Manipulation of Electric Charge on Vesicles by Means of Ionic Surfactants: Effects of Charge on Vesicle Mobility, Integrity, and Lipid Dynamics

Alexander A. Yaroslavov,* Oleg Y. Udalyk, Viktor A. Kabanov, and Fredric M. Menger*

Abstract: A combination of electrophoresis, dynamic light scattering, conductometry, and fluorescence spectroscopy was applied to investigate vesicles (both in the "solid" and "liquid" states) that had been imparted with electric charge through the incorporation of ionic amphiphiles. These amphiphilic compounds comprised cardiolipin (with two negative charges), sodium dodecyl sulfate (with one negative charge), and cetylpyridinium bromide (with one positive charge). By this means it was discovered that negative vesicles could be converted into neutral vesicles, and then into positive vesicles, by the addition of a cationic surfactant. The amount of cationic surfactant required for the conversion depended upon the mobility of the surfactant within the bilayer. Vesicles were found to be capable of absorbing large amounts of surfactant, both cationic and anionic, before ultimately disintegrating and releasing their contents. Mixtures of cationic and anionic vesicles were able to exchange surfactant, and thereby neutralize each other's charges, without any concurrent vesicle fusion. This phenomenon is reliable only if the vesicles are in the liquid state. Finally, a biphasic exchange process was observed in which a surfactant rapidly departs from one bilayer and then enters another, while a fluorescently labeled lipid travels the reverse path only slowly.

Keywords
liposomes · phospholipids · surfactants · vesicles

Introduction

The behavior of charged lipid vesicles can be analyzed within the framework of classical Derjaguin–Landau–Vervey–Overbeek (DLVO) theory.[1, 2] The theory postulates vesicle/vesicle interactions reflecting a van der Waals attraction that is countered by electrostatic repulsion. If, for example, the excess repulsive energy of two vesicles greatly exceeds their translational energy, the system will be stable. If, on the other hand, there is no electrostatic repulsion between the vesicles, the vesicles should coagulate.[3] In actual fact, however, uncharged vesicles do not spontaneously aggregate, a fact now attributed to an additional repulsive term, the hydration force.[4] In order for two vesicles to approach each other at close range (<4 nm), their surface headgroups must lose their shells of hydration. Thus, vesicles can fuse only at the cost of dehydration energy.

Electrostatic repulsion between charged vesicles can be modified by ion binding, and many previous studies have made use of this fact to control vesicle aggregation and fusion. For example, Ohki et al.[5] showed that large anionic vesicles (>100 nm) exhibit two modes of aggregation upon addition of monovalent cations. In the concentration range 0.1–0.4 M cation, aggregation takes place spontaneously, and Li^+ is more effective than Na^+. On the other hand, at a cation concentration >0.4 M, aggregation progresses only gradually with time, and Na^+ is more effective than Li^+. As might be expected, divalent cations (Ca^{2+}, Ba^{2+}, Sr^{2+}) are effective in inducing fusion of vesicles composed of an anionic lipid (phosphatidylserine).[6] There is a threshold amount of bound cation below which the fusion rate is small and above which the rate increases rapidly. The cation Mg^{2+} is unable to promote fusion but affects the aggregation kinetics when other divalent cations are present. These results, which are only a small sample of those available in the literature, suffice to illustrate the complexities that exist when vesicles are perturbed by the ionic composition of the medium. The complexities are, in part, the consequence of diverse ion–vesicle binding constants as well as unknown effects of the ions on the hydration forces that often dictate fusion events. The role of vesicle size and curvature in fusion processes adds to the difficulties.

Two papers, one of them very recent,[7, 8] deserve particular mention here because they are among the few that take a slightly different slant on the matter of charged vesicle behavior. In 1988, the group of Silvius et al.[7] reported on positively charged lipid vesicles prepared by mixing neutral phospholipids with low mole fractions of the cationic lipid analogue $CH_2(OCOR)$-$CH(OCOR)CH_2N(CH_3)_3^+$, where OCOR is a long oleoyl chain. Thus, positive charge was imparted to the vesicles by means of a cationic species that directly incorporated itself into the membrane. These vesicles were then mixed with negatively charged

