a narrow temperature range and particular starting conditions, such as tube volume and reactant quantities. Each crystal must, nevertheless, be fully characterized, particularly when polytypes exist (TaS_3 case). In addition, the closely related structures of the various phases make intergrowth phenomena likely. Intergrowth would lead to slight local displacements of metal and iodine atoms in their respective chains. Chain fragments of one type in another can strongly influence the depinning energy of CDWs in $NbSe_3$, although the role of chemical impurities has recently been recognized as being very important.[57]

The chemistry of low-dimensional solids is a particularly fertile domain, as is often the case for interdisciplinary fields. It illustrates the importance of ties that exist between coordination chemistry and the solid state.[58] The close relationship is manifested in remarkable similarities in terms of structures, orbital descriptions, and unusual physical properties (i.e., charge density waves, spin density waves, and low-dimensional magnetism). These similarities give one the impression that there is some underlying principle unifying the two fields. This approach, however, should not be developed beyond what is supported by experimental results.

This work was made possible thanks to the contributions of many outstanding colleagues and graduate students. I would especially like to acknowledge A. Meerschaut, R. Brec, M. Evain, L. Guemas, and P. Gressier, who work with me in Nantes, and M. Whangbo of the University of North Carolina at Raleigh, who spent some time here as a visiting professor.

(57) Thorn, R.; Cornell U. Personal communication.

(58) Sunshine, S. A.; Keszler, D. A.; Ibers, J. A. *Acc. Chem. Res.* **1987**, *20*, 395-400.

Polyelectrolyte-Sensitized Phospholipid Vesicles

JAMES L. THOMAS and DAVID A. TIRRELL*

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

Received January 29, 1992

Control of the structure and permeability of biological membranes is one of the most basic and least understood problems in contemporary biophysics. Diverse cellular processes require exquisite control of the membrane structure, including endocytosis and exocytosis, maintenance of the Golgi apparatus and the endoplasmic reticulum, and mitosis and meiosis, to mention just a few. Recently, significant progress has been made in controlling the permeability, and often the structures, of synthetic bilayer vesicles. These studies are especially important in that they help elucidate previously unimagined mechanisms for membrane control. Although no single model system is likely to utilize all of the rich capabilities exploited in nature, the synthetic systems can serve to identify concepts and mechanisms likely to be useful in a biological context. In addition, control of vesicular membrane systems may usher in novel chemistries for use in imaging, sensing, and therapeutic applications.

Particular attention has been given to the synthesis of vesicles that are responsive to their environment through increased permeability or fuseogenicity, since such vesicles have therapeutic potential as vehicles for targeted drug delivery[1,2] (Figure 1). Environmental cues associated with the target, such as the low pH in an endosome of a cell that has internalized a targeted vesicle, can be used to trigger vesicle permeabilization or fusion with the endosomal membrane. Targeting has been achieved by anchoring antibodies, specific for tumor or viral antigens, to the vesicle surface. Excellent reviews of this application of responsive vesicles have been published by Collins and Huang[3] and by Papahadjopoulos and Gabizon.[4]

pH-sensitive vesicles have also been used as tools for fundamental studies in cell biology. The capability of delivering foreign DNA, RNA, antibodies or other proteins, or fluorescent labels to the cytoplasmic compartment of cultured cells is necessary to gain an understanding of the complex chemistry and organization of the cytoplasm. An important step in the use of pH-sensitive vesicles for these applications has been made by Reddy et al.,[5] who showed that pH-sensitive vesicles could be used to deliver a foreign protein (ovalbumin) to the cellular cytoplasmic compartment more efficiently than osmotic loading, which has conventionally been used to introduce foreign materials into cultured cells. Another important application of pH-sensitive vesicles has been as "microsensors" to determine the kinetics of acidification in cellular endocytic compart-

James L. Thomas was born December 2, 1959, in Akron, Ohio. He attended Stanford University (B.S. Physics, 1982) and Cornell University (Ph.D. Physics, 1991). He is currently employed as a postdoctoral fellow in the Department of Polymer Science and Engineering at the University of Massachusetts at Amherst. His principal research interests are the biophysics of membranes and the conformations and chemistry of membrane-active macromolecules.

