Modification of the Surface Coat of Nanometer-Sized Magnetoliposomes by Phosphadidylethanolamines

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Abstract: - Using high-gradient magnetophoresis, the non protein-mediated transfer of dimyristoyl-phosphatidylethanolamine ($DC_{14:0}PE$) and dipalmitoylphosphatidylethanolamine ($DC_{16:0}PE$) between sonicated phospholipid vesicles and magnetoliposomes was followed. The latter structures consisted of nanometer-sized magnetite cores which are surrounded by a phospholipid bilayer. In accordance with the 'aqueous transfer model', it is shown that the transferability strongly depends on the fatty acyl side chain composition of the PE entity used. With $DC_{14:0}PE$ a halftime for transfer of 912 min is found, while, using $DC_{16:0}PE$ no transport occurred within the time scale of the experiment (3 days). The relevance of these observations to the production of colloids of biomedical importance is briefly discussed.

Key-Words: - Lipid exchange; Magnetizable biocolloids; Magnetoliposomes; Magnetophoresis; Membranes; Nanometer-sized iron oxide grains; Phospholipid transfer; Phospholipid vesicles

1 Introduction

Magnetoliposomes are biocompatible magnetizable nanocolloids. They consist of a magnetic core which is surrounded by either a monolayer or a bilayer of phospholipid molecules [1]. In the former case, the lipids are strongly attached, with their polar headgroup facing the iron oxide surface and the fatty acyl side chains oriented towards the solvent. As a result of the strong chemisorption forces, the lipid layer withstands desorption with detergents or organic solvents [1,2]. The bilayer coated particles, on the other hand, are formed by deposition of a second layer on top of the first one. This outer layer is more loosely bound, since interaction is mainly governed by the hydrophobic effect, and, as a result, can be easily removed by classical phospholipid extraction procedures [1,2].

A most interesting feature of the double layered coat is that it closely mimics the configuration of natural membranes. Consequently, it can act as a perfect host for intrinsic membrane proteins and enzymes [3]. In addition, many properties of these proteins are tuned by the nature and dynamics of the phospholipids that constitute the lipid envelope [4]. Therefore we feel that a study on the mobility of membrane phospholipids is worthwhile to be done.

2 Problem Formulation

The dynamics of membrane phospholipids deal both with intra- (e.g. lateral diffusion and flip-flop movements) and intermembrane effects (e.g. lipid transport between different membrane structures). The present work deals with the latter phenomenon.

3 Problem Solution

3.1 Transfer system

Lipid transport is monitored between small unilamellar sonicated vesicles, used as donors, and magnetoliposomes, used as acceptors (Fig. 1). Both types of biocolloids have a diameter of about 25 to 30 nm [1]. The lipid composition of their membranes consists of a mixture of the zwitterionic dipentadecanoylphosphatidylcholine [$DC_{15:0}PC$] and the anionic dipentadecanoylphosphatidyl-glycerol [$DC_{15:0}PG$]. In addition, the vesicle donors contain either dimyristoylphosphatidylethanol-amine [$DC_{14:0}PE$] or the longer chain length dipalmitoylphosphatidylethanolamine [$DC_{16:0}PE$] at a 10% molar ratio. It is the transfer potency of these two molecules that is followed in this study.

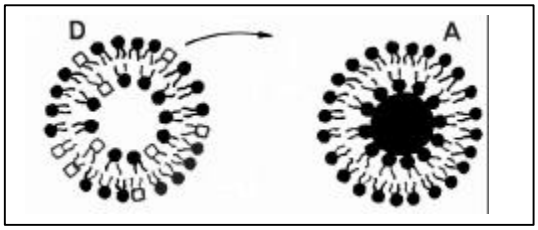

Fig. 1. Schematic representation (not to scale) of the set-up to transfer PE from donor vesicles (D) to magneto-liposome acceptors (A). The unfilled squares represent PE and the filled circles the host phospholipids ($DC_{15:0}PC$ and $DC_{15:0}PG$). The amount of transferred lipid was determined after separation of the two types of biocolloids by high-gradient magnetophoresis.

