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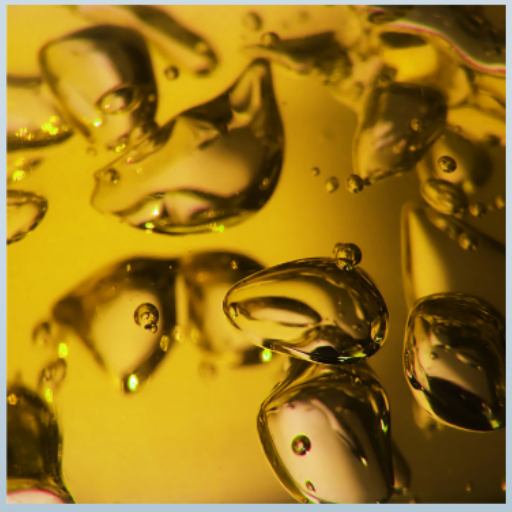

Lipids

Structure, physical properties and functionality

Kåre Larsson, Peter Quinn, Kiyotaka Sato and Fredrik Tiberg

An Oily Press title

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Lipids: Structure, physical properties and functionality

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Lipids: Structure, physical properties and functionality

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Foreword

Over ten years have passed since the publication of Kåre Larsson's *Lipids – Molecular Organization, Physical Functions and Technical Applications* (*LMO*) by The Oily Press, then based in Dundee, Scotland, and run by Dr William W. Christie. The book was soon recognized as a major contribution to the literature ("This is a book without comparison in the lipid literature...", Stig E. Friberg in the *Journal of Dispersion Science and Technology*, 1995, Vol.16, p.295 and "...the content is excellent", Philip W. Wertz in *Chemistry and Physics of Lipids*, 1994, Vol.74, p.99). Dr Christie's excellent choice of Kåre Larsson as the author was also confirmed ("His expertise in describing the various states of lipids is second to none", Edward G. Perkins in *INFORM*, 1994, Vol.5, p.1394 and "...written by an acknowledged world expert in his field", Fred B. Padley, *Lipid Technology*, 1994, Vol.6, p.102). Until the publication of *LMO* there had been no single, concentrated source of so much information on the subject: "The strength of this book – and it is enormous – is the fact that the author has been able to compile in one volume information otherwise found only in the most widely different kinds of scientific journals" (Friberg).

When *LMO* was published, Kåre Larsson was a Professor of Food Technology in the Chemical Centre at Lund University, Sweden. He is now cofounder of Camurus AB and Probi AB, and earlier of Biogram AB (which later became Bioglan AB), and serves as Chairman of the Board of the Camurus Lipid Research Foundation in Lund. Camurus is a provider of drug delivery systems and works closely with pharmaceutical manufacturers. Kåre Larsson is a Fellow of the Royal Swedish Academy of Science and the Academy of Engineering Science and has authored more than 200 original papers and five books covering areas of lipid biophysical chemistry, food science and nutrition, and biomedicine. He is also the named inventor on several patents, of which four have led to industrial products. In 2001 he won the Rhodia prize of the European Colloid and Interface Society for his discovery of cubosomes and hexosomes and explorative work on their applications.

When I asked Kåre Larsson to write a second edition, the comprehensive coverage of *LMO* became an obstacle – the subject area had expanded to such an extent that one author could not cover it alone. But we did not want to resort to the usual edited book with each chapter written by a different author. The solution was to invite three other well-known scientists in this field to act as coauthors – and it is a tribute to Kåre's reputation that all three agreed.

Peter Quinn, Professor of Biochemistry in the Department of Life Sciences at King's College London, UK, is renowned for his work on biological

membranes and their constituents, as evidenced by the publication of ten books, more than 400 research papers, and several patents.

Kiyotaka Sato, Professor in the Graduate School of Biosphere Sciences, Hiroshima University, Japan, is widely recognized as a leading expert in the physical chemistry and biophysics of fats and lipids and in the crystallization of biological soft materials. Among other awards, he was presented with the 2005 AOCS Stephen S. Chang Award for distinguished accomplishments in basic research that have been used by industry for the improvement or development of products related to lipids in the area of lipid crystallization and crystallography.

Fredrik Tiberg is President, Chief Executive Officer and Head of Research & Development at Camurus AB, and also Adjunct Professor of Surface and Colloid Chemistry at Lund University. He has published more than 80 original scientific papers, coauthored several books, and been named as inventor on a number of patents.

With some major changes in the areas covered, and with four authors instead of one, we were obliged to define the resulting publication as a new book with a new ISBN number, rather than as a second edition retaining the old number. Therefore we took the opportunity of also giving it a new and more appropriate title: *Lipids: Structure, Physical Properties and Functionality*. With the knowledge and experience of the above-named four authors behind it, I am confident that this new book will build on the reputation of *LMO* and be a valued source of information for many years to come.

