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Development and initial evaluation of PEG-stabilized bilayer disks as novel model membranes

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Abstract

We show in this study that stable dispersions dominated by flat bilayer disks may be prepared from a carefully optimized mixture of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethyleneglycol)-5000] [PEG-DSPE(5000)]. By varying the content of the latter component, the average diameter of the disks can be changed in the interval from about 15 to 60 nm. The disks show excellent long-term stability, and their size and structure remain unaltered in the temperature range between 25 and 37 °C. The utility of the disks as artificial model membranes was confirmed and compared to uni- and multilamellar liposomes in a series of drug partition studies. Data obtained by isothermal titration calorimetry and drug partition chromatography (also referred to as immobilized liposome chromatography) indicate that the bilayer disks may serve as an attractive and sometimes superior alternative to liposomes in studies aiming at the investigation of drug–membrane interactions. The disks may, in addition, hold great potential for structure/function studies of membrane-bound proteins. Furthermore, we suggest that the sterically stabilized bilayer disks may prove interesting as carriers for in vivo delivery of protein/peptide, as well as conventional amphiphilic and/or hydrophobic, drugs.

Keywords: Bilayer disks; Drug partitioning; Liposome; Model membrane; PEG lipid; Phospholipid; Immobilized liposome chromatography

1. Introduction

Recent developments in the area of drug discovery, including the use of combinatory chemistry in combination with highly automated synthesis systems, have created a need for methods that allow for fast screening of large sets of drug candidates. Because the majority of drugs have to cross one or several biological membranes in order to reach the target site, evaluation of drug–membrane interactions, and in particular the determination of drug partition and diffusion constants, constitute an important part of the screening procedure. Although predictions obtained by theoretical, in silico, methods [1,2] may serve as valuable tools during the initial selection of drug candidates, there is still a great demand for simple and reliable experimental methods. The validity of these methods relies to a large extent on the availability of suitable model membranes that accurately reflect the physicochemical properties of the relevant biological membranes.

Due to their structural similarity with biological membranes, phospholipid liposomes have been extensively used as model membranes, and during the last 10 years, several liposome-based methods for the determination of drug partitioning have been developed and tested. These include different potentiometric [3–5], chromatographic [6–12], electroforetic [13–15], and calorimetric [16,17] methods, as well as methods based on changes in the spectroscopic behaviour of the drug molecule [18]. The results of these studies indicate the good potential of phospholipid liposomes to serve as models for biological

Abbreviations: DSPC, 1,2-distearoyl-sn-glycero-3-phosphocholine; DPC, drug partition chromatography; EPC, egg phosphatidylcholine; ITC, isothermal titration calorimetry; PEG, poly(ethyleneglycol); PEG-DSPE(5000), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethyleneglycol)-5000].

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membranes in partition studies. A number of problems have been identified, however, that need to be solved in order to improve the performance and ease of handling of the liposome-based techniques. First, the current methods available for liposome preparation give rise to a significant amount of polydispersity in both the size and structure of the liposomes. Most notably, the preparations do as a rule contain an unknown fraction of bi- or multilamellar liposomes. Depending on the properties of the drug, as well as on the analytical technique used, this fact may give rise to considerable difficulties concerning the interpretation and quantification of the results. More specifically, unless ample time is given for equilibration between the different bilayers of the bi- and multilamellar structures, a proportion of the lipids will, in effect, be hidden from interaction with the analyte. Moreover, also in the unlikely event of a purely unilamellar preparation, the interpretation of data may be confused by the fact that initially only the outer leaflet of the membrane is accessible for direct interaction. Second, because phospholipid liposomes do not represent thermodynamically stable but merely kinetically trapped structures, they tend to aggregate and fuse with time. Eventually, the liposome sample will phase separate into a lamellar phase in excess water. This behaviour is manifested in an inherently poor long-term stability of conventional liposome preparations.

