Biochimie 95 (2013) 2018–2033

Contents lists available at SciVerse ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Research paper

Calcein release behavior from liposomal bilayer; influence of physicochemical/mechanical/structural properties of lipids

Behnoush Maherani [a,*], Elmira Arab-Tehrany [a], Azadeh Kheirolomoom [b], David Geny [c], Michel Linder [a]

[a] Laboratoire d'Ingénierie des Biomolécules (LIBio), Université de Lorraine, 2 Avenue de la Forêt de Haye, 54501 Vandoeuvre lès Nancy, France
[b] Department of Biomedical Engineering, 451 East Health Sciences Drive, University of California, Davis, CA 95616, USA
[c] Centre for Psychiatry and Neurosciences, INSERM U894, 2 ter rue d'alésia, 75014 Paris, France

ARTICLE INFO

Article history:
Received 1 May 2013
Accepted 8 July 2013
Available online 16 July 2013

Keywords:
Polar drugs
Diffusion
Hydrophobic thickness
Area modulus
Polar surface area

ABSTRACT

The design of the drug delivery depends upon different parameters. One of the most noticeable factors in design of the drug delivery is drug-release profile which determines the site of action, the concentration of the drug at the time of administration, the period of time that the drug must remain at a therapeutic concentration.

To get a better understanding of drug release, large unilamellar liposomes containing calcein were prepared using 1,2-dioleoyl-*sn*-glycero-3-phosphocholine, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine and 1,2-palmitoyl-*sn*-glycero-3-phosphocholine, and a mixture of them; calcein was chosen as a model of hydrophilic drug. The calcein permeability across liposomal membrane (with different compositions) was evaluated on the basis of the first-order kinetic by spectrofluorometer. Also, the effects of liposome composition/fluidity as well as the incubation temperature/pH were investigated.

Furthermore, we simulated the digestion condition in the gastrointestinal tract in humans, to mimic human gastro-duodenal digestion to monitor calcein release during the course of the digestion process. *In vitro* digestion model "pH stat" was used to systematically examine the influence of pH/enzyme on phospholipid liposomes digestion under simulated gastro-duodenal digestion.

The results revealed that calcein permeates across liposomal membrane without membrane disruption. The release rate of calcein from the liposomes depends on the number and fluidity of bilayers and its mechanical/physical properties such as permeability, bending elasticity. Chemo-structural properties of drugs like as partition coefficient (Log *P*), H-bonding, polar surface area (PSA) are also determinative parameter in release behavior.

Finally, stimulated emission depletion (STED) microscopy was used to study calcein translocation through liposomal bilayers.

Abbreviations: ASA, acetylsalicylic acid; Calcein, 3,3-bis[*N*,*N*-bis(carboxymethyl)-aminomethyl]fluorescein; CMC, critical micellization concentration; C_S^{-1}, compressibility modulus; CW STED, continuous wave stimulated emission depletion technique; *d*, hydrophobic thickness; D_m, membrane diffusion coefficient; DLS, dynamic light scattering technique; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-phosphorylglycerol; DSC, differential scanning calorimetry; FI, fluorescence intensity; FWHM, full width at half maximum; HB, H-bonding; HBD, hydrogen bond donors; HOMO, highest occupied molecular orbital; h_m, membrane thickness; ITC, isothermal titration calorimetry; K_A, area modulus; K_c, bending elasticity; K_m, water–lipid partition coefficient; K_p, liposome/water partition coefficient; *L*, membrane thickness; Log *D*, distribution coefficient; Log *P*, partition coefficient; LUMO, lowest unoccupied molecular orbital; LUVs, large unilamellar vesicles; MLVs, multilamellar vesicles; MW, molecular weight; NaC, sodium cholate; PDI, poly dispersity index; PEG, polyethylene glycol; P_m, permeability coefficient; $PC_{m/w}$, membrane/water partition coefficient; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; PSA, polar surface area; PMT, photomultiplier tube; QSAR, quantitative structure–activity relationships; Rhodamine-DHPE, Rhodamine B1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine,triethylammonium salt; SR, super-resolution; STED, stimulated emission depletion microscopy; T_m, phase transition temperature; TMA-DPH, 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene *p*-toluenesulfonate.

* Corresponding author. Tel.: +33 (0)3 22 82 74 73; fax: +33 (0)3 22 82 75 95.
E-mail addresses: bmaherani@gmail.com, behnoush.maherani@u-picardie.fr (B. Maherani).

0300-9084/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.biochi.2013.07.006

1. Introduction

Currently, many efforts in the field of drug delivery have been performed for developing the targeted delivery systems in which the drug is only active in the target site and formulating the sustained release systems in which the drug is released over a period of time in a controlled manner [1].

The goal of drug delivery system is also to administer a drug at a therapeutic concentration to a particular site of action for a specified period of time. The design of the final product for drug delivery depends upon different parameters. a) The drug must be administered by considering some factors which affect therapeutic action of the drug. These parameters include the site of action, the concentration of the drug at the time of administration, the period of time that drug must remain at a therapeutic concentration, and the initial release rate of the drug for controlled release systems. b) The drug must remain physically and chemically stable in the formulation for a defined time. c) The choice of delivery method must indicate the effective administration route for the drug [1,2].

