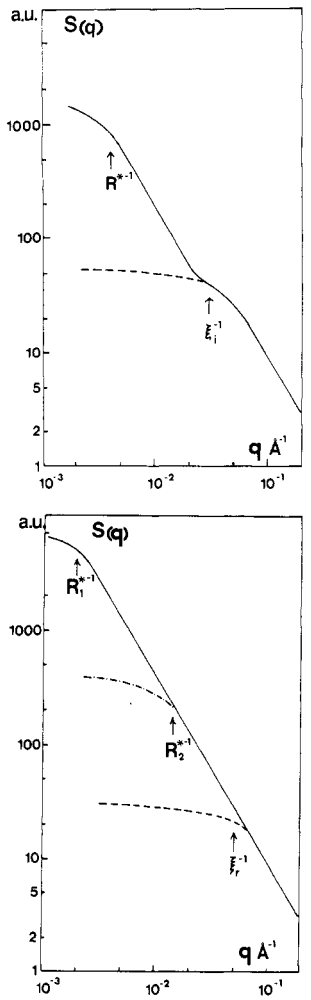

Figure 3. Schematic representations of possible scattered intensities by overswollen networks. (a) Nonnegligible contribution of the scattering by the interstitial medium at $q \simeq 1/\xi_i$ (ξ_i = screening length of the interstitial medium); (- - -) scattering by the interstitial medium alone. (b) Negligible contribution of the scattering by the intersitial regime at $q \simeq 1/\xi_i$; (- - -) scattering by the reaction bath; (—) scattering by very large frozen blob clusters, $P_b^1 \simeq P_c$, $R_1^* =$ typical size of the clusters; (–·–) scattering by smaller frozen blob clusters, $P_b^2 < P_c$, $R_2^* < R_1^*$. The slope in this regime is $(3 - \tau)/\nu \simeq 1.6$.

could be provided by scattering experiments.

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Jacques Bastide*
Institut Charles Sadron (CRM-EAHP)
6, rue Boussingault, 67083 Strasbourg-Cedex
France and Laboratoire Léon Brillouin, CEN Saclay
91191 Gif-sur-Yvette, France

Ludwik Leibler*
Laboratoire de Physico-chimie Macromoléculaire
Université Pierre et Marie Curie, ESPCI
10, rue Vauquelin, 75231 Paris Cedex 05, France

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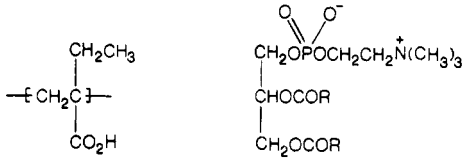
pH-Dependent Vesicle-to-Micelle Transition in an Aqueous Mixture of Dipalmitoylphosphatidylcholine and a Hydrophobic Polyelectrolyte

The capacity to respond to chemical and physical stimuli is an essential characteristic of biological membranes. Intercellular communication and recognition, response to the binding of drugs and hormones, photoreception, and many other critical cellular functions are in fact *membrane* processes in biology and are subject to control as a result of the responsive nature of the bilayer membrane.

We[1] and others[2] have demonstrated several means of preparing *synthetic* bilayer membranes that respond to signals in an analogous fashion. Our approach exploits conformational transitions in membrane-associated polyelectrolyte chains to modulate bilayer structure and permeability. In particular, we have found that addition of the hydrophobic polyelectrolyte, poly(2-ethylacrylic acid) (PEAA, **1**), to aqueous dispersions of natural or synthetic phosphatidylcholines (**2**), renders the lipid



1 **2a**, R = $C_{15}H_{31}$

membrane exceedingly sensitive to pH.[1a-d] pH sensitivity arises from the cooperative collapse of the polyelectrolyte chain from an expanded, hydrophilic state in basic solutions to a globular, hydrophobic coil upon acidification.[1d,3] Collapse of the chain is accompanied by rapid and quantitative release of vesicle contents,[16] and the fact that release occurs near physiological pH makes this a process of some potential interest in biology and medicine. We present herein evidence that the release of vesicle contents is a result of membrane rupture caused by a reorganization of the surfactant aggregate from vesicular to mixed micellar form.

