieties. The transfer of an acyl chain to LPS in *E. coli* by PagP is also specific for a chain length of 16 carbon atoms [6]. The present review will focus on the recognition of polyunsaturated acyl chains (PUFA) in the interaction of proteins and lipids.

1.2. Importance of polyunsaturated acyl chains (PUFA) in human nutrition

PUFA are essential components in the human diet as they cannot be synthesized *de novo* from saturated or monounsaturated fatty acids. These lipids have important roles in human health [7,8]. Docosahexanoic acyl chains (22:6) are particularly abundant in the brain where they appear to have an important role in brain function and development [9]. Arachidonoyl chains (20:4) also have an important role in providing free arachidonic acid that is a precursor for many potent prostanoid ligands [10]. The rate of production of these active signaling metabolites of arachidonic acid is determined

Abbreviations: PUFA, polyunsaturated fatty acids; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PI4P, phosphatidylinositol-4-phosphate; PIP_2, phosphatidylinositol-(4,5)-bisphosphate; DGK, diacylglycerol kinase; PI4P5K, phosphatidylinositol-4-phosphate-5-kinase

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by the availability of this PUFA as a free fatty acid, as a result of phospholipid hydrolysis catalyzed by phospholipase A2.

1.3. Non-uniform distribution of PUFA in different lipid classes

All fatty acids have membrane-disrupting, detergent-like properties in their anionic form at neutral pH. Hence, free PUFAs are found in biological specimens only at very low concentrations. Most PUFAs are found as acyl chain components that are covalently attached to phospholipids. There is selective distribution of PUFAs among different lipid classes and among different organs [11]. In particular, arachidonoyl chains are particularly enriched in phosphatidylinositols (PI) and in phosphatidylethanolamines (PE) [11,12]. In comparison, docosahexanoyl moieties are enriched in phosphatidylserines (PS) as well as PE [11]. The fraction of acyl chains that are docosahexanoic is greater in brain and heart, compared with other tissue, while the arachidonoyl moiety is predominant in the PI of most tissues [11].

1.4. Proteins that interact selectively with PUFA

The principal reason for the concentration of particular PUFA in certain lipid classes is the specificity of the interaction of certain enzymes with membrane lipids based on their acyl chain composition. There is a whole range of degrees of specificity in the interaction between proteins and lipids with different acyl chains, from cases in which the protein will interact with only a single molecular species, to cases in which there is a varying degree of preference of one acyl chain over another. In this review we will refer to proteins that do not distinguish between one acyl chain and another as "non-specific" and those that show varying degrees of preference for one acyl chain over another as "specific". The degree of specificity varies both among different proteins as well as being dependent on how different the acyl chain structures are that are being compared. Phospholipids that are rich in PUFA have a tendency to be segregated in membranes based on their exclusion from cholesterol-rich regions [13]. However, in addition, there are mechanisms to determine which lipid class these acyl chains are incorporated into. It is this latter aspect, based on the acyl chain specificity of protein–lipid interactions, that this review is based on.

2. Lipoxygenases: example of a well studied protein family with a binding site for PUFA

Our review focuses mainly on the interaction of proteins with phospholipid components of membranes that are directly responsible for the unequal distribution of PUFA among different lipid classes. Nevertheless, there are also examples of specific interaction of some proteins including certain isoforms of fatty acid binding proteins [14] and of lipoxygenases with PUFA as free fatty acids.

We will discuss lipoxygenases in more detail because these enzymes have been extensively studied for a number of years and the crystal structure of several lipoxygenases have been determined [15]. Lipoxygenases are non-heme iron-containing dioxygenases that catalyze the stereo-specific peroxidation of PUFA. The conventional nomenclature classifies animal lipoxygenases with respect to their positional specificity of arachidonic acid oxygenation as 5-LOXs, 8-LOXs, 11-LOXs, 12-LOXs or 15-LOXs. Proteins with sequence homology to mammalian lipoxygenase are found in all animal and plant species as well as most prokaryotes, but not in archea [15]. However, the function of lipoxygenase in prokaryotes is not well established. In addition, PUFA other than arachidonic acid, are found to be the best substrates for many non-mammalian forms of lipoxygenase. This presents a complication when applying the same nomenclature to non-mammalian lipoxygenase as that given above.

