

Lipids and Biomembranes of Eukaryotic Microorganisms



LIPIDS AND BIOMEMBRANES OF EUKARYOTIC MICROORGANISMS

Edited by JOSEPH A. ERWIN

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Preface

One of the most active fields of current research involves an attempt to construct a molecular biology of membranes. In this endeavor microbial systems are emerging as useful research tools, which they have been in other areas of biochemistry and cell biology. One universal fact has emerged from these studies: Membranes are composed of two types of molecules, proteins and lipids. While little has been learned in the area of membrane proteins in microorganisms, a great deal is now known about their lipids. However, the bulk of the literature on microbial lipids is both scattered and of relatively recent vintage; hence a book that synthesizes our knowledge of microbial lipids and relates this knowledge to microbial membranes is both timely and useful. This volume attempts such a synthesis for eukaryotic microorganisms.

Why does this study restrict itself to eukaryotic microorganisms? In part, practical considerations demanded a focus that was more contained than the entire microbial world. The recent literature on bacterial lipids and their possible function in membranes is so extensive that it would demand a separate volume.

While the upper limits of the microbial world cannot be easily defined (mushrooms and seaweeds, for example, could be classified as "microbial" only out of courtesy), microbial organisms fall into two very distinct categories; the prokaryotic, consisting of the bacteria and the blue-green algae, and the eukaryotic, consisting of all other algae plus yeasts, fungi, and protozoa (for a discussion of the two different cellular types, see Stanier *et al.*, 1963 and Stanier, 1970).^{*} This distinction appears to be one of the most fundamental to be found in the biological realm, and it is to a large degree based upon the subject matter of this book, membranes and lipids. Eukaryotic microorganisms, in common with the cells of higher plants and animals, characteristically contain a variety of intracellular membranous organelles,

^{*} Stanier, R. Y. (1970). *Symp. Soc. Gen. Microbiol.* **20**, 1-38.

Stanier, R. Y., Doudoroff, M., and Adelberg, E. A. (1963). "The Microbial World." Prentice-Hall, Englewood Cliffs, New Jersey.

many of which are self-reproducing. Such organelles are lacking in prokaryotes; the simple intracellular vesicles of photosynthetic bacteria and the mesosomes common to many bacteria are derivatives of the bacterial limiting or plasma membrane (Stanier, 1970). Even the plasma membrane of prokaryotes differs from that of eukaryotes. Endocytosis, for example (a term that embraces related phenomena such as pinocytosis, phagocytosis, and reversed or secretory pinocytosis), is a fundamental property of the plasma membranes of eukaryotes but is never found among prokaryotes (Stanier, 1970).

The chemical composition of eukaryotic and prokaryotic organisms is also strikingly different. Sterols and polyunsaturated fatty acids are ubiquitous components of all eukaryotic cells—plant, animal, and microbial (Chapters 1 and 2). In bacteria, however; polyunsaturated fatty acids are completely absent, and sterols, if present at all, are found only in trace amounts. [Blue-green algae are an intermediate group in that some species contain sterols and polyunsaturated fatty acids but others do not (Chapters 1 and 2).] Galactolipids and sulfolipids are the universal and principal components of the photosynthetic membranes of all eukaryotes but are absent in photosynthetic bacteria (see Chapter 5). Again, the glycerophospholipids—phosphatidyl choline, phosphatidyl serine, and phosphatidyl inositol—commonly found among eukaryotes (see Chapter 3) are rarely found among prokaryotes. In contrast, phosphatidyl glycerol, a major and common lipid component of prokaryotes, is either a minor component of eukaryotic lipids or absent altogether (Chapter 3). Similarly, lipids containing long-chain bases in lieu of glycerol (the ceramides and sphingolipids) are widespread among eukaryotes and are virtually never found in prokaryotes.

Thus different groups of eukaryotic microorganisms may display variations in membrane morphology and in lipid composition, but these are simply variations on a common theme characteristic of the entire eukaryotic world—plant, animal, and microbial. Hence the results of studies on the role of lipids in the formation and function of membranes in eukaryotic microorganisms are very likely to be applicable to the bulk of the biological world. We would have to employ considerably greater caution if we attempted to extrapolate the results of studies on the membrane systems of bacteria to the eukaryotic world, even though the apparent simplicity of the membrane systems of bacteria make them attractive as research tools.

