

Liposomes

a practical approach

Edited by
R R C New

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Preface

Over the last twenty years the liposome has changed its status from being a novel plaything for the laboratory worker to a powerful tool for the industrialist—with the gap between the ideal desired characteristics of liposomes and what is technically feasible becoming narrower all the time. The properties of membrane preparations have been researched extensively, and ingenious ways have been found of manipulating them to confer behavioural characteristics which stretch the imagination—sensitivity to heat, light, pH, magnetic field, and chemical structure. Few other areas of study can routinely bring into play such a wide range of phenomena.

Liposomes may be defined simply as lipid vesicles enclosing an aqueous space. They were brought to the attention of the scientific world by A.D. Bangham in 1965, and proposed as useful models for cell membranes. Indeed, using the definition above, even cells and organelles themselves may be considered to be just sophisticated types of liposome. Using these artificial membrane vesicles, great insight was brought to many aspects of cell physiology such as permeability, fusion, membrane-bound enzyme properties etc, and will continue to do so. More recently, the potential of liposomes in the medical field is slowly becoming realized, with several clinical trials in progress examining their use as drug delivery agents. Applications in the areas of diagnosis, immuno-modulation, and genetic engineering have been identified and developments will follow.

In spite of numerous books and papers written on the subject, many people are still unclear about what liposomes are, and how work employing them is carried out. The aim of this book is to dispel some of that mystery. It has been written with two groups of people in mind. Firstly, the laboratory worker, who wishes to have at his/her fingertips detailed, tried-and-tested methods which will be accepted by experienced workers in the field as giving results which are reliable and convincing. The methods presented here are not a comprehensive list of everything which can be, or has been done, but are a careful selection of the most useful and most easily applied methods for the general laboratory. The second category of reader is the graduate student who may have had little exposure to membrane techniques, and who will benefit from an understanding of the theory behind membrane processes. It is hoped that this may act as an introductory textbook for the basic principles of liposomology, before embarking on detailed study of more learned treatises.

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Roger R.C. New

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Abbreviations

ADC	analogue to digital converter
AM	ammonium molybdate
ANS	anilinonaphthalene sulphonate
APSA	<i>N</i> -(<i>p</i> -aminophenyl) stearylamine
α -T	α -tocopherol
α -TS	α -tocopherol succinate
BCECF	2,7-biscarboxyethyl-5(6)-carboxyfluorescein
BCIP	5-bromo, 4-chloro, 3-indolyl phosphate
BHT	butylated hydroxytoluene
BPS	biotinylated phosphatidyl serine
BSA	bovine serum albumin
CDI	carbodiimide
CF	carboxyfluorescein
CHEMS	cholesterol hemisuccinate
CL	cardiolipin
CMC	critical micelle concentration
DCCI	dicyclohexyl carbodiimide
DLPC	dilauroyl phosphatidyl choline
DMPC	dimyristoyl phosphatidyl choline
DMPG	dimyristoyl phosphatidyl glycerol
DODAC	dioctadecyl ammonium chloride
DOPC	dioleoyl phosphatidyl choline
DPPA	dipalmitoyl phosphatidic acid
DPPC	dipalmitoyl phosphatidyl choline
DPPG	dipalmitoyl phosphatidyl glycerol
DRV	dried-reconstituted vesicle
DSPC	distearoyl phosphatidyl choline
DTT	dithiothreitol
EDCI	1-ethyl-3-(dimethyl aminopropyl)-carbodiimide
ESR	electron spin resonance
F	fluorescence
FPL	French pressed liposomes
FTS	freeze-thaw sonication
G6PDH	glucose-6-phosphate dehydrogenase
HDL	high density lipoproteins
IUV	intermediate-sized unilamellar vesicle
LDL	low density lipoprotein
LPC	lyso-phosphatidyl choline
LUV	large unilamellar vesicle
MEL	micro-emulsification liposomes
Mes	morpholino ethane sulphonic acid
MLV	multi-lamellar vesicle
Mops	morpholino propane sulphonic acid
MPS	monocyte phagocyte system
MTT	3-[4,5-Dimethyl thiazol-2-yl]-2,5-diphenyl tetrazolium bromide
MVL	multi-vesicular liposome
NHSIA	<i>N</i> -hydroxysuccinimido-iodoacetate

PA	phosphatidic acid
PC	phosphatidyl choline
PCS	photon correlation spectroscopy
PE	phosphatidyl ethanolamine
PG	phosphatidyl glycerol
PHA	pulse height analysis
PI	phosphatidyl inositol
PMSF	phenyl methyl sulphonyl fluoride
PS	phosphatidyl serine
PTA	phosphotungstic acid
PTR	phase transition release
PVP	polyvinyl pyrrolidone
RES	reticular endothelial system
RET	resonance energy transfer
REV	reverse-phase evaporation vesicle
RSVE	reconstituted Sendai virus envelope
SAMSA	5-acetylmercaptosuccinic anhydride
SATA	succinimidyl-5-acetylthioacetate
SFFF	sedimentation field flow fractionation
SM	sphingomyelin
SMPB	<i>N</i> -succinimidyl (4-[<i>p</i> -maleimidophenyl])butyrate
SPDP	<i>N</i> -succinimidyl pyridyl dithiopropionate
SPLV	stable plurilamellar vesicle
SUV	small unilamellar vesicle
TAC	time to amplitude converter
TBA	thiobarbituric acid
TBS	Tris-buffered saline
TEA	triethylamine
TEP	1,1,3,3, tetraethoxypropane
TMS	trimethyl silane
TNBS	trinitrobenzene sulphonic acid
TNS	(6-[<i>p</i> -toluidinyl]naphthalene-2-sulphonate)
TO	triolein
WCOT	wall-coated open tubular