Most lipoxygenase enzymes have a single polypeptide chain that folds into two distinct domains, a small N-terminal β-barrel domain and a larger domain that is mostly helical and contains the catalytic site of the enzyme. The N-terminal domain composed of anti-parallel β-strands has been suggested to be important for membrane binding on the basis of mutagenesis and limited proteolysis experiments [16,17]. It remains possible, however, that the N-terminal segment affects the folding of the catalytic domain. The larger helical catalytic domain contains the substrate-binding pocket for binding PUFA and the catalytic non-heme iron. The non-heme iron of all lipoxygenase isoforms is octahedrically coordinated to five amino acid sidechains and a hydroxide ligand. The substrate binding pocket is a hydrophobic cavity that allows transfer of a PUFA from a membrane to this site on the protein situated near the membrane interface. Hydrophobic amino acid residues line the binding site but no direct structural information is available to specify the molecular details of the interaction of PUFA with this site. However, there are some suggested differences in the shape of this hydrophobic binding site among different forms of lipoxygenase enzymes. In addition, in some lipoxygenase enzymes this site is opened, while in others it is blocked by other segments of the protein that are opened by a conformational change [15].

The specificity of lipoxygenase enzymes for different fatty acids and for different positions on the fatty acid must be determined by the location of the susceptible bonds of the substrate in relation to the components at the enzyme active site required for catalysis. Mammalian forms of lipoxygenase preferentially oxidize arachidonic acid, while these enzymes from plants are generally specific for linoleic acid. There are also differences in which of the double bonds are oxidized. This specificity exists because of the targeted binding of the fatty acid in the hydrophobic pocket of the binding site, despite the fact that PUFAs are extremely flexible molecules. The principal binding forces between lipoxygenase and these substrates are hydrophobic, π-electron and ionic interactions. The entry of the substrate into the binding site is generally considered to proceed with the hydrophobic terminal methyl end of the fatty acid entering first. However, there maybe some cases in which the substrate enters with the polar carboxyl group first that can then bind to a cationic site on the enzyme. There is some evidence from X-ray diffraction studies for such an entry [18]. The difference between "head-first" and "tail-first" orientation of the substrate may depend on the nature of the substrate and on the reaction conditions [19–21].

5-Lipoxygenase is the enzyme that catalyzes the formation of both proinflammatory leukotrienes and anti-inflammatory lipoxins from arachidonic acid. Ca^{2+} is required for the membrane binding and enzymatic activity of human 5-lipoxygenase [22] and for nuclear translocation [23]. Ca^{2+} also enhances the membrane binding of some other forms of lipoxygenase. Human 5-lipoxygenase is sensitive to inactivation as a consequence of the oxidation of the catalytic iron by molecular oxygen [24]. This inactivation process was inhibited by mutation of the wild type enzyme, allowing for a detailed structural analysis of the enzyme by crystallography [25]. The crystal structure was solved to a 2.4 Å resolution that showed that although the active site residues were highly conserved with those of other lipoxygenase enzymes, there were distinct differences in the structure, suggesting that development of specific inhibitors for 5-lipoxygenase should be possible [25].

3. Enrichment of phosphatidylinositol-cycle intermediates with arachidonoyl chains: role of specific protein interactions

Phosphatidylinositol is the only lipid class that has a specific arachidonoyl-containing molecular species in every organ of the rat in which it has been investigated [11]. The predominant molecular species is 1-stearoyl-2-arachidonoyl phosphatidylinositol. Phosphatidylinositol is a component of the "PI-cycle" in which PI is continually synthesized and broken down. The PI-cycle is shown in Fig. 1. However, besides phosphatidylinositol, other lipid intermediates of the PI-cycle, diacylglycerol and phosphatidic acid are not enriched in particular acyl chains. This can be explained because there is only one predominant source of