[*] Prof. Dr. A. A. Yaroslavov, O. Y. Udalykh, Prof. Dr. V. A. Kabanov
Faculty of Chemistry, Moscow State University
Moscow 119899 (Russia)
Prof. Dr. F. M. Menger
Department of Chemistry, Emory University
Atlanta, Georgia 30322 (USA)
Fax: Int. code +(404)727-6586

0947-6539/97/0305-0690 $ 17.50+.50/0

vesicles containing an anionic lipid, phosphatidylserine, with the following results: a) Mixtures of vesicles with opposite surface charge readily aggregated with each other at low ionic strengths. b) Under certain conditions, the aggregated vesicles could be observed to mix their lipids as well as to exchange their aqueous contents. c) The composition of the vesicles, in terms of their neutral lipids, is critical in controlling both the aggregation and the mixing events that follow aggregation. For example, vesicles that contain high phosphatidylethanolamine/phosphatidylcholine ratios are more prone to exchange their lipids and to mix their aqueous interiors. It may be important in this regard that phosphatidylethanolamine is a lipid with relatively weak surface hydration forces.

Marchi-Artzner et al.[8] also examined lipid exchange in vesicles of opposite charge. In this case, vesicular membranes of egg lecithin plus cholesterol were supplied with either an amphiphilic cation (stearylammonium ion) or an amphiphilic anion (dicetyl phosphate). When the two types of vesicles were brought into contact, they began to exchange their charged lipids. The resulting progressive charge neutralization occurred in the absence of any direct fusion of the vesicles. Vesicle size was shown to play a major role in the kinetics and thermodynamics of vesicle adhesion and lipid exchange.

It is common to introduce an article on vesicles by citing their potential in the field of drug delivery. Often this refers to the process by which vesicles can fuse with cells and deliver their contents. But the possibility of fast lipid exchange between vesicles and cells, independent of fusion and accentuated by electrostatic effects as just described, offers an alternative mechanism for delivery. The fact that the outer monolayer leaflets of bacterial cell membranes are negatively charged relative to those of mammalian cells[9] might, for example, allow selective targeting to bacteria.

Our own work on charged lipids, described herein, used dipalmitoylphosphatidylcholine (DPPC) or egg lecithin (EL) as the main membrane constitutents. Since DPPC exists in the "solid" or "gel" state at 20 °C, while EL exists in the "liquid" or "liquid-crystalline" state at 20 °C, we were able to examine the effect of fluidity on the membrane behavior. The neutral vesicles were converted into negatively charged structures by incorporation of cardiolipin (CL^{2-}), a sort of "double phospholipid" bearing four long lipid chains plus two anionic phosphodiester groups. Vesicular charge could be further modified by addition of surfactants (either anionic sodium dodecyl sulfate SDS^- or cationic cetylpyridinium bromide CPB^+). The effects of charged components within the vesicles were examined by electrophoretic mobility, conductivity, dynamic light scattering, and fluorescence.

Results and Discussion

Vesicles (also called liposomes) were prepared by ultrasonicating films composed of either a neutral lipid (EL or DPPC) or an anionic lipid mixture (EL + CL^{2-} or DPPC + CL^{2-}). At ambient temperature, the EL vesicles were all in the "liquid" state, whereas the DPPC-based vesicles were below T_m and thus in the "solid" state. Doubly anionic CL^{2-} was always added to the extent of 5 mol% of the neutral lipid. Since the neutral lipid had a concentration of 1 mg g^{-1} (or about 1.4 mM), the CL^{2-} concentration was 0.07 mM throughout. Anionic and cationic surfactants (SDS^- and CPB^+) were added to the phospholipid vesicles and, in this manner, the vesicular charge was controlled. Lipid-to-surfactant ratios were 3:1 or greater, except in those experiments in which huge amounts of surfactant were used. Although high surfactant concentrations are known to solubilize phospholipids and destroy vesicles,[10] the concentrations used in our experiments were generally too low to do this (see below). NMR experiments have shown no detectable monomeric or micellar surfactant coexisting with the vesicular surfactant at high lipid-to-surfactant ratios.[11]

The electrophoretic mobility of vesicles composed of various lipid–additive mixtures was investigated by means of a laser microelectrophoresis method. Electrophoretic mobility is a powerful method for assessing vesicular charge. Figure 1 depicts the vesicle mobility as a function of anionic or cationic surfactant added to the system. Plot 1 shows EL vesicles, originally

Abstract in Russian:

Методами электрофореза, динамического светорассеяния, кондуктометрии и флуоресцентной спектроскопии исследовано взаимодействие нейтральных и отрицательно заряженных твердых и жидких везикул с ионными поверхностно-активными веществами (ПАВ) - додецилсульфатом натрия (ДСН) и цетилпиридиний бромидом (ЦПБ). Добавление положительно заряженного ЦПБ к отрицательно заряженным везикулам приводит вначале к нейтрализации, а затем к перезарядке везикул. Количество ЦПБ необходимое для нейтрализации везикул зависит от фазового состояния везикулярной мембраны. Целостность везикул сохраняется даже в значительных избытках обоих ПАВ. Жидкие смешанные везикулы способны обмениваться молекулами ПАВ и липидов. Скорость первого из этих процессов существенно превосходит скорость второго. Миграция молекул не сопровождается ни агрегацией ни слиянием частиц.

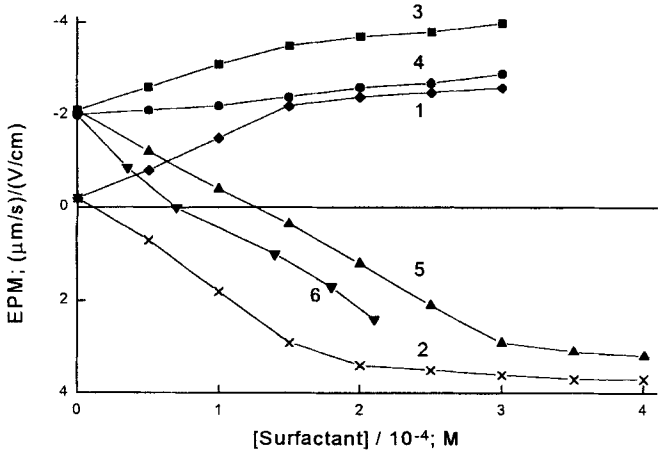

Figure 1. EPM of vesicles in the presence of surfactants. 1: EL vesicles + SDS^-; 2: EL vesicles + CPB^+; 3: EL/CL^{2-} vesicles + SDS^-; 4: DPPC/CL^{2-} vesicles + SDS^-; 5: EL/CL vesicles + CPB^+; 6: DPPC/CL vesicles + CPB^+. Lipid concentration 1 mg ml^{-1}; phosphate buffer (0.01 M, pH = 9.2), 20 °C.

neutral and immobile, becoming increasingly negative as SDS^- is added to the membrane. In Plot 2, the EL vesicles acquire a growing positive charge as CPB^+ is added. The third plot shows the EL + CL^{2-} vesicles negatively charged; this negative charge further increases upon addition of SDS^-. Curve 4 represents a similar situation for DPPC and CL^{2-} to which SDS^- is added, and Plot 5 shows an interesting crossover from negative to positive as negative EL + CL^{2-} lipid is mixed with CPB^+. The same effect is seen in Plot 6, in which DPPC + CL^{2-} are combined with CPB^+.

Consider now the concentrations used to obtain Plot 6: [DPPC] = 1.4 mM and [CL^{2-}] = 0.07 mM. Charge neutralization (i.e., the point at which the plot intersects the zero line) occurred when [CPB^+] was 0.07 mM. Since there is abundant evidence to prove that CL^{2-} distributes itself nearly uniformly between the inner and outer leaflets of the vesicular bilayer,[12, 13] the concentration of CL^{2-} in the outer leaflet is 0.035 mM. But each CL^{2-} contributes two anionic charges, giving a "charge concentration" of 0.07 mM on the outer vesicle surface. This corresponds exactly to the concentration of CPB^+ needed to neutralize the surface charge in curve 6. Thus, CPB^+ binds to the outer leaflet, presumably adjacent to the CL^{2-} anions, and does not "flip-flop" through the solid DPPC lipid to the inner leaflet within the timeframe of the experiment. The stoichiometry of the charge neutralization also proves that little surfactant remains in the bulk water outside the vesicles.

The situation was quite different when CPB^+ was added to a liquid membrane composed of EL and CL^{2-} (Plot 5). In this case, the charge neutralization occurred at 0.13 mM CPB^+ (twice the value found for the solid DPPC membrane). This suggests one of two possibilities: a) disruption of the vesicular membrane to bring CPB^+ into contact with both leaflets of the bilayer, or b) transmembrane migration of CL^{2-} or CPB^+ (or both) between the two leaflets of intact vesicles, thereby allowing CPB^+ to neutralize the total CL^{2-} content of the membrane. The following experiments were carried out to differentiate between these possibilities.

