CHEMICAL REVIEWS

EDITORIAL

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Introduction to Lipid Biochemistry, Metabolism, and Signaling

Lipids are ancient and ubiquitous molecules. Among the three *domains* of life on our planet, differences are found in the lipid chemistry of the predominant building blocks (e.g., L-glycerol vs D-glycerol, ester vs ether linkages, among others) between life forms, but even many viruses possess lipid envelopes until they are shed inside the host cell. The evolution of an outer membrane composed of a complex mixture of lipids, proteins, and carbohydrates is one of the defining characteristics of an organism. Lipids are generated from two basic biosynthetic pathways. The first involves the condensation of acyl carrier protein intermediates derived from malonyl-CoA and acetyl-CoA esters and a carbanion intermediate. This pathway leads to diverse classes of lipids that contain fatty acyl chains, including fatty acids, phospholipids, and glycerolipids. The polyketide biosynthetic pathway provides a similar pathway in plants. The second biosynthetic pathway involves the condensation of branched-chain five-carbon pyrophosphate intermediates and a carbocation intermediate. This latter pathway is the source of all lipid species identified in the Archaea domain as well as a number of species in the Bacteria and Eukarya domains, such as prenols, sterols, and arachaeal glycerolipids, glycerophospholipids, and sphingolipids. Appreciation of these biosynthetic sources has been suggested by some investigators to be a more enlightened definition of what molecules are lipids as opposed to classic definitions of solubility in an organic solvent (reviewed in Brown, H.A.; Murphy R.C. *Nat. Chem. Biol.* **2009**, *5*, 602−606). It is clear that lipids evolved from these biosynthetic pathways to become involved in the multitudes of biological processes used by living organisms. The comprehensive counting of the total number of lipid molecular species in nature has yet to be fully tallied, but when one considers chirality, precise locations of double bonds, attachments of various head groups, carbohydrates, and amino acids, and other potential chemical diversity, the numbers are in the thousands or beyond.

In this thematic issue of *Chemical Reviews*, we seek to represent the diversity of species and functions in which lipids participate. We focus on lipid species and pathways in mammalian cells and emphasize classes of lipids where misregulation plays a role in human diseases. This issue is one of the most comprehensive collections of reviews on the subject of lipid biochemistry to date. The first section includes contributions on lipid species that are generated by the metabolism of polyunsaturated fatty acids, such as arachidonate and docosahexaenoic acid. Subsequent sections include the metabolism and signaling pathways of glycerolipids, sphingolipids, glycerophospholipids, and sterols. The enzymes that regulate these pathways, the functions of the lipid metabolites, and recent advances in developing chemical modulators are points of special emphasis in this thematic issue.

ENZYMATIC SUBSTRATE OXYGENATION

Glycerophospholipids contain fatty acids esterified to the glycerol backbone at the *sn*-1 and *sn*-2 positions. In general, the *sn*-1 fatty acyl group is saturated whereas the *sn*-2 fatty acyl group is monounsaturated or polyunsaturated. Thus, there is an enormous concentration of unsaturated fatty acid residues in cellular membranes. The double bond geometries of the unsaturated fatty acids are nearly always *cis*, which introduces significant distortion into the membrane bilayer and contributes to its fluidity. Hydrolysis of the *sn*-2 ester releases polyunsaturated fatty acids that are substrates for multiple oxygenases, which introduce a single atom of oxygen, a single molecule of oxygen, or two molecules of oxygen into the carbon framework. Cyclooxygenases are hemeproteins that catalyze the double dioxygenation of arachidonic acid to form prostaglandin endoperoxides. The cyclic peroxide group of these products is converted to one of five different metabolites, each of which binds to one or more membrane-bound G protein coupled receptors. The prostaglandin or thromboxane products exert a broad range of biology through these receptors, and inhibition of cyclooxygenases by nonsteroidal anti-inflammatory drugs attenuates the production of these bioactive lipids. This is a major contributor to the pharmacological action of this important class of drugs. Smith, Urade, and Jakobsson describe the chemistry and enzymology of the cyclooxygenase cascade with focus on both the endoperoxide-generating cyclooxygenases and the endoperoxide-metabolizing isomerases and reductases. Of particular interest are the recent findings that cyclooxygenases act as functional heterodimers despite the fact that they are structural homodimers.