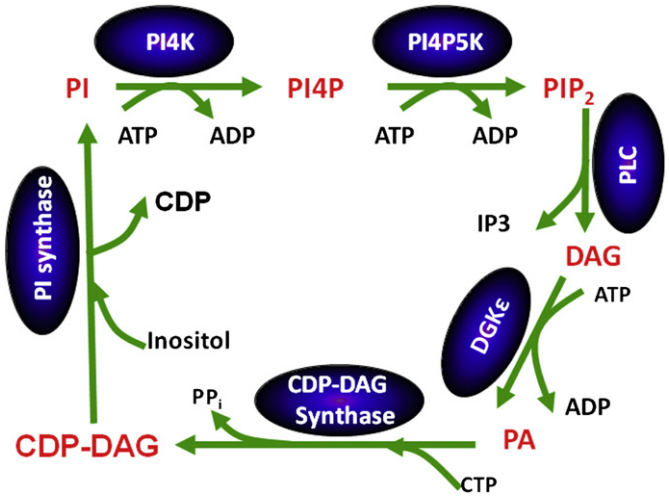

Fig. 1. Scheme for the phosphatidylinositol cycle. Enzymes catalyzing each of the individual steps are shown with their abbreviated names in a blue oval. Intermediates in the cycle are shown in red. PI4K, phosphatidylinositol-4-kinase; PI4P, phosphatidylinositol-4-phosphate; PLC, phospholipase C; DAG, diacyl glycerol; PA, phosphatidic acid; CDP-DAG, cytidine diphosphate diglyceride; other abbreviations are defined in the footnote for abbreviations.

phosphatidylinositol, through the coupling of CDP-diacylglycerol with inositol, while there are several metabolic pathways that give rise to diacylglycerol and phosphatidic acid [26]. Therefore if the PI-cycle is active, there must be a mechanism to select only the 1-stearoyl-2-arachidonoyl forms of diacylglycerol or of phosphatidic acid to participate in this cycle to produce principally one molecular species of phosphatidylinositol. Such selectivity of molecular species could be the consequence of spatial segregation of different lipids or as a result of enzyme specificity. I would like to discuss the existing evidence that certain enzymes of the PI-cycle preferentially convert lipid species having particular acyl chain compositions.

The PI-cycle can be stimulated by hormone activation of a PIP_2-specific phospholipase C by a variety of mechanisms including through both G-protein coupled receptors as well as receptor tyrosine kinases. The mechanism of this activation includes binding to G-protein subunits, by small GTPases from the Rho and Ras families, phosphorylation by both receptor and non-receptor tyrosine kinases as well as by membrane lipid components [27]. PIP_2-specific isoforms of phospholipase C will catalyze the formation of a diacylglycerol that is enriched in 1-stearoyl-2-arachidonoyl acyl chains [28]. The next step in the PI-cycle is the conversion of diacylglycerol to phosphatidic acid catalyzed by diacylglycerol kinase (DGK) [29]. Among the ten known mammalian isoforms of DGK, only one isoform, DGKε, exhibits specificity for substrates with particular acyl chains. The specificity of DGKε for arachidonoyl-containing diacylglycerol is well established [30–32]. In addition, DGKε preferentially phosphorylates diacylglycerol having a stearoyl group on the sn-1 position [33]. Thus, DGKε is selective for the molecular species of diacylglycerol that is produced in the PI-cycle, i.e. 1-stearoyl-2-arachidonoyl glycerol. In addition, there is evidence from lipidomic studies that DGKε is closely linked with PI-cycling, since in whole cells the absence of this enzyme lowers the arachidonoyl content of PI [12], even though PI is neither the substrate nor the product of the reaction catalyzed by DGKε.

The phosphatidic acid product of the DGKε-catalyzed reaction is then converted to CDP-diacylglycerol catalyzed by the enzyme phosphatidate cytidylyltransferase. There is some evidence that this enzyme exhibits specificity for 1-stearoyl-2-arachidonoyl phosphatidic acid [34]. Phosphatidylinositol is then formed from this CDP-diacylglycerol by reaction with inositol, followed by phosphorylation catalyzed by phosphatidylinositol-4-kinase. Both the alpha (accession number P42356) and the beta (accession number, Q9UBF8) isoforms of the human phosphatidylinositol-4-kinase have lipoxygenase-like motifs (see below), but the acyl chain selectivity of this enzyme has not been studied to our knowledge. The PI-cycle is completed in the final, rate determining step in the major route for the synthesis of PIP_2, the phosphorylation of phosphatidylinositol-4-phosphate catalyzed by phosphatidylinositol-4-phosphate-5-kinase. This enzyme has also been shown to have dependence on the acyl chain composition of the substrate with high activity observed with 1-stearoyl-2-arachidonoyl phosphatidylinositol-4-phosphate [35].