Peter J. Barnes
Publisher, The Oily Press
Bridgwater, UK

January 2006

Preface

The ambition behind the new edition of this book is to provide an up-to-date description of the diversity of lipid molecular arrangements in different physical states, as a basis for the understanding of lipid functionality in biological and technical systems. The first edition was published in 1994 with Kåre Larsson as author, and when he was asked by the publisher to revisit the text he realized that he could not cover this broad field alone. Three colleagues joined forces with him, and the present edition has therefore in many aspects been extended. In some cases the description is deeper with a more narrow focus. For example, the chapter on the solid state in the earlier edition covered all lipids, whereas in this new edition there is a very complete demonstration of the crystal structures and crystallization properties of fatty acids and fats. These general principles, however, can be applied to all lipids.

In biology, as well as in technical applications such as foods, we are dealing with soft matter. Lipids form aqueous phases alone or in conjunction with proteins and polysaccharides. The combination of short-range disorder and long-range order into liquid-crystalline structures plays a crucial role. A driving force is the dualistic properties of the molecules in relation to water. Molecular regions avoiding water contact, in combination with regions striving towards such contact and interaction, lead to self-assembly, and even in the liquid state to the formation of organized structures on the colloidal level. This new edition presents many new results, particularly on the structure and functions of dispersions of liquid-crystalline phases forming nanostructures and mesoporous systems.

With regard to the role of lipids in cellular and molecular biology, this book focuses on biophysical aspects, and discussion of lipid biochemistry is limited to a chapter on cell membranes.

Kåre Larsson, Lund, Sweden
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Kiyotaka Sato, Hiroshima, Japan
Fredrik Tiberg, Lund, Sweden

January 2006

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CHAPTER 1

Basic concepts

A. Classification of lipids

There is no strict definition of lipids. Extracts in organic solvents from biological tissues have been termed lipids for a long time. A definition more consistent with modern views, proposed by Christie (1987), says: "Lipids are fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds". From a physical point of view it is natural to divide lipids into two groups:

- polar lipids, which interact with water and form aqueous phases;
- non-polar lipids, which do not form aqueous phases.

These are the definitions we will use in this book. Interfaces, however, are a special case, where even non-polar lipids can interact with water and form monomolecular layers.

Simple fatty acids exhibit most of the structural states described in this book. Oleic acid, for example, behaves like oil in relation to water at room temperature. If titrated into soap, however, a wide variety of aqueous phases are obtained. This means that by lowering the pH, dissociated oleic acid – a polar lipid – becomes undissociated and a non-polar lipid. In this respect fatty acids represent a special case.

The structures of fatty acids in different states of order represent well the structures in the solid state, described in Chapter 2, as well as in the disordered liquid-crystalline phases, described in Chapter 3. The overall organization into bilayers in the solid state is driven by the carboxyl groups, which associate the molecules by hydrogen bonds into dimers or polymers. The hydrocarbon chains are extended to different degrees. In solids, planar zigzag conformations of the carbon–carbon bonds exist (all-*trans*), whereas in disordered states, occurring in liquid crystals and in melts, *gauche* conformations form dynamically along the chains. Recording of the *trans/gauche* ratio, for example by Raman spectroscopy, has demonstrated the conformational changes that occur during chain-melting transitions.

The raw materials used in lipid technology are fats and oils, with plant extracts dominating. About 120 million tonnes of vegetable oils were produced in 2001, the main types being soybean oil (29 million tonnes), palm oil (23 million tonnes) and rapeseed oil (14 million tonnes). The most important polar lipids, phospholipids, are obtained as by-products during the industrial refining of vegetable oils into pure triacylglycerols.

1. Non-polar lipids

General information on the chemistry of lipid molecules can be found in *The Lipid Handbook* (Gunstone *et al.*, 1994). Fatty acids can be saturated or unsaturated, and the predominant configuration of the double bonds is *cis*. One industrial approach to modifying the melting points of fats is hydrogenation, whereby unsaturated chains become saturated, and in parallel some of the double bonds may also be converted to the *trans* isomer.

Oils and fats (in what we call fats, at least a part is solid at room temperature) are *triacylglycerols*, formerly known as *triglycerides*. A triacylglycerol molecule is made up of three fatty acids (*acyl groups*) attached via ester linkages to a 3-carbon glycerol 'backbone' structure. Because both the types and the positions of the fatty acid components can vary widely, there are an enormous number of possible molecular combinations. Natural fats and oils are complex mixtures of triacylglycerols; butterfat, for example, contains on the order of 100 000 different molecular species.