David A. Tirrell was born January 10, 1953, in Easton, Pennsylvania. He attended MIT (B.S. Chemistry, 1974) and the University of Massachusetts (Ph.D. Polymer Science and Engineering, 1978), and was a postdoctoral fellow at Kyoto University, Japan, in 1978. He is presently Professor of Polymer Science and Engineering and Director of the Materials Research Laboratory at the University of Massachusetts and Editor of the *Journal of Polymer Science, Part A: Polymer Chemistry*. Professor Tirrell's research interests are in polymer synthesis and bioorganic polymer chemistry.

(1) Gabizon, A.; Papahadjopoulos, D. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6949-6953.
(2) Liu, D.; Huang, L. *Biochim. Biophys. Acta* **1990**, *1022*, 348-354.
(3) Collins, D.; Huang, L. In *Molecular Mechanisms of Membrane Fusion*; Ohki, S., Doyle, D., Flanagan, T., Hui, S., Mayhew, E., Eds.; Plenum Press: New York, 1988; pp 149-161.
(4) Papahadjopoulos, D.; Gabizon, A. In *Horizons in Membrane Biotechnology*; Nicolau, C., Chapman, D., Eds.; Wiley-Liss: New York, 1990; pp 85-93.
(5) Reddy, R.; Zhou, F.; Huang, L.; Carbone, F.; Bevan, M.; Rouse, B. *J. Immunol. Methods*, in press.

0001-4842/92/0125-0336$03.00/0

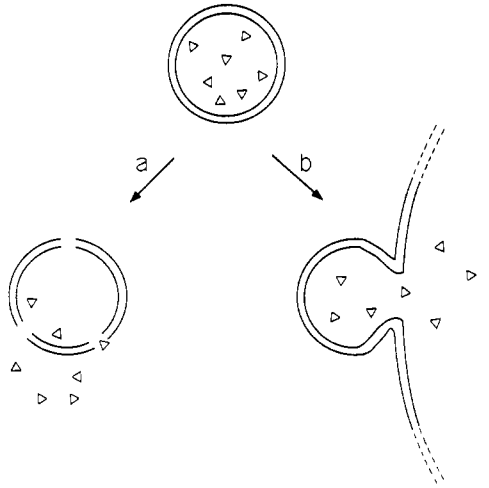


Figure 1. Environmentally sensitive phospholipid vesicles may respond to stimuli either by (a) increasing their permeability or (b) increasing their tendency to fuse with other membranes. Both responses may be useful in targeted drug delivery systems.

ments. Relief of self-quenching of an entrapped fluorescent dye indicates that endosomal pH has been depressed to the critical pH for vesicle fusion and/or leakage. By changing vesicle composition, and thence acid sensitivity, Collins and co-workers[6] examined the kinetics of several different stages in acidification in murine L929 cells.

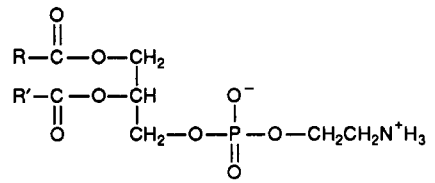
In vitro, responsive vesicles have been used for signal amplification in assays for small and large biomolecules. One approach, originally developed by Six et al.[7] and later by others,[8-11] utilizes the capability of the blood complement proteins to lyse antibody–coated (opsonized) vesicles. Vesicles are constructed with covalently-bound analyte on their surfaces and an entrapped self-quenching fluorescence indicator. These are then added to the test solution, and antibodies to the analyte are added. When little or no analyte is present in the test solution, the antibodies bind to the vesicles, and addition of complement proteins causes lysis and an increased fluorescence signal. If the analyte concentration is high, little antibody–vesicle binding will occur, and consequently the fluorescence will increase only slightly. A similar competitive binding assay has been developed by Pinnaduwage and Huang[12] using synthetic vesicles that rupture under the action of the enzyme β-galactosidase.

Thus synthetic responsive vesicles are becoming increasingly important, not only because of the insight they provide into membrane biology and chemistry but also because of the increasing number of practical applications for this technology. In this article, we shall briefly discuss several strategies used to create synthetic responsive vesicles, with an emphasis on the use of polymers to control the organization of phospholipid bilayers.