Thus, virtually every step in the PI-cycle results in some enrichment of the lipid intermediates with 1-stearoyl-2-arachidonoyl acyl chains. The fact that these metabolic interconversions correspond to a cycle means that each of the intermediates will be regenerated with each turn of the cycle. Consequently any partial enrichment of the lipid intermediates that occurs in one cycle will be amplified as a consequence of the cycle being repeated multiple times.

Another particular aspect of this system is that it exhibits specificity for both the sn-1 and sn-2 acyl chains of the lipids. This is shown by the high content of the1-stearoyl-2-arachidonoyl molecular species in the phosphatidylinositols and by the finding that DGKε is specific for both acyl chains of diacylglycerol. In the Introduction we commented that the interaction of proteins with membranes was more commonly specific for the headgroups. Not only does this example show specificity for the acyl chains, but it shows that they are so to the extent that interactions of this protein with lipids are selective for a single molecular species. This specificity results in an enrichment of a diacylglycerol with particular acyl chains and consequently other lipids of the PI-cycle have these same acyl chains. This is a consequence of the substrate specificity of DGKε as well as other enzymes of the PI-cycle.

4. Phosphatidylethanolamine: the lipid class with the highest enrichment of PUFA: pathways of PE synthesis

Although phosphatidylinositols are highly enriched with 1-stearoyl-2-arachidonoyl acyl chains, the lipid class most enriched with arachidonoyl chains is not phosphatidylinositols, but PE. What then is the mechanism of enrichment of PE with particular acyl chains?

There are some additional considerations with PE. While the only PUFA enriched in phosphatidylinositols is arachidonic acid, in the case of PE there is enrichment with both arachidonoyl and with docosahexanoyl groups, depending on the organ. Brain and heart muscle PE are particularly enriched with the docosahexanoyl group [11]. In addition, PE lipids are composed of substantial amounts of 1-alkyl-2-acyl forms as well as 1-alkenyl-2-acyl species in addition to the diacyl component. Finally in mammals there are two routes of synthesis of PEs one being through decarboxylation of phosphatidylserine and the other through the Kennedy pathway of the reaction of CDP-ethanolamine with diacylglycerol.

There have been some studies regarding the recognition of acyl chains by the enzymes involved in PE synthesis. The most specific example is with the activity of ethanolamine phosphotransferase that catalyzes the synthesis of PE from CDP-ethanolamine and diradylglycerols. That study, using an isolated microsomal preparation, showed that diradyl-glycerols having ether linkages at the sn-1 position of glycerol were much better substrates for this enzyme than diacylglycerols [36]. For the 1-alkyl-2-acyl glycerols or the 1-alkenyl-2-acyl glycerols, the nature of the acyl chain at the sn-2 position appeared to be of minor importance. However, for the diacylglycerols, although poorer substrates, the 1-palmitoyl-2-arachidonoyl glycerol was shown to a much better substrate than 1-palmitoyl-2-oleoyl glycerol [36]. Clearly additional studies are required to ascertain the nature of the acyl chain selectivity of this enzyme. There is also the pathway of PE synthesis by decarboxylation of PS. There is some specificity for the acyl chains of PS in mitochondrial samples

containing phosphatidylserine decarboxylase activity, but this specificity varies among different tissues [37]. In brain there is specificity for 22:6 acyl chains [37]. Some of the enrichment of PE with PUFA may come as a result of PS enrichment with PUFA, followed by less specific decarboxylation. There is a strong preference of phosphatidylserine synthase-1 (PSS1), the enzyme that catalyzes base exchange of choline for serine, for forms of PC with 22:6 acyl chains [38]. Thus, the PS substrate for the formation of PE by decarboxylation will already be enriched with PUFA. Combined with the known enrichment of PE in all tissues with PUFA [11], this suggests that there is specificity exhibited by the proteins involved in PE metabolism, to selectively bind to membranes containing PUFA.