Hydration of dipalmitoylphosphatidylcholine (DPPC, **2a**) at a concentration of 50 μg/mL in 50 mM phosphate

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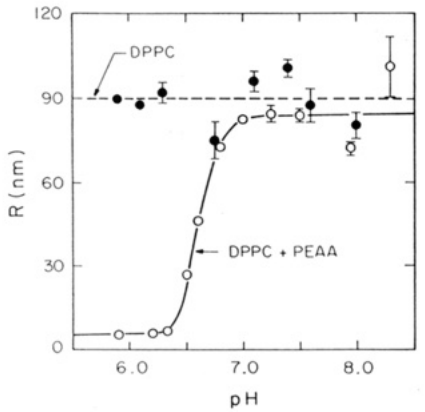


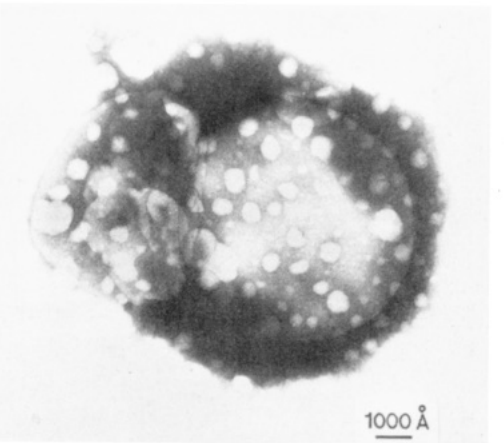
Figure 1. Average hydrodynamic radius (R_H) of aggregates of DPPC (50 μg/mL) suspended in (top) 50 mM phosphate buffers or (bottom) the same buffers containing 50 μg/mL PEAA. Preparation and analysis of samples as in ref 4. Radius plotted for DPPC + PEAA at pH <6.5 is the smaller radius obtained from the observed bimodal distributions.

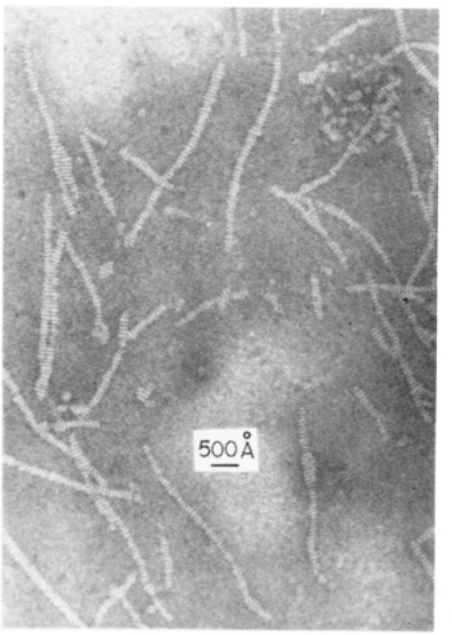
buffer, pH 7.4, affords a suspension of closed lipid vesicles. Analysis of such a suspension—subsequent to its passage through a Millipore filter of 0.45-μm pore diameter—reveals an average hydrodynamic radius (R_H) of 90 ± 10 nm by quasi-elastic light scattering (QELS).[4] The average hydrodynamic radius is found to be independent of the pH of the sample in the range of pH from 5.5 to 8.5 (Figure 1).

Similar treatment of DPPC suspensions that contain 50 μg/mL of PEAA reveals quite different behavior. While the observed particle sizes are similar to those found in polymer-free samples above pH 7.0, R_H decreases 15-fold, to ca. 5.5 nm, as the pH is depressed further (Figure 1). This very small particle size appears inconsistent with *vesicular* aggregation of DPPC in the acidic polyelectrolyte solution.

Negative-stain electron microscopy provides information consistent with that derived from light scattering.[5] Figure 2a shows the appearance of DPPC suspended in aqueous PEAA at pH 7.6. The sample under these conditions consists of closed vesicles and is virtually identical in appearance with the polymer-free control. After reduction of the pH to 5.5, however, the appearance of the sample is quite different (Figure 2b): Vesicles are absent, and the surfactant appears as extended stacks of disks of thickness approximately 5.5 nm and diameter approximately 16.0 nm. These images are strikingly similar to those obtained from preparations of nascent high-density lipoproteins and from recombinants of synthetic phospholipids and apolipoproteins.[6] The stacking in each case is almost certainly a result of the staining and drying steps required in preparing the sample for electron microscopy; the value of R_H determined by QELS is consistent only with the presence of scattering elements of the size of a single disk.[7] We reserve judgment on the question of possible distortion of aggregate shape on drying, but the consistency of our QELS and microscopic results leaves little doubt about the size—and the micellar nature—of the aggregates present in the acidic suspension.

A particularly intriguing image is obtained by slowing the vesicle-to-micelle transition by maintaining a moderately acidic (pH 6.4) suspension below the melting point (41 °C) of the DPPC bilayer. Structural reorganization is very slow under these conditions, so that the process can be captured at an early stage. Figure 2c was obtained in this manner[8] and shows two vesicles, apparently intact, with small stacks of micelles protruding from the membrane surface. Whether or not the immediate juxtaposition of structures is of any mechanistic significance remains to be determined.