3.2 High gradient magnetophoresis fractionation

Previous work in our laboratory has shown that high-gradient magnetophoresis is a powerful technique to separate vesicles from magneto-lipo-somes [1]. The high-gradient magnetophoretic set-up consists of tubings, filled with a plug of magnetizable steel fibers, and that are placed between the two poles of an electromagnet. Under the experimental conditions used (Bruker electro-magnet Type BE15; 15-cm diameter pole pieces put at a distance of 3 mm from one another; field strength 1.5 Tesla; sample flow rate : 12 mL/h), more than 99 percent of the magnetoliposome population is routinely captured from the kinetic incubation mixture. Quantification of the amount of PE that left the vesicles and accumulated in the magnetoliposomes is done by gas-liquid chromato-graphy after hydrolysis and methylation of the fatty acids [5].

3.3 Transfer potency of $DC_{14:0}PE$ and $DC_{16:0}PE$

Spontaneous transfer of $DC_{14:0}PE$ from $DC_{15:0}PC/DC_{15:0}PG/DC_{14:0}PE$ (molar ratios 8/1/1) vesicles to $DC_{15:0}PC/DC_{15:0}PG$ (9/1) magneto-liposomes is followed as a function of time at 25°C in 5mM 2-([tris(hydroxymethyl)methyl]amino)-ethanesulfonic acid [TES] buffer, pH 7.0. In terms of phospholipid content, the donor/acceptor molar ratio is 1, and the total lipid concentration is 0.80 µmol/mL.

The kinetic profiles[1] of the time-dependent $DC_{14:0}PE$ depletion of vesicles and $DC_{14:0}PE$ enrichment of magnetoliposomes are given in Fig. 2A. Surprisingly, at equilibrium, $DC_{14:0}PE$ is not equally distributed between donors and acceptors. This behavior can be rationalized by assuming that the kinetic energy barrier for intrabilayer inside-out flip-flop movements is too high [6]. As a result, for the highly curved structures which are used in this study, the inner leaflet phospholipids which comprise about 35 percent of the total phospholipid amount, do not participate in the overall transfer process [6].

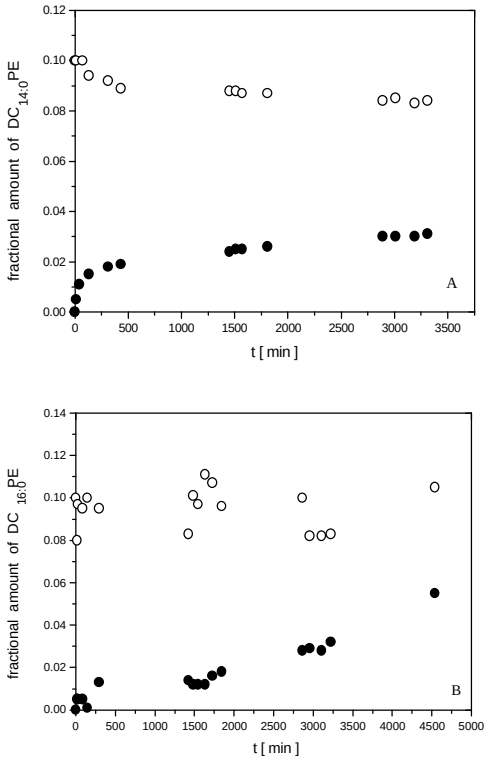

Fig. 2. Time course of the transfer to $DC_{15:0}PC/DC_{15:0}PG$ (molar ratio 9/1) magnetoliposome acceptors of (**A**) $DC_{14:0}PE$ from $DC_{15:0}PC/DC_{15:0}PG/DC_{14:0}PE$ (8/1/1) vesicle donors or (**B**) of $DC_{16:0}PE$ from $DC_{15:0}PC/DC_{15:0}PG/DC_{16:0}PE$ (8/1/1) donors. Vesicles are incubated at 25°C with magnetoliposomes in equimolar conditions at pH 7.0 in 5 mM TES buffer. The open circles represent the time dependent depletion of PE from the vesicles, while the filled circles show the PE taken up by the magnetoliposomes.