5. Development of an understanding of the features common to many proteins that bind to PUFA

Although there is detailed structural information only for the lipoxygenases, some common elements among the group of proteins that bind specifically to PUFA are beginning to emerge. One of these elements is the location of the binding site for PUFA. Although the substrates of these enzymes are hydrophobic and they will incorporate into membranes, their binding site on the protein is near the membrane interface. In the case of lipoxygenase, for example, removal of the most hydrophobic membrane-inserting loops, the β-barrel structure at the amino terminus, did not destroy the binding site for arachidonic acid [25,39]. The binding site for arachidonic acid is a channel formed within the α-helical segments in the remainder of the molecule. This can be appreciated from the structure of the enzyme derived from X-ray crystallography (Fig. 2). The arachidonic acid binding site of lipoxygenase is not inserted into the membrane where it can find the hydrophobic substrate; when bound to the enzyme, the arachidonic acid is completely surrounded by protein and has no contact with the membrane [40]. Thus, the process of arachidonic acid binding to lipoxygenase is better described as an extraction of the substrate from a membrane.

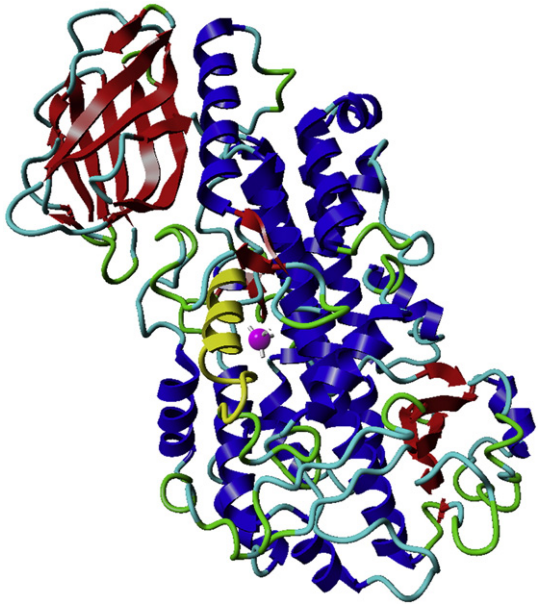

Fig. 2. The molecular structure of human 5-lipoxygenase constructed on the basis of the crystallographic structure deposited in the protein data bank (3O8Y). Structure of a single monomer. Note that the membrane-inserting segment is the β-barrel structure in the upper left-hand portion of the Figure. Arachidonic acid binds to a U-shaped tunnel within the core of the protein where the catalytic iron is the round, purple ball in the center of the structure. The protein segment corresponding to the arachidonoyl-recognizing motif is shown in yellow. α-helical segments, blue; β-structure, red; connecting segments, green.

We have also investigated the properties of the enzyme DGKε and the extent of insertion of the protein into a membrane. There is no atomic resolution structure available for this protein, hence some of the evidence is less direct, but it fits a model that is analogous in many respects to that of lipoxygenase. DGKε has only one segment that is sufficiently hydrophobic to be predicted to be a transmembrane helix, i.e. the segment comprising approximately residues 20–40. However, this segment of the protein can be removed without loss of enzymatic activity or specificity [41]. In fact there is evidence that the hydrophobic segment, with a FLAG-tag attached at the amino terminus, forms a re-entrant helix that enters and leaves the membrane at the same side, making it a monotopic protein [42]. *In silico* modeling studies indicate that this segment can easily interconvert between a transmembrane and re-entrant helix. Recent in vitro synthesis experiments suggest that the N-terminal FLAG tag contributes to the formation of a re-entrant helix [43]. Hence, the region of DGKε that penetrates the most into the membrane has no role in binding the arachidonoyl chain of diacylglycerol [41]. By contrast, however, we have identified a region in the accessory domain of DGKε [35] that has a similar pattern of amino acid residues as has been identified for lipoxygenases [40]. Mutation of any of the residues of DGKε that are equivalent to those found to be essential for arachidonoyl-binding by lipoxygenases results in a marked decrease in activity [35]. Furthermore, for two of the mutants that retained more activity, we demonstrated that their selectivity for arachidonoyl-containing diacylglycerol was diminished [35]. The lipoxygenase-like domain in DGKε spans residues 431–443 and is overlapping with a somewhat hydrophobic segment of residues 436–456. This hydrophobic segment appears as a peak in hydrophobicity plots but it is not predicted to be a transmembrane helix. However, its location in the sequence next to the lipoxygenase-like domain indicates the arachidonoyl binding site is close to the membrane interface.