In Chapters 1–4 each of the major classes of lipids—sterols, fatty acids, phospholipids, and sulfolipids—is considered separately. In each case an attempt has been made to provide a comprehensive summary and to evaluate critically the literature on the occurrence and biosynthesis of

these compounds in yeasts, fungi, algae, and protozoa. Physiological functions of these lipids, particularly their role in the membranes of the organisms, are discussed. In some cases attention has been called to the possible usefulness of lipids as taxonomic criteria.

A reading of these chapters reveals that much has been learned about eukaryotic microbial lipids and their biosynthesis during the last decade or so, but obvious vacuums of information exist. Perhaps the most serious of these are the paucity of data on the lipid composition of specific types of membranes in various eukaryotic microorganisms and our lack of understanding of the regulatory mechanisms governing the control of different lipids in these organisms (see Chapter 3).

In Chapters 5 and 6 experimental systems for studying the relation between the structure of lipids and their function in biomembranes are discussed. These systems include the photosynthetic membranes in organisms such as *Euglena*, *Chlorella*, and *Chlamydomonas* in which the formation of the chloroplasts is susceptible to experimental control (Chapter 5) and fatty acid auxotrophic mutants of yeasts and *Neurospora* in which the fatty acid composition of the membrane lipids can be altered by the experimenter (Chapter 6).

It is hoped that this volume will prove useful to lipid biochemists, microbial physiologists, taxonomists, and cell biologists who are interested in the molecular aspects of biomembranes.

JOSEPH A. ERWIN

A Note on Taxonomy and Biological Nomenclature

The members of the classic major groups of eukaryotic microorganisms (algae, fungi, yeasts, and protozoa) are highly heterogeneous and their boundaries are indistinct, which has led to jurisdictional disputes among taxonomists; for example, a number of groups of unicellular and colonial flagellated microorganisms such as euglenids, chrysomonads, dinoflagellates, and cryptomonads contain both photosynthetic and nonphotosynthetic forms. All of these are often termed phytoflagellates and are assigned by zoologists to the phylum protozoa (Honigberg *et al.*, 1964).^{*} Botanists, on the other hand, usually classify these same organisms as algae (Smith, 1950).[†] Similarly, the slime molds are considered to be protozoa by many zoologists, but botanists classify them as fungi (Cronquist, 1971).^{**} The fungi and the yeasts also present a taxonomic problem; it is not always possible to distinguish a true yeast from fungi that have yeastlike stages in their life cycle. It should be clear then that the designation of some groups of eukaryotic microorganisms as algae or protozoa or as yeasts or fungi is often arbitrary and largely a matter of personal taste. As editor of this volume I have made no attempt to impose my own tastes in this matter on the other authors. The nonbiologist should not be unduly upset to find the same organism called a protozoan in one chapter and an alga or fungus in the following chapter.

Microorganisms can also be categorized on the basis of their mode of nutrition (Lwoff, 1951).[‡] This has led to widespread use of a terminology that may sometimes be confusing to the nonbiologist. Organisms that

^{*} Honigberg, B. M., Balmuth, N., Bovee, E. C., Corliss, J. O., Gojdics, A., Hall, R. P., Kudo, R. R., Levine, N. D., Loeblich, A. R., Jr., Weiser, J., and Wenrich, D. A. (1964). *J. Protozool.* 11, 7.

[†] Smith, G. M. (1950). "The Fresh Water Algae of The United States." McGraw-Hill, New York.

^{**} Cronquist, A. (1971). "Introductory Botany," pp. 125-222. Harper, New York.

[‡] Lwoff, A. (1951). In "Biochemistry and Physiology of Protozoa" (A. Lwoff, ed.), Vol. 1, pp. 1-28. Academic Press, New York.

grow on mineral media in the light and that derive all their organic carbon compounds from the fixation of carbon dioxide via photosynthesis are designated as photoautotrophs. If such organisms require supplementation of their mineral media with vitamins, they are termed photoauxotrophs. If growth supported by photosynthesis is supplemented by utilization of substrate levels of organic compounds supplied in the medium, such growth is referred to as photoheterotrophic. Microorganisms that grow in the absence of light utilizing exogenously supplied organic compounds as sources of carbon and energy are termed heterotrophs; those that utilize only dissolved nutrients are osmotrophs, while those that can take up particulate matter (including smaller microorganisms) are phagotrophs.

