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SPONTANEOUS PHOSPHOLIPID TRANSFER BETWEEN ARTIFICIAL VESICLES FOLLOWED BY FREE-FLOW ELECTROPHORESIS

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SUMMARY

The application of continuous free-flow electrophoresis in the liposome field is described. The technique is able to distinguish between artificial phospholipid vesicles as a function of their surface charge. This made possible to follow intervesicular interactions between differently charged bilayer structures. As an example, we present evidence for a relatively fast, spontaneous phospholipid transfer between dimyristoylphosphatidylcholine and mixed dimyristoylphosphatidylcholine/dimyristoylphosphatidylglycerol vesicles.

INTRODUCTION

The exchange of lipid molecules between membranes is an event of primary importance in biology. In the case of phospholipid molecules, this transfer can be catalyzed by specific phospholipid exchange proteins (for a review see |1|). Evidence obtained from studies with artificial bilayers indicates that in certain conditions phospholipid molecules may also exchange in a spontaneous way |2|. Mostly, intervesicular phospholipid transfer has been monitored by following modifications of the physical properties of the vesicles (e.g. changes in density |3|, in size |4|, in melting behaviour |2,4|, et c.). Surprisingly, little attention has been focused on procedures which follow intervesicular phospholipid transfer on the basis of changes in surface charge. Only the application of ion-exchange chromatography has been reported in the case of protein-mediated phospholipid exchange |5|, but, unfortunately, with this method the material is not quantitatively recovered.

Abbreviations

DMPC : dimyristoylphosphatidylcholine ; DMPG : dimyristoylphosphatidylglycerol ; pyranine, 8-hydroxy-1,3,6-pyrenetrisulfonate ; MES : 2-(N-Morpholino)ethanesulfonic acid.

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In this paper, the application of the continuous free-flow electrophoresis technique in the liposome field is described for the first time. In the past, this method has been proved to be a powerful and suitable tool for the separation of proteins, cells and cell organelles [6]. Here, we first checked the potency of the free-flow electrophoresis technique to distinguish between vesicles according to their surface charge. We also present evidence for a fast, spontaneous phospholipid transfer between two populations of fluid vesicles, bearing a different charge.

MATERIALS AND METHODS

Materials

DMPC was obtained from Sigma (St. Louis, MO, USA) and DMPG was synthesized from it as described [7]. Pyranine was purchased from Eastman-Kodak (Rochester, N.Y., USA) and cholesterol[1-^{14}C]oleate from the Radiochemical Centre (Amersham, England).

Preparation of lipid vesicles

Lipid vesicles were prepared by ultrasonication (150 W MSE desintegrator) at 30° C for 15 min of 10 ml of a phospholipid dispersion (20 mg phospholipid in 5 mM MES, 10 mM KCl, pH 6.0). Probes (pyranine and cholesterol [1-^{14}C] oleate) were cosonicated. The resulting vesicles were centrifuged at 30° C for 30 min at 20.000 x g to remove metal particles from the sonication tip. Non-encapsulated pyranine was removed by gel filtration on Sephadex G-75. Vesicle preparations were stored at 33° C. Thin-layer chromatographic analysis of the lipid in vesicle preparations after sonication showed no detectable amounts of degradation products such as lysophospholipids.

Free-flow electrophoresis

The electrophoretic characterization of the vesicles was performed in a continuous free-flow electrophoresis apparatus (FF48 type, Desaga, Heidelberg, GFR), according to Hannig [6]. The electrophoresis buffer consisted of 5 mM MES, 10 mM KCl, pH 6.0. As electrode buffer the same medium, but 10 fold concentrated, was used. The electrophoretic runs were carried out at 420 V (60 V/cm) and 150 mA. Temperature of the cooling liquid was set at 28° C so that during electrophoresis the temperature inside the separation chamber was about 33° C. (To avoid cooling of the vesicles in the sample compartment, the whole apparatus was installed in an incubation room at 33° C). The flow rate was 225 ml x h^{-1} for 48 fractions. The vesicle samples to be separated were injected continuously into the streaming buffer at the cathode side (above fraction 39) at a rate of 0.74 ml x h^{-1}.

Detection and characterization of the vesicles

Fluorescence and light scattering measurements were performed on an Aminco Bowman spectrophotofluorometer. Transition temperatures were

measured on the same apparatus by the intramolecular excimer forming probe, 1,3-bis(β-naphthyl)propane [8] .
Dimensions and architecture of the vesicles were checked by Sepharose CL-2B column chromatography and by electron microscopy. It was found that mainly small unilamellar vesicles were formed with a diameter of about 30 nm.