Vesicles comprising EL + CL^{2-}, loaded with 1.0 M NaCl, were placed in a borate buffer of equal ionic strength (see Experimental Section). Addition of CPB^+ to this system at a concentration equivalent to charge neutralization (1.4 mM) did not result in a significant increase in conductivity. Since no NaCl leaked out into the bulk medium, the integrity of the liquid vesicles must remain intact upon addition of the CPB^+. One can conclude, therefore, that the complete charge neutralization of the EL–CL^{2-} vesicles by CPB^+ was achieved by the charged components (CL^{2-} and/or CPB^+) moving freely across the liquid bilayer. This can be envisioned as occurring in three ways: a) transfer of CL^{2-} molecules from the inner leaflet to the outer leaflet; b) transfer of CPB^+ molecules from the outer leaflet to the inner leaflet; c) a combination of the two. "Flip-flopping" of CL^{2-} from inside to outside seems extremely unlikely, because this is a slow process, even for a simple double-chained lipid;[14] CL^{2-} has four chains and two anionic charges, and it would thus be expected to migrate even more reluctantly. By default, one must conclude that the charge neutralization is ascribable to the CPB^+. In summary, CPB^+ neutralizes CL^{2-} on both sides of a liquid EL membrane but only on the outer side of a solid DPPC membrane.

The above experiments offered an opportunity to study the effects of much higher surfactant concentrations—concentrations potentially large enough to damage the vesicles. The data are presented in Figure 2. It can be seen that no increase in

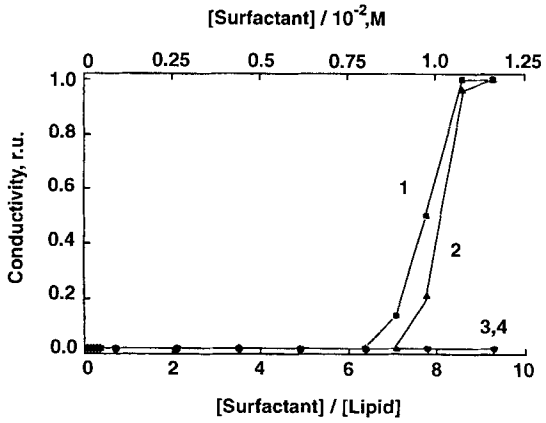

Figure 2. Relative conductivity of systems containing vesicles loaded with 1 M NaCl after addition of surfactants. 1: EL/CL^{2-} vesicles + SDS^-; 2: EL/CL^{2-} vesicles + CPB^+; 3: DPPC/CL^{2-} vesicles + SDS ; 4: DPPC/CL^{2-} vesicles + CPB^+.

conductivity occurred with EL–CL^{2-} vesicles unless the ratio of SDS^-/EL (Plot 1) or CPB^+/EL (Plot 2) exceeded 6. At ratios of 7 ± 1, the conductivity rose sharply to a level corresponding to complete disruption of the vesicles. Since the value of about 7 is the same for both SDS^- and CPB^+, the destruction of the anionic liquid vesicles seems to be insensitive to the charge on the surfactant. No doubt the charge imparted by bound surfactant overwhelms that provided by the relatively small concentration of CL^{2-}. Large quantities of surfactant would be expected to greatly alter bilayer packing even at ratios where the vesicles manage to remain intact.

When the surfactant-perturbed vesicles were monitored by dynamic light scattering (Figure 3a, Plots 1 and 2), the vesicle size was found to remain constant up to a SDS^--to-EL or CPB^+-to-EL ratio of about 7. Above this ratio, large uncharacterized aggregates were formed with diameters greater than