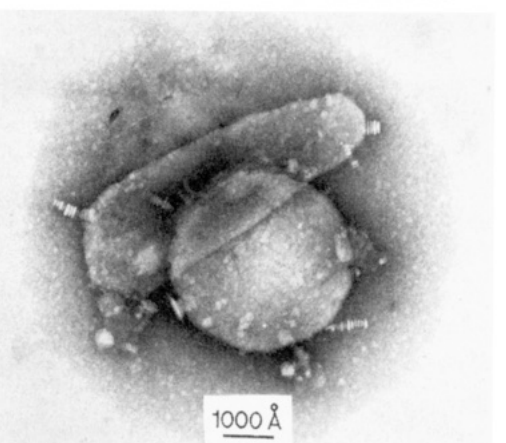


Figure 2. (a, top) Negative-stain electron micrograph of DPPC suspended in phosphate-buffered aqueous PEAA, pH 7.6. Sample prepared as in ref 5. (b, middle) Negative-stain electron micrograph of DPPC suspended in phosphate-buffered aqueous PEAA, pH 5.5. Sample prepared as in ref 5. (c, bottom) Negative-stain electron micrograph of DPPC suspended in phosphate-buffered aqueous PEAA, pH 6.4. Sample prepared as in ref 8.

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(4) DPPC was obtained from Sigma Chemical Co. (99%) and used as received. DPPC was placed in a glass vial and dissolved in a minimum volume of chloroform. The solvent was removed by rotary evaporation to leave a lipid film. Buffer (Na_2HPO_4/NaH_2PO_4/NaCl, ionic strength = 50 mM) was added to bring the lipid concentration to 50 μg/mL. In samples that contained PEAA, the polymer was predissolved in the hydration buffer at a concentration of 50 μg/mL. The lipid was hydrated at 65 °C by 3–5-min temperature equilibration, 10 s of vortex agitation, 1 min of sonication at 65 °C (30 W, Branson Sonifier 185), and 1 h of incubation at 65 °C. Samples were then cooled to 25 °C and filtered into the scattering cell for QELS measurement in a conventional spectrometer using an argon ion laser (approximately 200 mW) and digital correlator (Langley Ford Instruments Model 1096). Correlation functions were analyzed by using second cumulants to obtain average size and by Laplace inversion to obtain the size distribution.

(5) Samples for electron microscopy were prepared in the following manner. DPPC was deposited as a lipid film as described in ref 4 and hydrated at a concentration of 2 mg/mL in phosphate buffer (cf. ref 4) with repeated vortex agitation at 50 °C. An equal volume of a 2 mg/mL solution of PEAA in the same buffer was added, and the mixture was heated to 50 °C for 30 min with periodic vortex agitation, cooled to room temperature (22 °C), and allowed to stand for several hours. Formvar- and carbon-coated Cu grids were treated with 5 μL of the polymer–lipid mixture. After 30 s, excess liquid was wicked away by using the edge of a filter paper. The samples were stained for 30 s with 5 μL of 2% aqueous phosphotungstic acid, the pH of which was adjusted to correspond to that of the sample. Again, the excess liquid was wicked away. The grids were then examined in a JEOL 100S transmission electron microscope at 80 kV.

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(7) Under the conditions used in this work, the calculated diffusion coefficient of an oblate ellipsoid of major and minor axes 16.0 nm and 5.5 nm, respectively, is 3.6×10^{-7} cm^2/s. The measured diffusion coefficient was 4.0×10^{-7} cm^2/s. Thus the hydrodynamic radius implied by the microscopic images is within 10% of that actually measured, if one assumes no aggregation of disks prior to drying.

(8) This sample was prepared as follows. A solution of PEAA (2 mg/mL) in 50 mM Na_2HPO_4/NaH_2PO_4 buffer, pH 6.4, was added to an equal volume of a 2 mg/mL suspension of DPPC in the same buffer. The mixture was subjected to vortex agitation for 5–10 min at room temperature (22 °C) and allowed to stand for several hours before transfer to Cu grids as described in ref 5.

† Department of Polymer Science and Engineering, University of Massachusetts.

‡ Department of Physics and Astronomy, University of Massachusetts.

§ Eastman Kodak Company.

Keith A. Borden,† Ki Min Eum,‡ Kenneth H. Langley,‡ Julia S. Tan,§ David A. Tirrell,*,† and Connie L. Voycheck§

Department of Polymer Science and Engineering and Department of Physics and Astronomy University of Massachusetts Amherst, Massachusetts 01003

Research Laboratories, Eastman Kodak Company Rochester, New York 14650

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