Each species is defined by the fatty acids in the 1-, 2- and 3-positions of the glycerol backbone. For example, soybean oil is dominated by two species: LiLiLi, in which linoleic acid (Li) occupies all three positions on the glycerol backbone; and LiLiO, in which oleic acid (O) occupies the 3-position. It should also be mentioned that some ether analogues of triacylglycerols occur in nature. Marine lipids, for example, can contain an ether-linked alkyl group in the 1-position and ester-linked acyl groups in the 2- and 3-positions of the glycerol backbone. From a physical point of view they still behave like triacylglycerols.

Diacylglycerols, formerly known as *diglycerides*, contain two fatty acid groups, which can be located at different positions on the glycerol backbone. The same diacylglycerol molecule can exist as two different isomers, depending on whether the acyl groups are in the 1,2-position (note that this is equivalent to the 2,3-position) or the 1,3-position. Acyl migration takes place easily, and during processing an equilibrium mixture consisting of approximately equal amounts of the two isomers is usually formed.

Sterols occur in free form or as fatty acid esters. The molecules are characterized by a steroid skeleton, which makes most of the molecules rigid. The most important member is *cholesterol* – a lipid of great importance as a constituent of the cell membranes in our body. There are also sterols in plants, which are closely related to cholesterol, such as stigmasterol. Cholesterol is transported in the circulation as cholesterol esters within lipoproteins. In the liver, cholesterol is transformed into bile acids, which are polar lipids that function like detergents in the intestinal digestion of fats.

Cholesterol has for a long time been regarded as a crucial lipid for the functioning of mammalian cell membranes. A recent study of knockout mice lacking the ability to synthesize cholesterol was therefore most surprising, as

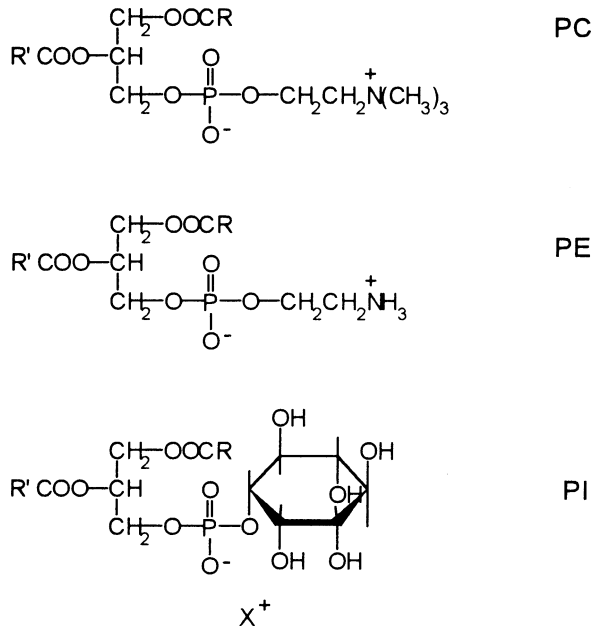

Figure 1.1 Chemical formulae of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI). The counter-ion (usually sodium) is denoted by X^+.

they were viable even though cholesterol was replaced by another sterol (Wechsler *et al.*, 2003). An important mechanism induced by cholesterol in the cell membranes is phase separation of the bilayer into cholesterol-rich domains (lipid rafts and caveolae; see also Chapters 3 and 9) and cholesterol-poor regions. Perhaps this segregation was still achieved in the knockout mice by the alternative sterol.

2. *Polar lipids*

Monoacylglycerols or *monoglycerides* are the simplest type of polar lipids, and are the dominant functional additives used in industrial food processing. They are produced on a large scale to high purity by molecular distillation. Of the two isomers, the 1-isomer dominates in the equilibrium mixtures formed by acyl migration (about 90% 1-isomer and 10% 2-isomer).

Phospholipids or *phosphoglycerolipids* associate spontaneously into lipid bilayers in water, which is the basic mechanism behind the formation of biological membranes. They have acyl groups ester-bound in the 1- and 2-positions of the glycerol backbone, and a polar group involving phosphate in the 3-position. The most important members of this lipid class are phosphatidylcholine (PC), phosphatidylethanolamine (PE) and

phosphatidylinositol (PI), all shown in Figure 1.1. Other phospholipids that occur commonly are phosphatidylglycerol (PG), phosphatidylserine (PS) and phosphatidic acid (PA). The aqueous interactions of phospholipids are closely related to their ionic character. Whereas PC and PE are zwitterionic (containing one positive group and/or one negative group, depending upon the pH), the others (i.e. PI, PG, PS, and PA) are all anionic at physiological pH.