Strategies for the Design of Environmentally Responsive Phospholipid Vesicles

Four basic strategies have been utilized in generating responsive synthetic vesicles. These involve the following: (i) conversion of the membrane-forming lipid to a nonlamellar phase, (ii) formation of channels or defects, (iii) controlled membrane fusion, or (iv) induced lipid micellization. Each of these approaches is discussed in turn below.

Nonlamellar Lipids. The most common strategy for controlled membrane destabilization is to make use of the tendency of certain phospholipids, particularly phosphatidylethanolamines (PE) (1), to form nonlamellar phases. At elevated temperatures and pH below



1

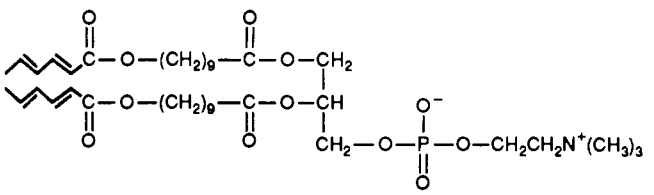
about 9, the preferred structure for PE is an inverted hexagonal phase, designated H_{II}.[13] Stable liposomes containing PE can be prepared by incorporating other amphiphiles into the bilayer. The ability of an amphiphile to stabilize PE bilayers depends on the headgroup charge, molecular shape, and miscibility with PE. Both oleic acid[14,15] and diacyl succinylglycerols[16] can stabilize PE when charged, i.e., at high pH. At lower pH, the protonated acids are unable to stabilize the membrane, and vesicles become fuseogenic and/or leaky. (Interestingly, fuseogenicity of PE liposomes is actually decreased at elevated temperatures,[17] perhaps because a leaky intermediate is stabilized.)

The charge of a stabilizing amphiphile can also be altered enzymatically. The β-galactosidase-sensitive vesicles mentioned above were constructed from a mixture of the ganglioside G_{M1} with PE. The β-galactosidase enzyme cleaves a charged galactosyl moiety from G_{M1}, which leads to membrane destabilization.[12]

The miscibility (in PE) of the stabilizing amphiphile can be manipulated to provide membrane photosensitivity. Frankel et al.[18] included a photopolymerizable derivative of phosphatidylcholine (bis-sorb PC) (2) in PE bilayers. Irradiation at 256 nm led to polymerization and microphase separation of the bis-sorb PC. The PE-enhanced regions were unstable, and leakage of vesicle contents ensued.

The PE-based responsive systems are particularly well suited for intracellular delivery applications, since (1) they require interbilayer contact, which occurs

(6) Collins, D.; Maxfield, F.; Huang, L. *Biochem. Biophys. Acta* **1989**, *987*, 47–55.
(7) Six, H. R.; Uemura, K.; Kinsky, S. C. *Biochemistry* **1973**, *12*, 4003–4011.
(8) Yasuda, T.; Naito, Y.; Tsumita, T.; Tadakuma, T. *J. Immunol. Methods* **1981**, *44*, 153–158.
(9) Martin, F. J.; Papahadjopoulos, D. *J. Biol. Chem.* **1982**, *257*, 286–288.
(10) Ishimori, Y.; Yasuda, T.; Tsumita, T.; Notsuki, M.; Koyama, M.; Tadakuma, T. *J. Immunol. Methods* **1984**, *75*, 351–360.
(11) Gaber, B. P.; Ligler, F. S.; Bredehorst, R. *Biotechnological Applications of Lipid Microstructures*; Gaber, B. P., Schnur, J. M., Chapman, D., Eds.; Plenum Press: New York, 1988; pp 209–214.
(12) Pinnaduwage, P.; Huang, L. *Clin. Chem.* **1988**, *34*, 268–272.
(13) Allen, T.; Hong, K.; Papahadjopoulos, D. *Biochemistry* **1990**, *29*, 2976–2985.
(14) Straubinger, R.; Duzgunes, N.,; Papahadjopoulos, D. *FEBS Lett.* **1985**, *179*, 148–154.
(15) Collins, D.; Connor, J.; Ting-Beall, H.-P.; Huang, L. *Chem. Phys. Lipids* **1990**, *55*, 339–349.
(16) Collins, D.; Litzinger, D.; Huang, L. *Biochim. Biophys. Acta* **1990**, *1025*, 234–242.
(17) Ellens, H.; Bentz, J.; Szoka, F. C., Jr. *Biochemistry* **1986**, *25*, 285–294.
(18) Frankel, D.; Lamparski, H.; Liman, U.; O'Brien, D. *J. Am. Chem. Soc.* **1989**, *111*, 9262–9263.