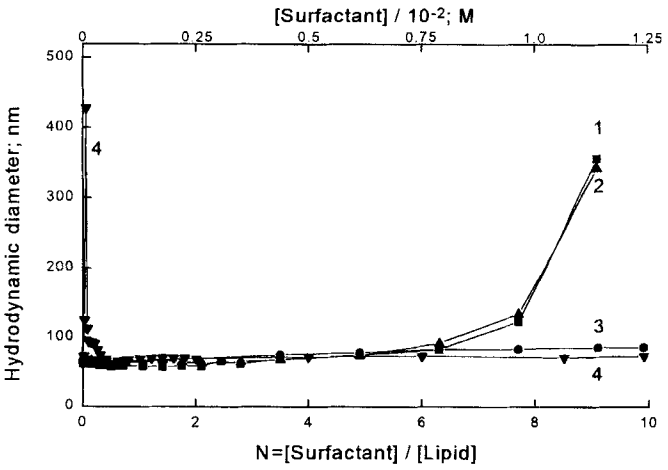

Figure 3. Hydrodynamic diameters of vesicles in the presence of surfactants. 1: EL/CL^{2-} vesicles + SDS^-; 2: EL/CL^{2-} vesicles + CPB^+; 3: DPPC/CL^{2-} vesicles + SDS^-; 4: DPPC/CL^{2-} vesicles + CPB^+.

0947-6539/97/0305-0692 $ 17.50+.50/0 Chem. Eur. J. 1997, 3, No. 5

300 nm. These are, most likely, structureless mixed micelles. It is surprising that adding a great deal of surfactant to the vesicles below a ratio of 7 had such a small effect on vesicle diameter. We suspect that vesicle growth from the added material is compensated by the contraction of the vesicle's hydration shell owing to the creation of charge. Thus, the overall hydrodynamic radius remains unaltered. It is also surprising that as many as seven surfactant molecules per lipid are required to fully destroy the bilayers. This result testifies to the "elasticity" of the lipid bilayer, a feature that allows the phospholipid membrane to accept large numbers of bound proteins and other guests in natural systems.

Solid DPPC–CL^{2-} vesicles again behaved very differently from the liquid EL vesicles. The former were not destroyed by even a 10-fold excess of SDS^- over EL (Figure 2, Plot 3), and the size of the vesicles remained constant upon addition of SDS^- (Figure 3, Plot 3). Addition of a huge excess of CPB^+ also had no effect upon the integrity of the vesicles (Figure 2, Plot 4). It will be noted from this same plot that there was a precipitous drop in the size of the particles (from about 420 nm to a more normal 60 nm) near CPB^+ concentrations corresponding to the charge neutralization point. The simplest explanation for this phenomenon is that below the charge neutralization point the vesicles have aggregated. Perhaps the CPB^+ behaves as a "molecular adhesive" with its headgroup in one vesicle and its tail in another. In any event, once there is sufficient CPB^+ to impart a positive charge to the vesicles, the intervesicular electrostatic repulsion causes the vesicles to desegregate into their normal size. Clearly, the combination of electrophoretic mobility, conductivity, and light scattering is a potent tool for elaborating the detailed structural changes occurring among the vesicles.

To summarize our results thus far: addition of CPB^+ to liquid EL + CL^{2-} vesicles led to rapid adsorption of the surfactant in which, owing to a "flip-flop" process, the CL^{2-} became charge-neutralized on both sides of the bilayer. Ultimately, the vesicles were converted from anionic into cationic entities. When large excesses of CPB^+ were added, the vesicles were destroyed and their content released. Solid DPPC + CL^{2-} vesicles behaved differently. Only the CL^{2-} in the outer leaflet was charge-neutralized by absorbed CPB^+. And even a CPB^+-to-DPPC ratio of 10 could not destroy the vesicles.

We next addressed the important question of molecular exchange beween vesicles. Cationic EL + CPB^+ and DPPC + CPB^+ vesicles and anionic EL + SDS^- and DPPC + SDS^- vesicles were prepared with 1 M NaCl inside them. Next, vesicles of opposite charge were mixed: EL + CPB^+ vesicles with EL + SDS^- vesicles, and DPPC + CPB^+ vesicles with DPPC + SDS^- vesicles. The systems were again examined by electrophoretic mobility, conductivity, and light scattering.