In the case of DGKε, the substrate is diacylglycerol which is much more hydrophobic than the substrate for lipoxygenases, arachidonic acid. Therefore, we would not expect the diacylglycerol to be pulled out of the membrane into a protein binding site as happens with arachidonic acid with lipoxygenases. We rather suggest that in the case of 1-stearoyl-2-arachidonoyl glycerol, only the sn-2 chain of the diacylglycerol is removed from the membrane and not the entire lipid molecule. The arachidonoyl chain is less hydrophobic than a saturated acyl chain. The energetics of pulling this single chain of diacylglycerol out of the membrane may not be much different from the case of lipoxygenases binding to free arachidonic acid. The suggestion that the arachidonoyl group binds specifically to DGKε is supported by our observations that there is no other acyl chain besides arachidonoyl that can be present in the sn-2 position of diacylglycerol and result in a substrate with over about 30% of the activity of the best substrate, 1-stearoyl-2-arachidonoyl glycerol. In contrast, substituting the preferred stearoyl group on the sn-1 position with arachidonoyl to make1,2-diarachidonoyl glycerol, gives a substrate that has activity that is comparable to 1-stearoyl-2-arachidonoyl glycerol (unpublished results). This and other similar results, indicate that the specificity for a particular acyl chain at the sn-1 position is not as great as that for the sn-2 position. Nevertheless, the acyl chain at the sn-1 position does exhibit some selectivity, with the stearoyl group being favored over either longer or shorter saturated acyl chains [33]. This indicates that the role of the sn-1 acyl chain is not simply to provide hydrophobicity or membrane partitioning, but may serve to determine the depth of insertion of the entire diacylglycerol substrate into the membrane, determining the alignment of the hydroxyl group with the active site of the enzyme.

We suggest that there is a similar amino acid pattern in many proteins that interact with PUFA. The pattern of residues in the arachidonoyl-recognizing motif for each of these proteins is similar, but not identical. These groups of required amino acid residues make up a binding site that we propose has some similarity to that of lipoxygenases. However, the binding sites for all these enzymes are clearly not identical. First of all, the substrates for the large group of

PUFA-recognizing proteins can be quite different. For example, it is diacylglycerol for DGKε and free arachidonic acid for lipoxygenases. As an example, there is a cationic residue in lipoxygenases that could bind the carboxyl group, but such an interaction would not be relevant for enzymes acting on diacylglycerols. In addition, the nature of the chemical reaction and the geometry of the transition state will be different for different enzymes. Nevertheless, the amino acid pattern that is part of the arachidonoyl-recognition site is similar for proteins interacting with different PUFA. For DGKε and for mammalian lipoxygenase the enzymes recognize arachidonic acid. However, for plant lipoxygenases, the favored substrate is linoleic or linolenic acid [44]. The specificity of enzymes for different PUFAs is most dramatically seen by comparing two lipoxygenase isozymes from maize *(Zea mays)*. One of these isoforms exhibits high activity with α-linolenic acid but is essentially inactive with arachidonic acid, while the other isoform has specificity for linoleic acid [45]. The PUFA that will be the best substrate depends on the specific structure of the binding site of the enzyme, which is determined by more than just the presence or absence of this amino acid pattern. Thus the presence of this lipoxogenase-like pattern of amino acids does not determine the nature of the PUFA that will be acted upon, but it does indicate that the protein will bind better to a lipid substrate containing a polyunsaturated acyl chain rather than a saturated or monounsaturated one. The amino acid pattern is not a unique one, but can be defined by the sequence L-$X_{(3-4)}$-R-$X_{(2)}$-L-$X_{(4)}$-G, in which -$X_{(n)}$- represents n residues of any amino acids in this domain [35]. The first residue of this sequence is L, while for lipoxygenases it is I. Although this pattern is found in about 2% of known protein sequences, including many proteins that do not interact with PUFA, it has allowed us to identify sites in other enzymes that are responsible for interaction with PUFA. The segment of the protein identified as having a lipoxygenase-like motif is found in a region that is highly conserved in evolution as shown by several sequence alignments that have been recently presented [35].