Many microscopic algae and fungi, most yeasts, and some protozoa can be cultured in the laboratory in pure culture (free of all other living organisms). Such cultures are often termed axenic and are usually clonal (they are derived from a single cell via multiplication by asexual means and hence are essentially genetically homogeneous). Cultures that contain more than one type of living organism are termed nonaxenic. Cultures containing a single type of algae (usually clonal) but contaminated with bacteria are designated by the botanists as unialgal.

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CHAPTER 1

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I. Distribution

A. ALGAE

1. Introduction

The major sterols in leafy material of higher plants are frequently sitosterol (I) and stigmasterol (II), which are accompanied by a complex

mixture of minor sterols that can vary considerably from species to species. Compared with cholesterol (III), which until recently was considered a typical animal sterol but which is now known to occur widely in traces in higher plants, these two sterols exhibit structural features characteristic and widespread in phytosterols: (1) an additional side chain, which can be methyl or methylene (1-C) or ethyl or ethylidene (2-C), at C-24, and (2) a double bond at C-22. The major sterols present in the different algal classes vary much more considerably, and this much greater variety is also reflected in the carotenoid distribution in algae compared with higher plants.

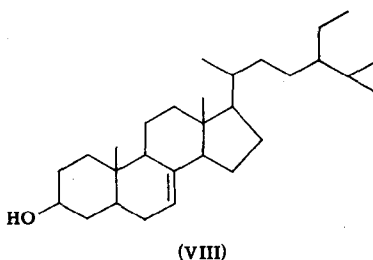
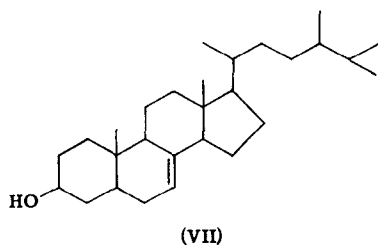
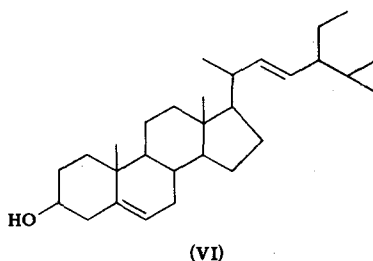
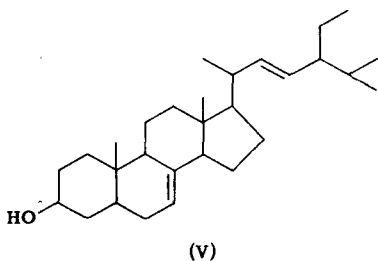
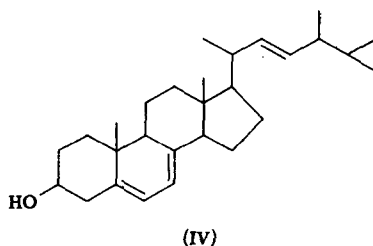
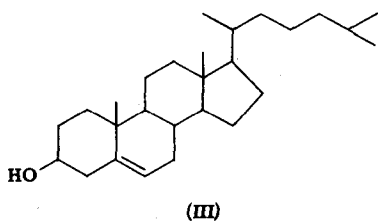
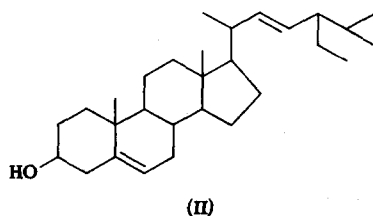
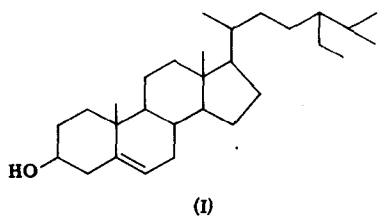
Recently, analytical techniques for detecting sterols have improved greatly and have become much more sensitive. In particular, mass spectrometry combined with gas-liquid chromatography has provided the means of identifying very small amounts of sterols with a reliability and confidence that was unknown 5 years ago. Some earlier identifications must therefore be considered today with some caution; they are summarized by Miller (1962).

