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Biochimica et Biophysica Acta 1714 (2005) 63 – 70

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

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Lipid transfer between cationic vesicles and lipid–DNA lipoplexes: Effect of serum

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Received 22 April 2005; received in revised form 20 May 2005; accepted 25 May 2005
Available online 24 June 2005

Abstract

Differential scanning calorimetry was used to examine the lipid exchange between model lipid systems, including vesicles of the cationic lipoids ethyldimyristoylphosphatidylcholine (EDMPC), ethyldipalmitoylphosphatidylcholine (EDPPC) or their complexes with DNA (lipoplexes), and the zwitterionic lipids (DMPC, DPPC). The changes of the lipid phase transition parameters (temperature, enthalpy, and cooperativity) upon consecutive temperature scans was used as an indication of lipid mixing between aggregates. A selective lipid transfer of the shorter-chain cationic lipoid EDMPC into the longer-chain aggregates was inferred. In contrast, transfer was hindered when EDMPC (but not EDPPC) was bound to DNA in the lipoplexes. These data support a simple molecular lipid exchange mechanism, but not lipid bilayer fusion. Exchange via lipid monomers is considerably more facile for the cationic ethylphosphatidylcholines than for zwitterionic phosphatidylcholines, presumably due to the higher monomer solubility of the charged lipids. With the cationic liposomes, lipid transfer was strongly promoted by the presence of serum in the dispersing medium. Serum proteins are presumed to be responsible for the accelerated transfer, since the effect was strongly reduced upon heating the serum to 80 °C. The effect of serum indicates that even though much lipoplex lipid is inaccessible due to the multilayered structure, the barrier due to buried lipid can be easily overcome. Serum did not noticeably promote the lipid exchange of zwitterionic liposomes. The phenomenon is of potential importance for the application of cationic liposomes to nonviral gene delivery, which often involves the presence of serum in vitro, and necessarily involves serum contact in vivo.

Keywords: Liposome; Lipid exchange; cmc; Monomer; Cationic lipid; Serum

1. Introduction

Lipid vesicles have been regularly and successfully used as experimental biomembrane models. The exchange of lipid molecules between membranes is a common process of significant importance to a proper functioning of cells and organisms. The mechanisms of intracellular lipid transfer are manifold, including membrane fusion, spontaneous redistribution between membranes by lipid monomer equilibration through aqueous phase, molecular transfer during collision, and redistribution controlled by lipid transfer proteins. Membrane fusion has been widely discussed and recognized as an important biological event for such key cell functions as compartmentalization, endocytosis, secretion, synaptic transmission, etc. [1]. Most membrane lipids, including phospholipids and sterols, have a finite, low monomer concentration in the aqueous phase. By being in equilibrium with the interface, monomer lipids have a tendency to redistribute spontaneously between membranes [2–10]. In general, spontaneous transfer of natural phospholipids by monomer diffusion proceeds very slowly and is expected to be of little consequence to intercellular lipid transfer [6].

The usual approach to lipid mixing includes preparation of molecular mixtures in organic solvent, with subsequent solvent removal and hydration. This procedure is believed to produce essentially equilibrium mixtures, but it is not quite biologically relevant, since in biological systems, lipid exchange can take place in aqueous medium.

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doi:10.1016/j.bbamem.2005.05.009

Because of their potential application in gene therapy and drug delivery applications [11,12], positively charged lipoids are now being intensively synthesized and studied (the term 'lipoid' is appropriate since 'lipid' refers to natural products, whereas 'lipoid' is used to molecules that are similar to lipids in structure and in properties but not natural products). However, comprehensive knowledge on the physico–chemical characteristics of their aggregates is still deficient. Among the numerous cationic lipoids synthesized and tested recently, phosphatidylcholine triesters (Fig. 1) [13–15] are the only membrane lipid derivatives shown to be metabolized by cells. They are thus considered promising nonviral transfection agents. These compounds are chemically stable, hydrate well and form uni- or oligolamellar liposomes. The saturated ethyl-phosphatidylcholines have been shown to exhibit gel–liquid crystalline phase transitions at temperatures close to those of the parent phosphatidylcholines [13,16–18].