Figures 4a and 4b show the EPM profiles for pure EL + SDS^- vesicles and EL + CPB^+ vesicles, respectively. The two charge types were mixed at equal concentrations, and within five minutes only one type of vesicle, with a neutral charge, could be observed (Figure 4c). The size of the new vesicles equaled that of the original components, and no leakage of NaCl was evident in their formation. One can conclude that the surfactant molecules incorporated within the liquid vesicular membrane can transfer rapidly from one vesicle to another,

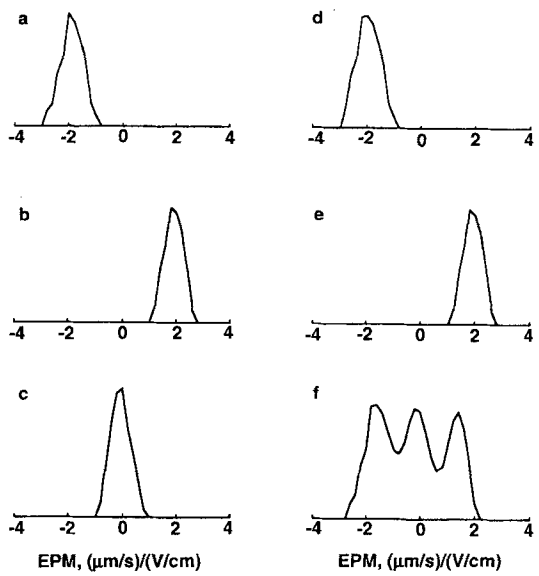

Figure 4. EPM scans of charged vesicles. a) EL/SDS^{2-} vesicles; b) EL/CPB^+ vesicles; c) EL/SDS^{2-} vesicles + EL/CPB^+ vesicles 5 min after mixing; d) DPPC/SDS^{2-} vesicles; e) DPPC/CPB^+ vesicles; f) DPPC/SDS^{2-} vesicles + DPPC/CPB^+ vesicles 5 min after mixing.

creating vesicles in which there is a uniform distribution of both SDS^- and CPB^+ throughout the vesicle population.

In contrast, a mixture of DPPC + SDS^- vesicles (Figure 4d) and DPPC + CPB^+ vesicles (Figure 4e) produced a broad, irregular profile that was stable with time (Figure 4f). Partial leakage of NaCl occurred simultaneously with the transformation. It appears as if interaction between solid vesicles of opposite charge leads to formation of defects and, probably, to outright fusion.

Liquid EL vesicles were also able to exchange lipid molecules, although the process was much slower than that of surfactant exchange. This was demonstrated by a fourth method of analysis, fluorescence spectroscopy, carried out as follows: EL vesicles were prepared with 0.1 wt% of a fluorescently labeled lipid, dipalmitoylphosphatidylethanolamine fluoresceinthiocarbamoyl (PEA*). When CPB^+ was added to the vesicles, the surfactant entered the bilayers and quenched the fluorescence in a matter of a few seconds. The extent of quenching depended upon the ratio of CPB^+ to PEA* (Figure 5). Cationic EL–CPB^+ vesicles were then added to an equal concentration of neutral EL vesicles labeled with PEA*, and the degree of quenching monitored as a function of time. The data are presented in Figure 6 at three temperatures (20°, 40°, and 65 °C, corresponding to Plots 1, 2, and 3, respectively). One can see that the kinetics are biphasic at all three temperatures. The first stage was complete within about 1 min for all three plots. The second stage, on the other hand, was highly temperature dependent. At 20 °C, no further change in fluorescence was observed over and above the initial decrease for a period up to 40 min (Plot 1). At 40 °C, the fluorescence showed a slow second stage, and at 65 °C the minimum intensity was reached within 10 min (Plots 2 and 3, respectively).

How might these results be explained? Under the conditions of our experiments, the formal ratio of CPB^+ to PEA* (distributed initially, as mentioned, between two vesicle popula-

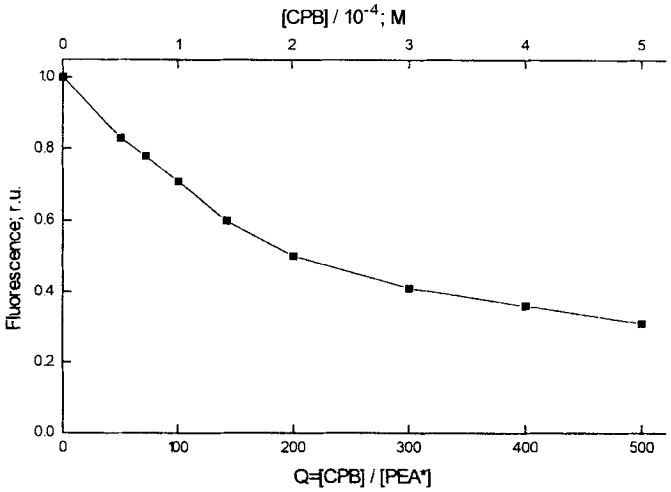

Figure 5. Relative fluorescence intensity of labeled EL/CL^{2-} vesicles in the presence of CPB^+.