2. Chlorophyta

a. Sterols. The main microalgae examined in this class belong to the order Chlorococcales and are *Chlorella* sp. They tend to fall into three main categories in which the major sterols are ergosterol ($\Delta^{5,7}$) (IV), chondrillasterol (Δ^7) (V), and poriferasterol (Δ^5) (VI). The additional minor sterols are indicated in Table I. *Scenedesmus obliquus* (two strains) falls into the third group in accumulating chondrillasterol, ergost-7-enol (VII), and chondrillast-7-enol (VIII) in about the same relative amounts as do the *Chlorella* sp. (Patterson, 1967). Earlier investigations had indicated a different distribution in *S. obliquus* (Bergmann and Feeney, 1950). The absolute configuration at C-24 is β in all sterols in *Chlorella* sp. This contrasts sharply with the C-24 alkylated sterols in higher plants, which always have the α configuration at C-24; compare, for example, poriferasterol (VI) with stigmasterol (II). One member of the Chlorococcales, *Hydrodictyon reticulatum*, is said to synthesize spinasterol (XI), which has the 24- α configuration (Hunek, 1969), but the identification is not unequivocal.

The colonial Chlorophyta so far examined are limited, but results indicate a sharp differentiation from the Chlorococcales. In the Uvales, *Ulva pertusa* contains 0.12% dry weight of sterol, of which 74% is cholesterol, 2.5% 24-methylene cholesterol (XII), and 14.5% fucosterol (XIII) (Ikekawa *et al.*, 1968), while the major component of *U. lactuca* was 28-isofucosterol (XIV) (Δ^5 -avenasterol) (Knights, 1965; Gibbons *et al.*,

1968). Earlier, sitosterol had been reported as the main sterol of *U. lactuca* (Heilbron *et al.*, 1935), but sitosterol and 28-isofucosterol were not separable by the techniques then available. Similarly, the major sterol of *Enteromorpha intestinalis* (Gibbons *et al.*, 1968) and *E. linza* (K. Tsuda and Sakai, 1960) is 28-isofucosterol, and this is probably true for *E. compressa*,



which was reported in 1935 to contain sitosterol. *Monostroma nitidum* is said to contain haliclonasterol (XV), a C-20 isomer of campesterol (K. Tsuda and Sakai, 1960). In the only member of the Cladophorales so far examined, *Chaetomorpha crassa*, the major sterol is sitosterol (I), and it is accompanied by smaller amounts of cholesterol (III), 24-methylene cholesterol (XII), campesterol (XVI), and brassicasterol (XVII) (Ikekawa *et al.*, 1968). A sterol glycoside is reported in *Oedogonium* sp. (Heilbron *et al.*, 1935).

b. 4-Methyl and 4,4-Dimethyl Sterols. Cycloartenol (XVIII) and 24-methylene cycloartanol (XIX) but not lanosterol (XX) have been detected in small amounts in *Enteromorpha linza* and *Ulva lactuca* (Gibbons, 1968), and 24-methylene lophenol (XXI) and 24-ethylidene lophenol (XXII) have been identified in *E. intestinalis* (Gibbons *et al.*, 1968).

TABLE I
STEROLS OF *Chlorella* SP. (% OF TOTAL STEROL)^{a, b}

Species	$\Delta^{5,7}$ -Sterols		Δ^7 -Sterols			Δ^5 -Sterols		
	1	2	3	4	5	6	7	8
<i>C. vannielii</i>	76	24						
<i>C. sorokiniana</i>	75	25						
<i>C. nocturna</i>	66	34						
<i>C. simplex</i>	70	30						
<i>C. candida</i>	76	24						
<i>C. protothecoides</i>	35	65						
var. <i>nannophila</i>								
<i>C. protothecoides</i>	16	84						
var. <i>communis</i>								
<i>C. ellipsoidea</i>						28	56	16
<i>C. saccharophila</i>						30	60	7
<i>C. vulgaris</i> ^c			25	65	10			
<i>C. glucotropha</i>			15	75	10			
<i>C. fusca</i>			27	59	12			
<i>C. emersonii</i>			28	62	9			
<i>C. miniata</i>			16	59	9			

^a From Patterson (1971).

^b Key to numbers: 1, ergosterol (IV); 2, unidentified $\Delta^{5,7}$ -sterols; 3, ergost-7-enol (VII); 4, chondrillasterol (V); 5, ergost-5-enol (IX); 6, chondrillast-7-enol (VII); 7, poriferasterol (VI); 8, elionasterol (X).

^c See also Tomita *et al.* (1970).