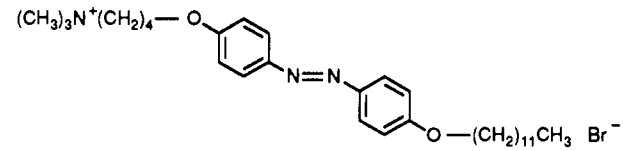


2

during endocytosis, and (2) they do not just become permeable, they become fuseogenic. Fuseogenicity is useful in these applications because an internalized vesicle will be surrounded by an endosomal membrane. Vesicle permeabilization will only release the encapsulated drug into the endosome, but vesicle fusion with the endosomal wall will deliver the drug to the cytoplasm, which is more frequently the desired destination. For many other applications, however, vesicle permeability is the central goal, and often interbilayer contact is not realized. For such applications, other strategies for synthesizing responsive vesicles may be more useful.

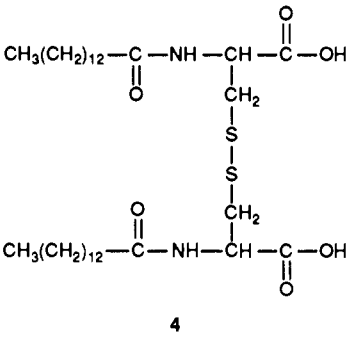
Channels and Defects. A straightforward approach is to use molecules that form channels or defects in the bilayer in response to the desired stimulus. A 30 amino acid amphipathic peptide synthesized by Subbarao and co-workers[19] undergoes a pH-dependent α-helix random coil transition. At low pH, the helical form incorporates into bilayers and aggregates to form aqueous channels.[20]

Photosensitive vesicles have been synthesized by including an alkyl ammonium salt with a pendant azobenzene chromophore (3) in phosphatidylcholine (PC) bilayers.[21,22] Irradiation at 366 nm converts the extended trans form of azobenzene to the cis form, which is kinked. The cis form appears to act as a membrane defect: membranes containing the cis form show enhanced permeability to water and to the indicator dye bromothymol blue. Other photoisomerizations[23,24] have been used in a similar manner.

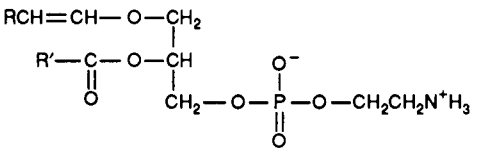


3

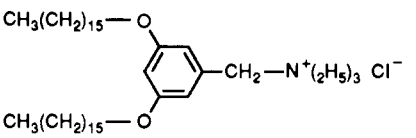
Single-chain derivatives of phospholipids (e.g., lysolecithin) often act as permeabilizing or solubilizing agents. Vesicles can be rendered sensitive to any chemical agent that can cleave one acyl chain from a diacyl lipid; for example, ordinary PC vesicles are sensitive to the enzyme phospholipase A2, which cleaves the acyl chain ester linkage on C2, yielding lysolecithin and a fatty acid. Double-chain cystine lipids, e.g., 4, can be cleaved with the reducing agent dithiothreitol; the partially water-soluble single-chain products cannot persevere in a vesicular structure.[25] In the presence of a photosensitizer, vesicles containing plasmalogen (5) are permeabilized by 630-nm irradiation, most likely due to cleavage of the *sn*-1-vinyl ether linkage.[26] Removal of headgroup charge can also destabilize liposomes: photolytic cleavage of a benzylammonium salt (6)[27] or a benzenediazonium lipid (7)[28] has been used to disrupt vesicles.