Lipoxygenases introduce a single molecule of O_2 into the carbon framework of polyunsaturated fatty acids, and the hydroperoxide products are transformed into a multiplicity of metabolites. In the case of arachidonic acid, this includes an epoxide called leukotriene A_4 that is hydrolyzed to leukotriene B_4 or conjugated with glutathione to form leukotriene C_4. Unique receptors exist for these products that contribute to the inflammatory response and allergic hypersensitivity. Haeggström and Funk review the chemical biology of this pathway of lipid mediator generation. The structures of the enzymes in this pathway are fascinating because the lipoxygenases are nonheme iron proteins structurally unrelated to cyclooxygenases; the leukotriene A hydrolase is a Zn^{2+} containing enzyme that also exhibits aminopeptidase activity; and the leukotriene C4 synthase is a glutathione transferase structurally related to a lipoxygenase-activating protein that lacks enzymatic activity.

Much of the focus of lipid oxygenase biochemistry has been on the oxidation of free fatty acid substrates. However, over the past 15 years a number of laboratories have reported that unsaturated fatty acid esters and amides are also substrates for oxygenation. The oxygenation products are similar to those produced from the free fatty acids, but their biological effects appear to differ. 2-Arachidonoylglycerol and 2-arachidonoylethanolamide are two such alternate substrates that are biologically interesting in their own right. They are the first known endogenous ligands for the cannabinoid receptors and are referred to as endocannabinoids. They are naturally occurring analgesic and anti-inflammatory compounds that are metabolized by hydrolysis to arachidonic acid or by oxygenation to prostaglandin- and leukotriene-related

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derivatives. Rouzer and Marnett review the biochemistry of endocannabinoid oxygenation by cyclooxygenase, lipoxygenases, and cytochromes P450 and the signaling properties of these novel metabolites.

The pharmacology of nonsteroidal anti-inflammatory drugs and their ability to inhibit cyclooxygenase enzymes has inferred that oxidized lipids are important mediators of inflammation. Indeed, there is significant prostaglandin production during the development of inflammation in various animal models and high levels of prostaglandins are found in inflamed tissue. However, oxidized lipids are also important mediators of the resolution of inflammation as reviewed by Serhan and Petasis. Multiple classes of pro-resolving lipids exist including resolvins, protectins, and maresins. These are polyhydroxylated derivatives of arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid that are formed by sequential oxygenation by multiple lipoxygenases. They exert extremely potent pro-resolving activity and may account for the beneficial effects of the ω-3 fatty acids found in fish oil.

NONENZYMATIC FATTY ACID OXIDATION

The elegant chemical transformations that give rise to prostaglandins, leukotrienes, and resolvins *inter alia* borrow heavily from the autoxidation of polyunsaturated fatty acids. In fact, one can consider their biosynthesis by cyclooxygenases and lipoxygenases as enzyme-controlled autoxidations. The fundamental chemistry of fatty acid autoxidation is reviewed by Yin, Xu, and Porter. They not only articulate the chemical precedents for enzyme catalysis but also highlight the destructive potential of lipid autoxidation with regard to membrane integrity and cellular homeostasis. Lipid autoxidation is an efficient radical chain process that is optimal when the fatty acids are arrayed in a monolayer, as they are in cell membranes. Interruption of these radical changes underscores the importance of vitamin E as the principal membrane-bound antioxidant.

Autoxidation of arachidonic acid was found in the 1960s to produce small amounts of prostaglandins that lacked the stereochemical control displayed by enzyme catalysis. This fact was rediscovered in the early 1990s but in the context of living tissue. These nonenzymatic oxygenation products, called isoprostanes, were found to be present in extracts from intact animals and healthy humans. This discovery unequivocally established that lipid oxidation occurs spontaneously in human beings and that it can be modulated by pro- or antioxidants. Milne, Yin, Hardy, Davies, and Roberts review the occurrence of lipid autoxidation products in animal models and humans, the biological effects of these novel metabolites, and their use as biomarkers of diseases associated with oxidative stress including inflammation and cardiovascular disease.