Here, we describe lipid transfer in mixtures of cationic phospholipid (ethylphosphatidylcholine) dispersions. We found that monomer molecular exchange through the aqueous phase represents the major mechanism of transfer between aggregates; furthermore, lipid mixing was strongly promoted by the presence of serum in the dispersing medium.

2. Materials and methods

2.1. Lipids

The cationic phospholipids were the following: dimyristoyl- and dipalmitoyl-*O*-ethylphosphatidylcholine (EDMPC and EDPPC, respectively), as triflate salts [13] or chloride salts [Avanti Polar Lipids]. The zwitterionic phospholipids were the following: dimyristoyl- and dipalmitoylphosphatidylcholine (DMPC and DPPC, respectively) [Avanti Polar Lipids].

Dispersions of the pure cationic lipoids were prepared in water by vortexing at a temperature above their gel–liquid crystalline phase transition. Samples of 2–5 mM concentrations were routinely used; for some control measurements, 1 mM and 10 mM lipid concentrations were also tested. These lipoids spontaneously generate unilamellar (or oligolamellar) liposomes, with a mean diameter of ~200 nm, as estimated by dynamic light scattering [13]. Dispersions were optically clear; short sonication did not change their calorimetric behavior. In order to prepare similar unilamellar vesicle dispersions of the zwitterionic DMPC and DPPC, they were additionally subjected to bath-sonication in the liquid crystalline phase for 10 min (until optical clarity). To some samples, herring sperm DNA [Invitrogen] (at 1:1 charge ratio) and/or fetal bovine serum [Gibco] (20 vol.%) were added at a temperature above their gel–liquid crystalline phase transition 1 h prior to the measurements.

Dispersions of different lipids and/or lipoplexes were mixed at low temperatures (in the gel phase), at equimolar lipid ratio, and immediately loaded into the calorimeter cell, precooled to 5 °C.

Samples for the construction of EDMPC/EDPPC phase diagram were prepared from mixtures of EDMPC and EDPPC as chloroform solutions; after solvent removal with a stream of argon and subsequent vacuum treatment, the samples were hydrated at 50 °C.

2.2. DSC measurements

Repeated heating and cooling scans were performed with the samples mixed as aqueous dispersions, using a VP-DSC Microcalorimeter (MicroCal Inc., Northampton, MA) at 30 °C/h over the temperature interval 5–50 °C, unless

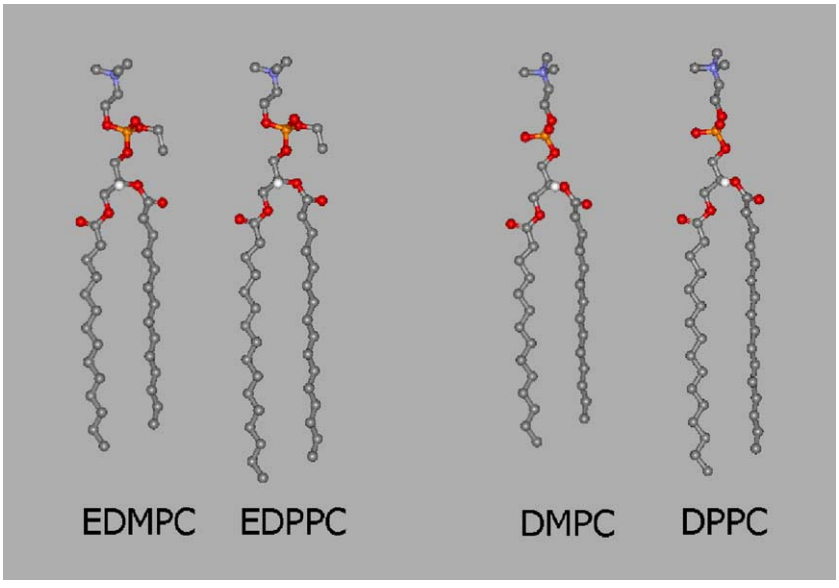

Fig. 1. Molecular structures of the cationic and zwitterionic phospholipids studied.