In recent years, the study of controlled release of drugs and other bioactive agents from carrier systems has attracted many researchers from around the world. Attempt to develop an efficient liposome for drug delivery with low leakage of the encapsulated molecules during the circulation is an essential prerequisite to minimize the unwanted side effects. However, once the liposomes have reached their destination, e.g. the interior of a target cell, the encapsulated molecules need, for most applications, to be quickly and efficiently released. The need for a quick release arises from the fact that (1) the active substance is not able to exert its therapeutic effect while being trapped in the lipid carrier and (2) internalization of liposomes via the endocytotic pathway may due to destroy the biological activity of the delivered substance [3].

Indeed, properties of liposomes vary significantly with composition, size, surface charge and preparation method. It is obvious that the design and development of drug carriers is a difficult issue because they have to behave as biocolloidal systems after administration.

Furthermore, physicochemical properties of drugs are also a critical subject in the design of the delivery systems [4,5]. The partition coefficient of bioactive also depends on vesicle size, number of liposomal bilayers and relates to differences in the curvature and the area compressibility of different vesicle structures [1].

A main process in bioactive delivery and targeting using liposome technology is the mechanism of material transfer through the liposomal lipid bilayer. The release of efficacious dose of liposome-entrapped bioactive depends on the permeability of the liposomal formulation with respect of the entrapped bioactive [1].

Several approaches are employed in order to obtain non-leaky liposomes during circulation and also capable of rapidly releasing their contents upon reaching their destination by a variety of mechanisms. The rationale behind the approaches varies but they have generally one thing in common; the liposomes are made from lipid components, or lipid mixtures, that in response to a given change in conditions develop a propensity to form leaky or non-lamellar structures. Recently, a number of methods have been developed for the modification of the liposome surface to enhance the temperature/light/pH sensitivity of the vesicles which release their loading in response to external stimuli [6].

Diffusion-controlled drug release model which controls the drug release by the drug solubility and diffusion coefficient in the release medium, is an alternative approach. At the steady state process, the drug release rate remains constant to result in a zero-order (constant) release. In this system, a drug is usually dispersed inside liposomal bilayer, and released without any rate-controlling barrier layer. During the migration of drug molecules from the surface to longer distances, the drug release rate decreases over time and resulting in non-zero-order release. In recently developed controlled drug delivery technologies, it was identified that the zero-order release would be more desirable than other methods of drug release. In these systems, structural properties of carriers, drug solubility and diffusion coefficient in the release medium are the rate-limiting steps. The release of the entrapped drug from the liposome also depends on the fluidity and number of bilayers and its permeability [7–9]. However, a common mechanism for entrapped molecules permeation across the lipid membrane has not been thoroughly discussed.

In this study, we used calcein as hydrophilic marker which has been notably used as a model for interactions drug/liposome and determining the encapsulation efficiency. Calcein is a water soluble, fluorescent and self-quenching probe that is widely used in studies of cell viability and mitochondrial function by microscopy fluorescence imaging [10]. Calcein-release phenomena have been utilized as an effective index to characterize the membrane properties of (model) biomembranes and to evaluate their stability in a variety of conditions.

The purpose of this study was to determine the basic characteristics of calcein permeation as a model of polar-hydrophilic drug from the liposome to apply them in design of a drug-delivery system. In this study, the permeability of calcein across some liposome membranes was evaluated on the basis of the first-order kinetics. The neutral phospholipids liposomes were used to intercept of the electrostatic interaction of lipid membranes with negatively charged calcein. Second, the composition/fluidity effect of liposome as well as the temperature effect was investigated.

Furthermore, we have used an *in vitro* model designed to mimic closely human gastro-duodenal digestion to monitor calcein release during the course of the digestion process. Simulation of gastrointestinal conditions is essential to predict the *in vivo* behavior of water-soluble drugs. We simulated the digestion process in the gastrointestinal tract in humans, in a simplified manner by applying physiologically based conditions. *In vitro* digestion model "pH stat" was used to systematically examine the influence of pH/enzyme on phospholipid liposomes digestion under simulated gastro-duodenal digestion.

Finally, in an attempt to obtain a better understanding of calcein translocation through the liposomal bilayers, we introduced stimulated emission depletion (STED) microscopy in this study. Unlike conventional microscopy, STED overcomes the normal diffraction limit and allows us to investigate calcein distribution within the confined medium.

2. Materials and methods

2.1. Materials

Phospholipids used in this study were 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), all purchased from Avanti Polar Lipids (Alabaster, AL, USA). 3,3-bis[*N*,*N*-bis(carboxymethyl)-aminomethyl] fluorescein (calcein), 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene *p*-toluenesulfonate (TMA-DPH) were acquired from Invitrogen (France). Rhodamine B1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine,triethylammonium salt (Rhodamine-DHPE) was purchased from Interchim (Paris, France). Enzymes from a single lot were provided as freeze-dried powders: pepsin from porcine gastric mucosa (activity: 3300 U/mg of protein), porcine pancreatic trypsin (activity: 13,800 U/mg of protein), bovine pancreatic -chymotrypsin (activity 40 U/mg of protein), bile salts (Bile extract porcine) and Bowman-Birk trypsin-chymotrypsin inhibitor, were acquired from Sigma–Aldrich (Paris, France). Phosphate buffer solution, monobasic sodium phosphate and dibasic sodium phosphate, were purchased from Sigma–Aldrich