The spontaneous autoxidation of polyunsaturated fatty acids not only generates isoprostanes but also yields molecules (e.g., α,β-unsaturated aldehydes and ketones) that are electrophiles and couple to intracellular nucleophiles. Covalent modification of nucleic acid produces adducts that block DNA replication, cause cell toxicity, and induce genetic mutations. Covalent modification of DNA or protein induces changes in cell signaling that enable a cell to respond productively to a low-level challenge or commit suicide at high levels of modification. In addition to electrophilic fatty acids produced by autoxidation, electrophilic fatty acid derivatives can be produced by coupling to reactive nitrogen species to form nitro or nitroso fatty acids. Schopfer, Cipollina, and Freeman outline the pathways of electrophile generation and the cellular responses that they induce. This relatively new branch of lipid oxidation-dependent cell signaling is an interesting complement to the well-characterized signaling by lipid mediators through G protein coupled or nuclear receptors.

PHOSPHOLIPASES AND LIPID KINASES

Lipids are frequently used as substrates to form second messengers. Among the most studied signaling pathways are enzymes that hydrolyze a lipid substrate at the ether linkage between the glycerol backbone or phosphodiesterases that cleave the phosphate group to release a free head group and form a bioactive lipid species. The first contribution in this section is a comprehensive review from Long and Cravatt on the serine hydrolase superfamily, which consists of more than 200 enzymes in humans. The review focuses on the metabolic serine hydrolases that include enzymes both well-known and obscure to most readers. The enzymes in this superfamily are involved in almost every imaginable (patho)physiological process, including perception, inflammation, oxidative stress, and infectious diseases. The authors group the various enzymes with regard to both enzymatic classification as well as consideration of sites of action. This is followed by a series of reviews on three of the better known phospholipases. We begin at the head group and work toward the glycerol backbone. Selvy, Lavieri, Lindsley, and Brown describe the diverse members of the phospholipase D (PLD) superfamily of enzymes. Emphasis is placed on those members that contain an HKD motif, but others are described as well. The ubiquitous nature of PLD is indicated by the breadth of biological organisms in which isoenzymes are found, including prokaryotic, fungal, plant, and mammalian (*inter alia*). A detailed history of the field is provided as a basis for describing the enzymology and the various roles PLDs play in cellular signaling. Recent development of isoenzyme-selective, small-molecule inhibitors is described as well as potential targets for therapeutic use in human diseases.

The next review moves to a site of action across the phosphate bridge toward the glycerol backbone. Harden, Waldo, Hicks, and Sondek describe the phospholipase C (PLC) isoenzymes that catalyze conversion of phosphatidylinositol (4,5)-bisphosphate into inositol (1,4,5)-trisphosphate and diacylglycerol. The soluble inositol head group mediates release of intracellular calcium that broadly activates signaling targets in waves across the cell. Harden and colleagues focused their review on the PLC-β isoenzymes that are activated by the Gα-subunits of the Gq heterotrimeric family of GTPases. Recent mechanistic insights into the activation and inactivation of this signaling complex are discussed. As we continue away from the head group and up the glycerol backbone, we find the next site of regulated, enzymatic cleavage on the *sn*-2 carbon of the glycerophospholipid substrate. The review by Dennis, Cao, Hsu, Magrioti, and Kokotos provides a comprehensive coverage of the broad family of phospholipase A_2 (PLA_2). This includes enzymes that are secreted both as aids to digestion and powerful venoms as well as one of the canonical targets in cellular signaling, cytosolic PLA_2, which serves as the wellspring of much of the arachidonic acid that is released as a precursor to the eicosaniod class of signaling lipids and is the focus of other reviews in this thematic issue. Dennis and colleagues provide a detailed overview of small-molecule inhibitors and the relative specificity across the isoenzyme classes. This section concludes with a review on diacylglyerol kinases (DGKs) by Shulga, Topham, and Epand. In contrast to the previous

reviews in this section that describe hydrolysis of lipid substrates to generate signaling products, lipids are also targets of phosphate transfer through the action of specific kinases. In this case diacylglycerol is phosphorylated to form phosphatidic acid. This is a distinct pathway with differences in the molecular species preferences from that of the PLD reaction described above. Epand and colleagues discuss the various isoenzymes of DGKs, physiological roles, and the diverse functions in which these enzymes are involved. The interrelationships of DGKs with the PLC and PLD signaling pathways provide interesting insights into the complex networks that exist in cellular signaling as well as discussing the acylation and deacylation pathways of diacylglycerol metabolism. Together this section provides a comprehensive overview of the history and recent key advances in developing chemical modulators of lipid-signaling enzymes.