RESULTS AND DISCUSSION

Electrophoretic mobilities of phospholipid vesicles

Zwitterionic phosphatidylcholines do not migrate in an electric field at pH 6.0 [9]. In the free-flow electrophoresis separation chamber however, we observed (fig. 1 and 2) that pure DMPC vesicles slightly move towards the anode Since the non-charged [^{3}H]inulin behaves in a similar way, this behaviour is probably caused by liquid current effects during operation [6].

Cosonication of DMPC with increasing amounts of DMPG considerably enhances the electrophoretic mobility. The relationship however is not linear (fig. 1). This is due -at least in part- to the fact that the actual mobility depends on

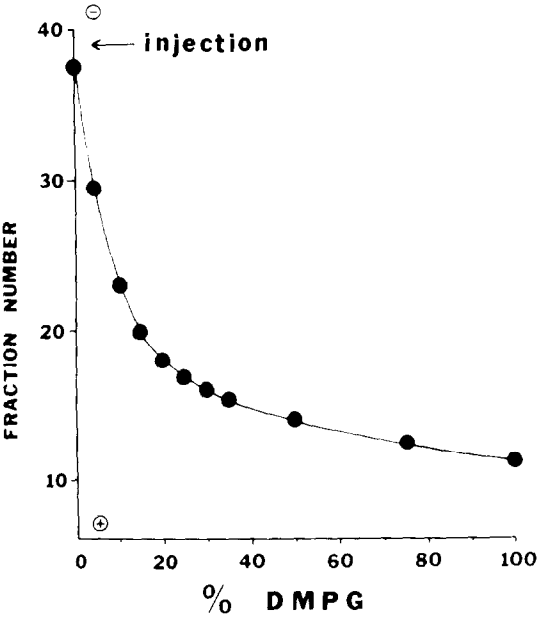

Fig. 1. Electrophoretic migration of DMPC vesicles, containing different amounts of DMPG. Electrophoresis is performed as described (see Materials and Methods). Vesicles are detected by light scattering measurements (excitation and emission wavelength are set at 400 nm). Arrow indicates that injection occured above fraction 39.

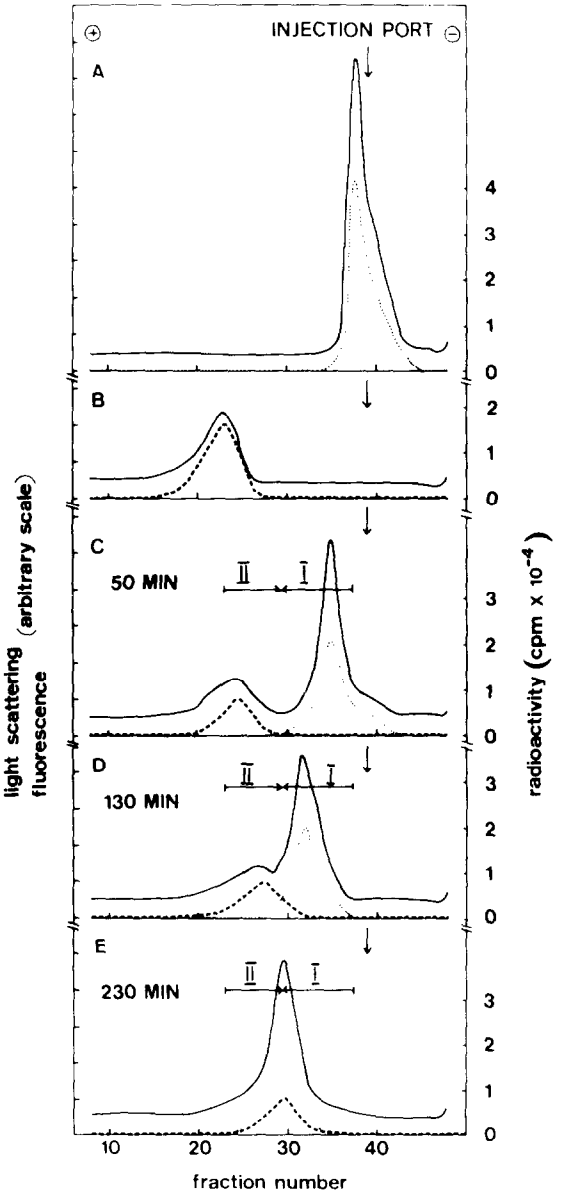

Fig. 2. Time-course of phospholipid exchange.
Electrophoresis is performed as described in the text. The fractions are analyzed for light scattering (excitation and emission at 400 nm) (——), pyranine-fluorescence (excitation 405 nm ; emission at 505 nm) (----) and cholesterol|1-^{14}C|oleate radioactivity (.....).
Electrophoretic distribution patterns of DMPC (A) and DMPC/DMPG (molar ratio 90:10) vesicles (B) before mixing.
Electrophoretic distribution patterns of equimolar mixtures of DMPC and DMPC/DMPG (molar ratio 90:10) as a function of incubation time (C-E). Numbers I and II refer to vesicle population I and II (see text).