In this study, we introduce an alternative model membrane, the sterically stabilized bilayer disk, which evades the abovementioned problems associated with conventional liposomes.

In addition to their use in drug partition studies, we believe that the sterically stabilized disks may find other important biochemical, biotechnical, and pharmaceutical applications. In particular, the potential use of the disks as carriers for drug delivery deserves attention. A brief discussion on this matter can be found in the Conclusions and future aspects section. In this first communication, we focus, however, on preparation, characterization, and evaluation of sterically stabilized disks intended for drug partition experiments.

2. Materials and methods

2.1. Materials and solutions

Glass columns (HR 5/2 and 5/5 I.D. 5 mm), Superdex 200 prep grade gel and Sephadex G-50 superfine were purchased from Amersham Biosciences (Uppsala, Sweden), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC, >99%), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethyleneglycol)-5000] (PEG-DSPE(5000), >99%), and cholesterol (>98%) from Avanti Polar Lipids (Alabaster, AL, USA), heptyltriphenylphosphoniumbromide and hexyltriphenylphosphoniumbromide from Labora (Stockholm, Sweden), egg phosphatidylcholine (grade 1) from Lipid Products (Nutfield, U.K.), 5- and 6-carboxyfluorescein from Molecular Probes (Leiden, The Netherlands), and alprenolol, atenolol, chlorpromazine, corticosterone, cortisone, hydrocortisone, metoprolol, oxprenolol, pindolol, prednisolone, promethazine, sulfasalazine, verapamil, tetraphenylphosphoniumchloride, and Triton X-100 (reduced) from Sigma (St. Louis, MO, USA). Desmethyldiazepam, diazepam, flunitrazepam, nitrazepam, and oxazepam were gifts from Smith Kline-Beecham (King of Prussia, PA, USA), and diclofenac, diflunisal, flurbiprofen, ibuprofen, indomethacine, indoprofen, lidocaine, loperamide, naproxen, phenytoin, piroxicam, propranolol, theophylline, and warfarin from AstraZeneca (Södertälje, Sweden). Chemicals not listed were of analytical grade or as stated in given references. The buffer used was 150 mM NaCl, 1 mM Na_2EDTA, 10 mM Tris/HCl, pH 7.4.

2.2. Preparation of liposomes and disks

All preparations contained 40 mol% cholesterol and different proportions of DSPC and PEG-DSPE(5000) unless otherwise stated. Cholesterol, DSPC, and PEG-DSPE(5000) were codissolved in chloroform. The chloroform was evaporated under N_2(g), and the remaining chloroform was removed under vacuum for at least 12 h. The lipid film was then hydrated in the buffer at 50 °C and vortexed vigorously. When indicated, the material was extruded 15 times through a polycarbonate filter (100 nm pore size). A LiposoFast membrane extruder from Avestin (Mannheim, Germany) was employed for this procedure.

2.3. Cryo-transmission electron microscopy

Thin (10–500 nm) sample films were prepared by a blotting procedure performed under controlled temperature (+25 °C) and humidity conditions within a custom-built environmental chamber. A small drop of sample was placed on an electron microscopy (EM) copper grid covered with a holey polymer film, and excess solution was removed by blotting with a filter paper. The film was vitrified by plunging the grid into liquid ethane held just above its freezing point, and the grid was thereafter transferred to a Zeiss EM 902 transmission electron microscope (Oberkochen, Germany) for examination. To prevent sample perturbation and the formation of ice crystals, the specimens were kept cool (below −165 °C) during both the transfer and viewing procedures. All observations were made in zero-loss bright-field mode and at an accelerating voltage of 80 kV. The technique has been described in detail elsewhere [19].

2.4. Estimation of entrapped volume

In order to get an estimate of the amount of liposomes present in the disk preparations, we used an assay based on the fluorescence increase due to release, and subsequent dilution of liposome entrapped carboxyfluorescein (CF). A liposome sample containing 5 mol% DSPE-PEG(5000) was