LIPIDS AS LIGANDS FOR RECEPTORS AND POST-TRANSLATIONAL MODIFICATIONS

Lipids not only are structural components of membranes and intracellular second messengers but also serve as ligands for receptors as well as covalently attached post-translational modification to proteins. Four reviews in this section outline different types of receptors at which lipids mediate information about changes in the environment or coordinate local responses to stimuli. The first contribution in this section by Hirata and Narumiya reviews prostanoid receptors. The structure, ligand binding properties, allosteric modulation, and a comprehensive description of the various receptor subtypes is described by the authors. These receptors are stimulated by oxygenated lipid molecules generated through the metabolism of the unsaturated 20-carbon fatty acids through the cyclooxygenase pathway. The generation and metabolism of this bioactive lipid signaling pathway is described in several reviews in the first section of this issue. The next contribution describes a distinct class of receptors from a different branch of the 20-carbon lipid family. Nakamura and Shimizu provide an in-depth review of leukotriene receptors, including the generation of the ligands, subtypes of receptors, mechanisms of activation, and a detailed description of synthetic ligands for receptor subtypes. The descriptions of the plethora of diseases in which these receptors have been implicated make this an especially valuable review. The next review in this section transitions from ligands derived from fatty acids to receptors that are activated by sphingosine 1-phosphate and lysophosphatidic acid. Blaho and Hla provide a detailed description of the synthesis of the ligands by enzymatic pathways and the pharmacological specificities of receptor subtypes. The roles of these receptors in immune, nervous, and reproductive functions and a synopsis of recent advances in inhibitor development provide an outstanding summary of this dynamic field in a period of rapid discoveries. The contribution from Harmon, Lam, and Glass moves us away from the cell surface and focuses on an important class of nuclear receptors. Particular focus is given to the peroxisome proliferator-activated receptors (PPARs) and their roles as master regulators of glucose metabolism, cellular differentiation, and immune response. Various lipid activators of PPARs are discussed as well as recent exciting findings on the existence of endogenous lipids that function as antagonists of these receptors. To conclude this section Hang and Linder review covalent attachment of lipid groups to proteins. These lipidation reactions include *N*-myristolation, *S*-palmitoylation, and prenylation. They emphasize recently developed chemical tools being used to study these pathways and the biological consequences of these modifications.

GLYCEROLIPID, SPHINGOLIPID AND STEROL PATHWAYS

The next section consists of three reviews that cover major lipid metabolic and signaling pathways. The first contribution by Coleman and Mashek reviews the pathways involved in the synthesis and lipolysis of triacylglycerol (TAG) in mammals. The authors systematically progress from the acylation of glycerol-3-phosphate through the branch points of the major classes of glycerophospholipids and TAG synthesis. It is informative with respect to the rich history of the biochemistry that elucidated these pathways and provides updates on identification of key genes and enzymes. The regulated breakdown of these species, potential roles in cell signaling pathways, and contributions to diseases are also discussed. The second contribution comes from Alfred Merrill on the sphingolipid and glycosphingolipid metabolic pathways. The review has a strong emphasis on recent contributions from the field of lipidomics in identifying nodes of pathway modulation. Analytical determinations using state-of-the-art mass spectrometry and tissue imaging that allows spatial resolution of sphingolipid species are described. Visualization tools and mathematical modeling are explained to facilitate a truly systems level analysis of these pathways. The final contribution in this section is by David Nes on the biosynthesis of cholesterol and sterols. Sterol nomenclature, stereochemistry, and variances found on sterol construction are detailed in this highly informative review. The review provides examples where the functional-genomics approach and sequencing of model organism has been integrated to elucidate the structural genes for enzymes at key steps of the biosynthetic pathways. This contribution includes a detailed and systematic analysis of sterol formation enzymes organized according to specificity, mechanism, inhibition, and mutagenesis studies. This provides a useful review of the contributions to the chemical biology of sterol pathways.