The initial kinetic data points, showing the decrease in PE content of the vesicles (Fig. 2), can be fitted by first-order reaction mathematics (Fig. 3) according to the following formula :

[1] Of course, the matrix lipids $DC_{15:0}PC$ and $DC_{15:0}PG$ are also moving, but these lipids undergo a bidirectional symmetric exchange and, thus, this phenomenon is not detectable in the present set-up.

$$\log \frac{[X_t - X_{eq}]}{[X_o - X_{eq}]} = -\frac{k_1 \cdot t}{2.303} \qquad (1)$$

where X refers to the amount of $DC_{14:0}PE$ associated with the vesicles before transfer starts (X_o), at any time during the transfer process (X_t), and at equilibrium (X_{eq}). From the slope of the straight line, a k_1 value of 0.00076_7 min^{-1} is calculated for $DC_{14:0}PE$ depletion from the vesicles. This corresponds to a halftime value ($t_{½}$) of 912 min. In contrast, the initial PE accumulation in the magnetoliposome population seems to occur at a much faster rate (Fig. 2A). In fact, the initial data points can not be fitted to an exponential function. Since, at 25°C, the matrix phospholipids are well below their gel-to-liquid crystalline phase transition temperature (T_t = 33°C for both $DC_{15:0}PC$ and $DC_{15:0}PG$ – ref. 7), the enhanced pseudo PE accumulation in the magnetoliposome fraction is probably caused by vesicle-magnetoliposome aggregation. Separate experiments (not shown), indeed, proved that the phospholipid content of the magnetoliposome fraction increased steadily as a function of time.

As shown in Fig. 2B, when the behavior of the longer chain length $DC_{16:0}PE$ is followed in a similar fashion, analysis of the vesicle population does not yield evidence for the occurrence of transfer within the time-course of the experiment (about 3 days). In the retentate fraction (magneto-liposomes), the $DC_{16:0}PE$ content slowly increases, most probably as a result of colloid clustering (see above).

4 Discussion

Although diacylphospholipids are known to be only sparingly soluble in water, spontaneous interbilayer fluxes have been frequently observed, in particular between artificial membranes [8-10]. The present work demonstrates that these movements are highly dependent on the lipid's fatty acyl side chain length. This behavior can be rationalized in terms of the so-called 'aqueous transfer model' [8-10], which assumes that the lipid molecules first jump out of the membrane, then travel through the aqueous phase and are subsequently taken up by the acceptor magnetoliposomes. During their transaqueous journey, the lipids must encounter a relatively high energy barrier. Most probably, the transient or activated state complex corresponds to a configuration in which the lipid dangles into the

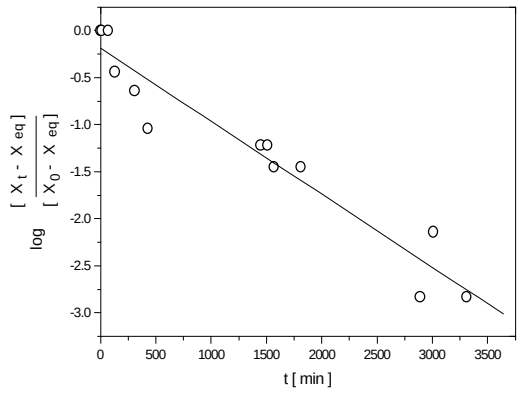

Fig. 3 First-order kinetic plot of $DC_{14:0}PE$ depletion from the donor vesicles, as illustrated in Fig. 2A. The straight line was drawn by a least squares fit through the early data points. The meaning of the y-axis label is explained in the text.

aqueous phase, but is still fixed to the donor surface by its terminal $–CH_3$ groups. As a result, transfer of $DC_{16:0}PE$ will be accompanied by a larger, un-favorable change in activation enthalpy, compared to that of $DC_{14:0}PE$ [8]. In this context, Massey *et al*. [9] and McLean and Phillips [10], indeed, have shown that in the case of diacylphospholipids the transfer rate increases by a factor of 5 to 10 when the fatty acyl chain is shortened by one $-CH_2–$ group.

5 Perspectives for applications

Phospholipids are the building stones of natural membranes. Consequently, they are excellent candidates to cover colloids, rendering them biocompatible. However, for specific *in vivo* applications, the usefulness of biocolloids is very often strongly dependent on the phospholipid type(s) used. Our results show that modification of the coat and the rate at which this occurs can be carefully controlled by selecting the length of the apolar chains of the phospholipids.

In the present work, diacylPEs were used as transferable molecules. A main feature of this lipid type is that its terminal $-NH_2$ group can be easily activated to bind polymers and/or proteins (e.g. antibodies). In this way, promising bio-colloidal structures can be produced, e.g. for drug targeting purposes [11,12]. Further work in this line is presently underway in our laboratory.

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