One of these proteins is phosphatidylinositol-4-phosphate-5-kinase (PI4P5K). There are three isoforms of this enzyme as well as products of alternative splicing. All forms of this enzyme have a lipoxogenase-like motif. The human isoform 1α has specificity for brain PI4P which is mainly the 1-stearoyl-2-arachidonoyl form compared with a dipalmitoyl form of PI4P, that is phosphorylated much more slowly [35]. In addition, mutation of specific essential residues of the lipoxogenase-like motif of this protein results in a marked loss of activity [35].

6. Conclusions

Recognition of specific free fatty acids or acyl chains of lipid structures must be a common phenomenon in mammalian cells. Oxidized forms of arachidonic acid are potent signaling lipids and their formation requires the specific recognition of arachidonic acid over other polyunsaturated fatty acids. In addition, the finding that PUFA are unequally distributed in different organs and in different lipid classes is another demonstration of the specific recognition of these groups.

The binding of arachidonic acid by lipoxygenases has been well studied and the crystal structures of many forms of lipoxygenase are available. It is clear in this case that the protein binds to a membrane and from that location is able to favorably compete with the membrane bilayer for binding to arachidonic acid. The arachidonic acid substrate enters into a channel in the protein that is outside of the membrane but is lined with hydrophobic residues. For many forms of lipoxygenase from many different species there is a common pattern of amino acid residues in the channel that binds arachidonic acid. This amino acid pattern is I-$(X)_4$-R-$(X)_2$-L-$(X)_4$-G. We have shown that similar features are found in DGKε, which is the only isoform of mammalian DGKs that is known to have specificity for PUFA. For DGKε, the segment that is most hydrophobic and partitions into the membrane, i.e. residues 20–40, is not required for substrate binding or catalytic activity [41]. Therefore the arachidonoyl moiety of the diacylglycerol substrate must be extracted from the membrane to bind to DGKε. In addition, among the 10 mammalian isoforms of DGK, DGKε is the one having a pattern of amino acid residues most closely resembling that required by lipoxygenases for binding arachidonic acid. The pattern for DGKε is L-$(X)_3$-R-$(X)_2$-L-$(X)_4$-G. It differs from the pattern in lipoxygenases by having L as the first residue and by having only 3, rather than 4 residues between the first residue and the required R. Although the two motifs are not identical, the nature of the substrates and the reaction that is catalyzed by the DGKε and the lipoxygenases are also quite different, so perhaps one should expect some differences in the nature of the arachidonoyl binding site. The argument that the identified motif is required for arachidonoyl recognition was shown by the fact that it is highly conserved among DGKε of different species and mutation of essential residues in this segment leads to loss of activity and in some cases loss of selectivity for the arachidonoyl group. The argument is also supported by the fact that a modest increase in arachidonoyl specificity can be introduced into another isoform of DGK, DGKα, by introducing the same pattern of required amino acid residues as found with DGKε [35].

It remains to be determined how general this motif is for proteins that recognize PUFA. We have presented evidence that PI4P5K has both arachidonoyl specificity as well as a pattern of residues identical to that of lipoxygenases, except that L, rather than I, is the first residue [35]. Certainly not all proteins with this or a similar amino acid pattern will recognize PUFA as one can find examples of non-membrane proteins with this pattern also, although the pattern is found in only 2% of known proteins and therefore is not common. However, we have allowed some small variation in the pattern among the three examples we have discussed, i.e. lipoxygenases, DGKε and PI4P5K. In addition, it appears from the example of plant lipoxygenases and others, that other PUFA, besides arachidonic acid, can have the highest binding affinity to a protein with a similar amino acid pattern to those discussed above. This simple predictive method is insufficient to determine which PUFA binds most avidly. However, the presence of this motif in a protein known to have specificity for PUFA allows for the identification of a segment of the protein important for substrate binding. Because of the importance of PUFA in biology, further investigation of these relationships is important to carry out. However, what the findings already demonstrate is that there are some common elements among proteins that are unrelated, except for their property of binding PUFA. These common elements include a binding site for PUFA that is outside of the membrane, requiring extraction of the substrate or part of the substrate from the bilayer to bind to a hydrophibic channel in the protein. This binding site also has an amino acid pattern equal to or similar to that found in lipoxygenases that forms part of the substrate binding site.

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