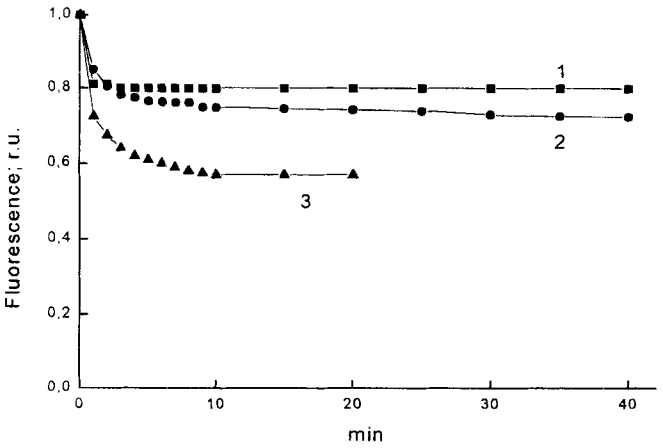

Figure 6. Relative fluorescence intensity of labeled EL vesicles after addition of EL/CPB^+ vesicles as a function of time. 1: 20 °C; 2: 40 °C; 3: 65 °C. Lipid concentration = 5 mg ml^{-1}; phosphate buffer (0.01 M, pH = 9.2).

tions) was 140. Based on the data in Figure 5, this should lead to a minimum relative fluorescence intensity of 0.6. A CPB^+-to-PEA* ratio of only 70 would, on the other hand, give a fluorescence intensity of 0.8. Now fluorescence intensities of 0.8 and 0.6 were exactly those observed as minimum values for the first (fast) and second (slow) stages of the biphasic behavior in Figure 6. The implications are clear. When the EL–CPB^+ vesicles are mixed with an equal number of EL–PEA* vesicles, there is an immediate transfer of CPB^+ to the EL–PEA* vesicles such that the CPB^+ is equally distributed among all vesicles (Figure 7). The ratio of CPB^+ to PEA* is 70 within the half of the vesicles that contain both CPB^+ and PEA*. As a consequence, the fluorescence is diminished to 0.8 of its original value in the first stage of the kinetics. There next ensues a second stage of slow migration of PEA* from the half of the vesicles that contains the probe to the other half that does not. This enhances the CPB^+-to-PEA* ratio from 70 to 140 as the fluorescence slowly decreases to 0.6, a value that is expected from uniform distribution of both CPB^+ and PEA* among all vesicles (Figure 7). The double-switched mechanism is, to our knowledge, newly reported.

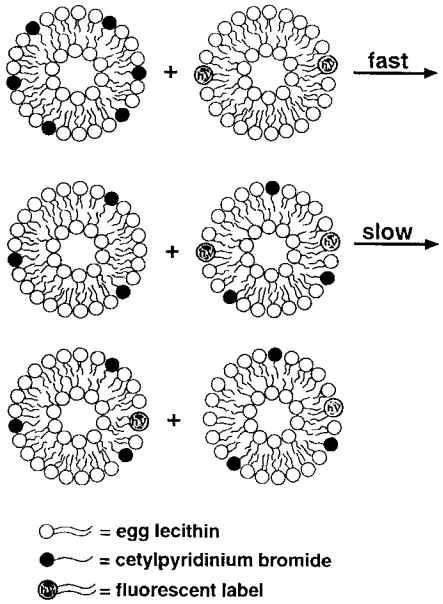

Figure 7. Fast vesicle-to-vesicle transfer of CPB^+ followed by slow vesicle-to-vesicle transfer of the fluorescent label PEA*, consistent with the biphasic behavior shown in Figure 6.

In conclusion, our work emphasizes the role of electric charge in vesicle behavior and in the ease with which charge can be manipulated by means of amphiphilic cations and anions. The results define some of the properties of amphiphilic ions, and one may hope that ultimately the information can be used for practical applications when vesicular systems come into contact with charged biological membranes.