The fatty acid in the 1-position of a phospholipid can be split off by the phospholipase enzyme PLA_1, and the fatty acid in the 2-position is released by PLA_2. In this way lysophospholipids are obtained. These are as polar as soaps and can therefore solubilize the components of cell membranes, causing lysis of the cells.

Galactolipids or *galactosylglycerolipids* originate mainly from the thylakoid membranes of plants, where photosynthesis takes place. Therefore they are extremely important and probably the most commonly occurring lipids on earth. The two major classes of galactolipids are monogalactosyl-diacylglycerols (MGDG) and digalactosyl-diacylglycerols (DGDG).

Sphingolipids contain a long-chain amine termed *sphingosine* linked by an amide bond to a fatty acid. Such simple amides are called *ceramides*, and occur for example in the skin. Free sphingosine, which is formed enzymatically, is a cationic detergent-like molecule. The sphingosine molecule contains hydroxyl groups along the hydrocarbon chain, and one hydroxyl group next to the amine group can be linked to a polar group. If this group is phosphatidylcholine, the molecule is rather similar to PC and is called *sphingomyelin*. Sphingomyelin associated with cholesterol forms phase-separated domains in mammalian cell membranes (the lipid rafts and caveolae mentioned above) with specific biological functions.

Gangliosides are bioactive ceramides occurring mainly in the brain and in milk. Milk contains two types, called GM3 and GD3, in which either one (GM3) or two (GD3) sialic acid residues are linked to a galactose unit that, together with a glucose unit, is bound to the ceramide molecule. Another type of sphingolipid containing a sugar group, which also occurs in the brain, is the cerebrosides. A subgroup is the sulfatides, which have a sulfate group attached to this sugar.

The physical properties of lipids and their relation to molecular shape and amphiphilic character are central themes in this book. The dualistic properties of the molecules in relation to water, with flexible hydrocarbon chains avoiding water contact and a polar head group that tends to orient towards water defines the amphiphilicity. From a physical point of view, lipid molecules are therefore often illustrated simply by a line representing the hydrocarbon chain axis, attached to a circle representing the polar head group. This schematic representation of structures, not the detailed chemical architecture, will be used in structural descriptions of the liquid state and the aqueous phases, which are highly disordered.

In industrial applications of lipid functionality (see also Section B below), the molecules are usually modified by chemical processes. The most important group of such functional lipids is *food emulsifiers*, which have many applications in addition to emulsification. Distilled monoacylglycerols and phospholipids dominate these emulsifiers. Phospholipids in pharmaceutical applications are usually prepared from egg yolk, whereas those used in food applications are by-products from the refining of vegetable oils. After extraction from soybeans, rapeseed or other oil crops, the free fatty acids are first removed as soaps by a sodium hydroxide solution. The next step (called degumming) is removal of the phospholipids by water due to the formation of an aqueous liquid-crystalline phase. This crude mixture is usually termed lecithin. Alternatively the word lecithin is used in the case of purified fractions (and even pure PC is sometimes termed lecithin). Other food emulsifiers are diacetyl-tartaric esters of monoacylglycerols (under the name DATEM), sodium and calcium salts of stearoyl-lactylates (termed SSL and CSL, respectively), and polylactoyl esters of stearic acid. Some food emulsifiers are also used in the formulation of drugs and cosmetics. Important examples include ethoxylated lipids, such as polyoxyethylene monoacylglycerols and polyoxyethylene sorbitan monostearate.

Finally, some other types of important lipids should be mentioned. If we consider *fat-soluble vitamins*, the significance of the physical state is illustrated by the fact that their bioavailability depends on their solubilization in triacylglycerols and phospholipids in the food. We may in this context apply the simple physical description mentioned above. On this level of description, vitamin D resembles cholesterol, with a polar head group and a rigid hydrocarbon chain, whereas each of the others – vitamin A (retinol), vitamin E (tocopherol), and vitamins K_1 and K_2 – can be represented by a polar head group and a flexible hydrocarbon chain. With regard to the complex physiological functions of vitamins, such a description illustrates the gap between lipid biochemistry and the physical aspects we deal with in this book. This is particularly evident when we consider the effects of small chemical modifications on the diversity of biological signalling by different *eicosanoids* (prostaglandins, thromboxanes and leukotrienes). This lipid family is derived from arachidonic acid, a 20-carbon fatty acid with four double bonds ($C_{20:4}$). The physical description of their disordered structures, which represents their functional states, is at present very primitive.

B. Structure, physical properties and functional properties

Lipids are frequently used in technical applications in order to obtain a specific function. One example is the emulsification of oil in water. In order to obtain an emulsion, we can add a polar lipid to an oil–water mixture, and after a mechanical agitation process, an emulsion is obtained. The polar lipid used is