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ABBREVIATIONS

xv

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CHAPTER 1

Introduction

ROGER R.C. NEW

1. AIM OF BOOK

This book is intended both as a compilation of methods which may act as a useful reference source for workers already in the field of liposomes, and as a simple guide to workers outside the field who are wondering whether liposomes might have some application to their own speciality, and if so, what sort of liposome is best to use. Consequently, for most of this volume the emphasis is not on the applications liposomes can be put to, since it is assumed the reader will already have his/her own uses in mind—uses which may be entirely original, and could not be anticipated by the authors of this book. Instead, we hope to make readers sufficiently well-informed about liposomes that they may choose for themselves the best methods to adopt for their purpose, and in attempting this, we have concentrated heavily on methodology as the means of classifying different areas of the subject.

The first half of the book describes in detail the different ways of making, modifying, purifying, and characterizing liposomes, while the last half discusses ways in which information can be obtained about the behaviour of the finished product in different biological systems. This introductory chapter gives a very simple guide to what liposomes are, and outlines the general principles involved in choosing a given type of liposome for a particular application.

2. STRUCTURE OF LIPOSOMES

Liposomes are simply vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecules (usually phospholipids). They form spontaneously when these lipids are dispersed in aqueous media, giving rise to a population of vesicles which may range in size from tens of nanometres to tens of microns in diameter. They can be constructed so that they entrap quantities of materials both within their aqueous compartment and within the membrane. The value of liposomes as model membrane systems derives from the fact that liposomes can be constructed of natural constituents such that the liposome membrane forms a bilayer structure which is in principal identical to the lipid portion of natural cell membranes—the 'sea of phospholipids' in the Singer and Nicholson model. The similarity between liposome and natural membranes can be increased by extensive chemical modification of the liposome membrane, and may be exploited in areas such as drug targeting or immune modulation, both *in vivo* and *in vitro*, where the ability to mimic (or to improve upon) the behaviour of natural membranes, and also to be degraded by the same pathways, makes them a very safe and efficacious vehicle for medical applications. Alternatively,

Introduction

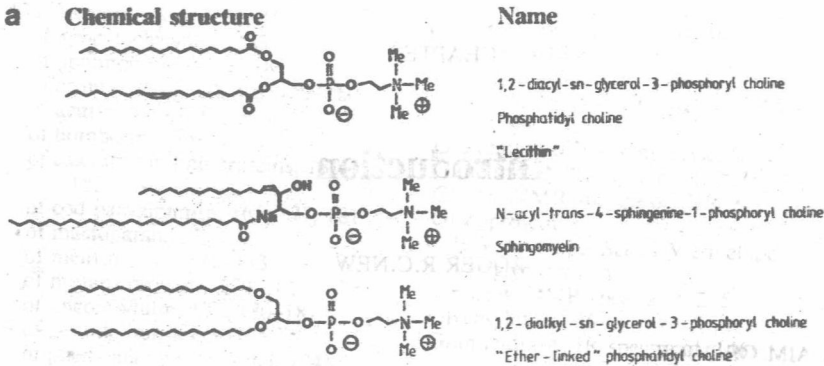


Figure 1(a) Three main classes of choline-containing phospholipids. Choline-containing phospholipids are the most abundant phospholipids in nature. In phosphatidyl choline (lecithin), a three-carbon glycerol bridge links two long chain fatty acids with a phosphoryl choline moiety; by convention, the fatty acids are said to occupy the 1 and 2 positions of the glycerol bridge while the polar headgroup is in position 3. The bridge carbon in position 2 (the middle carbon of the three-carbon glycerol bridge) is asymmetric—i.e., it displays optical activity because each of the four bonds is joined to a different chemical group. Natural phospholipids can have many different fatty acids conjugated in positions 1 and 2, usually with the longer and/or more unsaturated chain in position 2 as shown here (see also Table 2). Sphingomyelin consists of a single fatty acyl chain conjugated via an amide linkage to the nitrogen of sphingosine, which is again linked to phosphoryl choline. The lipid portion without the headgroup is known as ceramide. In addition to sphingomyelin, this ceramide residue is found in molecules known as gangliosides and cerebroside, which contain polysaccharide headgroups in place of phosphoryl choline. Hydrocarbon chains can also be joined to the glycerol bridge via ether linkages as in the third class of phosphocholine lipids, of which a synthetic analogue useful in liposome formulations is shown here. In nature, diether phospholipids usually have a glycerol headgroup, while choline or ethanolamine headgroups are more usually found in molecules containing one each of the acyl and ether linkages (e.g. in plasmalogens). These lipids are considerably less prevalent in nature than the diacyl phosphatidyl choline.