Experimental Section

Materials: Egg lecithin (EL), dipalmitoylphosphatidylcholine (DPPC), cardiolipin (CL^{2-}), cetylpyridinium bromide (CPB^+, 9×10^{-4} M), and sodium dodecyl sulfate (SDS^-, 8×10^{-3} M) were all obtained from Sigma. All work was carried out with double-distilled water that had been treated with a Milli-Q system.

Vesicle Preparation: EL or DPPC, alone or mixed with CL^{2-}, was dissolved in methanol and evaporated to a thin lipid film in a flask under reduced pressure. The lipid film was then dispersed in a borate buffer (pH = 8.0) with the aid of a Cole Palmer 4700 ultrasonic homogenizer. For EL, this was done while the mixture was cooled in an ice bath, and for DPPC while the mixture was warmed to 55 °C. The resulting vesicle preparations were cooled to 20 °C, at which the EL membranes were in the liquid-crystalline state ("liquid") while the DPPC membranes were in the gel state ("solid"). All vesicle preparations were subjected to centrifugation to remove titanium dust from the sonicator probe and then used within one day. SDS^- or CPB^- was always added externally to the vesicle systems.

Fluorescent Vesicles: EL vesicles containing a fluorescent lipid (dipalmitoylphosphatidylethanolamine fluoresceinthiocarbamoyl, PEA*, purchased from Sigma) were prepared as described above, except that the lipid film contained 0.1 wt% of labeled lipid.

Vesicles with NaCl: PC + CL^{2-} and DPPC + CL^{2-} vesicles containing NaCl were prepared and the lipid film was dispersed in 1 M NaCl as above. The vesicle preparations were then dialyzed against borate buffer for 6–8 h.

Instrumentation: Hydrodynamic diameters were determined by photon correlation spectroscopy with an Autosizer 2c instrument (Malvern, UK). Electrophoretic mobilities (EPM) were obtained by the laser microelectrophoresis

0947-6539/97/0305-0694 $ 17.50+.50/0 Chem. Eur. J. 1997, 3, No. 5

method with a Zetasizer 2c instrument (Malvern, UK). Fluorescent work was carried out with a Hitachi F-4000 fluorescence spectrophotometer. Conductivity experiments used a Radiometer CDM 83 conductivity meter.

Acknowledgments: This work was support by a National Institutes of Health grant to F. M. M.. Jason Keiper (Emory University) is thanked for preparing Figure 7. All experimental work was carried out at the Moscow State University.

Received: October 29, 1996 [F 510]

[1] B. W. Ninham, *Pure Appl. Chem.* **1981,** *53*, 2135–2147.
[2] A. M. Carmona-Ribeiro, L. S. Yoshida, H. Chaimorich, *J. Phys. Chem.* **1985**, *89*, 2928–2933.
[3] E. Evans, D. Needham, *J. Phys. Chem.* **1987**, *91*, 4219–4228.
[4] A. C. Cowley, N. L. Fuller, R. P. Rand, V. A. Parsegian, *Biochemistry* **1978**, *17*, 3163–3168.
[5] S. Ohki, S. Roy, H. Ohshima, K. Leonards, *Biochemistry* **1984**, *23*, 6126–6132.
[6] J. Bentz, N. Düzgünes, *Biochemistry* **1985**, *24*, 5436–5443.
[7] L. Stamatatos, R. Leventis, M. J. Zuckermann, J. R. Silvius, *Biochemistry* **1988**, *27*, 3917–3925.
[8] V. Marchi-Artzner, L. Jullien, L. Belloni, D. Raison, L. Lacombe, J.-M. Lehn, *J. Phys. Chem.* **1996**, *100*, 13844–13856.
[9] G. Deng, T. Dewa, S. L. Regen, *J. Am. Chem. Soc.* **1996,** *118*, 8975 8976.
[10] G. W. Stubbs, B. J. Litman, *Biochemistry* **1978,** *17*, 215–219.
[11] K. Nelson, F. M. Menger, unpublished results.
[12] A. A. Yaroslavov, V. E. Kul'kov, A. S. Polinsky, B. A. Baibakov, V. A. Kabanov, *FEBS Lett.* **1994**, *340*, 121–123.
[13] A. A. Yaroslavov, A. A. Efimova, V. E. Kul'kov, V. A. Kabanov, *Polym. Sci.* **1994**, *36*, 264–270.
[14] W. C. Wimley, T. E. Thompson, *Biochemistry* **1991**, *30*, 1702–1709.