the ζ-potential which does not represent the real charge of the surface of the vesicles. In addition, phosphatidylglycerol is preferentially localised in the outer shell of a mixed phosphatidylcholine/phosphatidylglycerol bilayer |10|. As a result (particularly at low DMPG concentrations), the surface charge density and thus the electrophoretic mobility will be greater than expected on the basis of the actual DMPG content.

Evidence for spontaneous phospholipid transfer

In the lower zone of DMPG concentrations, small differences in charge are easily distinguished in the electropherogram (cf. fig. 1). This observation prompted us to study possible interactions between equal amounts of DMPC/DMPG (molar ratio 90:10) and DMPC vesicles in a time-dependent way. Incubation (for different time intervals) and analysis were performed at 33° C which is well above their transition temperatures (23-24° C). It is immediately clear from fig. 2 that the peaks corresponding to the original vesicle types disappear. Concomitantly, two new populations with intermediate electrophoretic mobility are formed. The first (which we design as population I) continuously increases ir mobility. Undoubtedly this population originates from the DMPC vesicles. Support for this assumption comes from the fact that the non-exchangeable marker cholesterol|1-^{14}C|oleate |11|, which is initially embedded within the bilayer of the DMPC vesicles, remains completely associated with the population I vesicles. Alternatively, the second vesicle population (designed as population II), gradually decreases in mobility. Since pyranine, which serves as a marker for the matrix of the DMPC/DMPG (molar ratio 90:10) vesicles, remains completely entrapped by the population II vesicles, we believe that this population originates from the DMPC/DMPG (molar ratio 90:10) vesicles. After about 4 hours the peaks corresponding to population I and II have merged, suggesting that their surface charge density has become identical. Moreover, the electrophoretic mobility of these hybrid particles is indistinguishable from that of vesicles, prepared by cosonication of DMPC and DMPG in a molar ratio of 95:5 (cf. fig. 1).

The above described behaviour is completely compatible with a spontaneous intervesicular lipid transfer mechanism, apparently without affecting the autonomous character of the structures. Neither fusion nor aggregation processes can be responsible for the phenomenon observed. Otherwise, a time-dependent association of pyranine with population I and of cholesterol|1-^{14}C|oleate with population II would be expected. Furthermore, within the time of the experiment, we observed neither an increase in light scattering, nor the appearance of larger structures by Sepharose CL-2B chromatography or electron microscopy.

Until now, the underlying molecular mechanism of the non-protein-mediated phospholipid exchange remains poorly understood. Disruption of vesicles in fragments, followed by resealing to generate small hybrid particles is excluded in the present conditions. Indeed, pyranine is not liberated into the external medium and cholesterol|1-^{14}C|oleate remains embedded in the population I vesicles. Probably, transfer occurs by collision of vesicles |4| or by a transfer of lipid molecules either in a molecular or in a micellar intermediate state |2, 12, 13| .

In comparison with reports in the literature |2, 12|, the rate of spontaneous lipid transfer which we observed is relatively fast. Differences in experimental conditions however make it difficult to make strict comparisons. May be in our set-up the exchange process is favoured by the short fatty acyl side chains of the dimyristoylanalogues |2, 12|. Also, the transbilayer flip-flop rate of the phospholipid molecules, which is reported to be considerably enhanced in asymmetrically perturbed bilayers |14|, is probably an important factor in the transfer phenomenon |15|.

CONCLUSIONS

The free-flow electrophoresis technique allows separation of differently charged phospholipid vesicles under non-destructive conditions. In contrast to the ion-exchange chromatography technique, no change in the external

medium is needed to separate and to quantitatively recover all the phospholipid vesicles applied. Also, the electrophoretic separation does not seem to be affected by the size of the phospholipid structures. Indeed, we found that both multilamellar liposomes and single-shelled vesicles of equal phospholipid composition migrate with a similar electrophoretic velocity. Thus, free-flow electrophoresis can be a useful supplement to other techniques for studying phospholipid vesicles.

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