BIOINFORMATICS AND NEW TECHNOLOGIES IN LIPID RESEARCH

Finally, we conclude this thematic issue with two reviews that describe recent innovations in lipid biochemistry. Subramaniam, Fahy, Gupta, Sud, Byrnes, Cotter, Dinasarapu, and Maurya contribute a review on bioinformatics and systems biology approaches to defining lipomes. The authors describe recent efforts to update classification, ontology, and nomenclature of lipids that were essential to developing searchable databases of quantitative lipid species analysis. They discuss the practical aspects of making such large-scale collections of data of use to the broader research community and provide detailed descriptions of a variety of web-based tools that are available. A number of these resources were developed by Subramaniam and coauthors as part of the LIPID MAPS large-scale collaborative initiative. The review includes examples of how such data can be used in developing quantitative kinetic models of lipid metabolism and shares insights into how the field of lipidomics will facilitate investigators in answering biochemical questions. The final contribution is an exciting overview of new innovations in imaging lipid species in tissues by mass spectrometry. Zemski-Berry, Hankin, Barkley, Spraggins, Caprioli, and Murphy provide an insider's guide to the development of instrumentation and matrices that have allowed a spatial

dimension to be added to analytical lipid determinations. The composition of tissues is now being mapped for localization and relative concentrations of various lipid species, and this provides valuable insights into changes that occur in (patho)-physiological processes and in response to injury. The provocative images generated by this new technology are clearly transforming the ways that lipids will be studied and potentially leads to future innovations that will allow subcellular lipid species dissection.

In closing, we note with sadness the passing of Christian R. H. Raetz (George Barth Geller Professor of Biochemistry at Duke University Medical Center). Chris was an outstanding scientist, a leader in the field of lipid biochemistry, and a valued collaborator. Chris was working on a contribution for this thematic issue on *E.coli as a model for bacterial systems* when his untimely passing prevented its completion.

H. Alex Brown and Lawrence J. Marnett
Guest editors
Departments of Pharmacology, Chemistry, and Biochemistry, Vanderbilt Institute of Chemical Biology, Vanderbilt University School of Medicine

BIOGRAPHIES

H. Alex Brown is Professor of Pharmacology and Chemistry at Vanderbilt University School of Medicine. Alex received his Ph.D. degree in 1992 from the University of North Carolina at Chapel Hill working in the laboratory of T. Kendall Harden and then pursued postdoctoral training in the Department of Pharmacology at the University of Texas—Southwestern Medical Center with Paul Sternweis. Alex joined the faculty at Cornell University in 1996 with appointments in Pharmacology and Biochemistry Molecular & Cell Biology. Alex received the Sidney Kimmel Foundation for Cancer Research Scholar award in 1997. Working with Fred McLafferty at Cornell, Alex developed the field of computational lipidomics in his laboratory utilizing electrospray ionization mass spectrometry. Al Gilman, then the director of the Alliance for Cellular Signaling (AfCS), invited Alex to use this emerging technology to contribute to the AfCS research program. In 2002, Alex was recruited to Vanderbilt University School of Medicine as the Ingram Professor of Cancer Research in Pharmacology. Alex has been the director of the glycerophospholipid core for the LIPID Metabolites and Pathways Strategy (LIPID MAPS) consortium since 2003. He has served on the editorial board for the *Journal of Biological Chemistry*, NIH-NIGMS & NIHLB study section, ASBMB publications committee, and as associate editor of *Molecular Pharmacology* from 2006 until 2009. In addition, Alex was editor of a three-volume series on Lipidomics and Bioactive Lipids for *Methods in Enzymology*, coeditor with Lawrence Marnett of a thematic issue on lipid biochemistry for *Chemical Reviews* in 2011, and has organized multiple international conferences on lipid metabolism and signaling. Alex received the Vanderbilt Ingram Cancer Center (VICC) "High Impact Publications Award" in 2010, as well as the Vanderbilt University Medical Center "Leadership of a Multi-Investigator Team Award" together with Craig Lindsley in 2011. Alex is currently Metabolomics co-director for the Scripps-Vanderbilt Human Chemical Sciences Institute and the associate director of System Analysis for the Vanderbilt Institute of Chemical Biology (VICB). His current research is focused on understanding the roles of lipid molecular species and phospholipases in cellular functions and human disease.

Lawrence J. Marnett received his B.S. in Chemistry from Rockhurst College (1969) and his Ph.D. in Chemistry from Duke University (1973). After postdoctoral training at Karolinska Institute and Wayne State University, he joined the faculty in Chemistry at Wayne in 1975. He moved to Vanderbilt University in 1989 as Mary Geddes Stahlman Professor of Cancer Research and Professor of Biochemistry and Chemistry. His research interests are in the chemical biology of oxygenation of polyunsaturated fatty acids. Dr. Marnett is Editor-in-Chief of *Chemical Research in Toxicology* and Director of the Vanderbilt Institute of Chemical Biology. He is the author of over 400 publications.

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