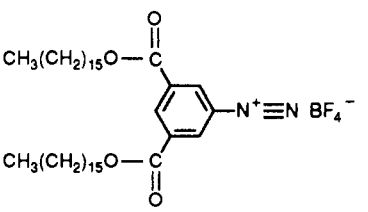
4



5



6



7

Macromolecule-Induced Fusion. A third strategy for modifying membrane behavior is to exploit the tendency of vesicular aggregates to undergo fusion. Some phospholipids form vesicles that have an "intrinsic" environmental sensitivity: phosphatidylserine vesicles aggregate and fuse in the presence of millimolar calcium, for example. The range and strength of stimuli that affect the fuseogenicity of model membranes can be controlled through interactions with exogenous macromolecules. Several natural and synthetic amphipathic polypeptides are known to enhance rates of vesicle fusion in vitro, often in a pH-dependent manner; a thorough review of pH-dependent vesicle fusion, both with and without macromolecular mediators, has been written by Düzgünes and co-workers.[29]

Polyelectrolyte-Induced Micellization. As mentioned above, the nature of the response of a vesicle to an environmental cue can take several forms—an in-

(19) Subbarao, N. K.; Parente, R. A.; Szoka, F. C., Jr.; Nadasdi, L.; Pongracz, K. *Biochemistry* **1987**, *26*, 2964–2972.
(20) Parente, R. A.; Nir, S.; Szoka, F. C., Jr. *Biochemistry* **1990**, *29*, 8720–8728.
(21) Kano, K.; Tanaka, Y.; Ogawa, T.; Shimomura, M.; Okahata, Y.; Kunitake, T. *Chem. Lett.* **1980**, 421–424.
(22) Kano, K.; Tanaka, Y.; Ogawa, T.; Shimonura, M.; Kunitake, T. *Photochem. Photobiol.* **1981**, *34*, 323–329.
(23) Pidgeon, C.; Hunt, C. A. *Photochem. Photobiol.* **1983**, *37*, 491–494.
(24) Morgan, C. G.; Thomas, E. W.; Sandhu, S. S.; Yianni, Y. P.; Mitchell, A. C. *Biochim. Biophys. Acta* **1987**, *903*, 504–509.
(25) Ringsdorf, H.; Schlarb, B.; Venzmer, J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 113–158.
(26) Anderson, V. C.; Thompson, D. H. In *Polymers at Interfaces: Macromolecular Assemblies*; Stroeve, P., Salazs, A., Eds.; American Chemical Society: Washington, DC, in press.
(27) Haubs, M.; Ringsdorf, H. *Nouv. J. Chim.* **1987**, *11*, 151–156.
(28) Fuhrhop, J. H.; Bartsch, H.; Fritsch, D. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 804–805.
(29) Duzgunes, N.; Straubinger, R. M.; Baldwin, P. A.; Papahadjopoulos, D. In *Membrane Fusion*; Wilschut, J., Hoekstra, D., Eds.; Marcel Dekker: New York, 1991; pp 713–730.

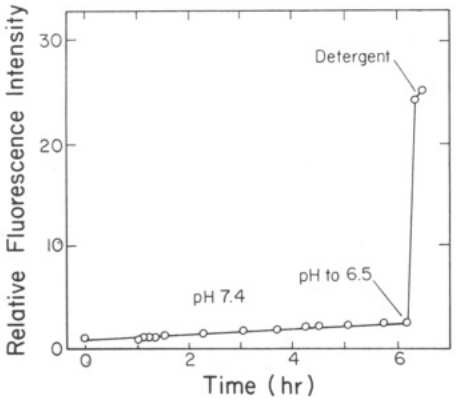
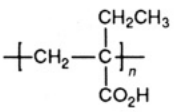


Figure 2. Efflux of 6-carboxyfluorescein from sonicated phosphatidylcholine vesicles suspended in 50 mM Tris-HCl, 100 mM NaCl, 0.03% PEAA, at indicated pH. Reprinted with permission from ref 31. Copyright 1985 New York Academy of Sciences.

creased tendency to fuse with other vesicles, permeabilization to ions or large molecules, or even complete membrane disruption and solubilization.

Earlier work in this laboratory on the interactions of synthetic macromolecules with phospholipid membranes has led to the observation that certain amphiphilic polyelectrolytes can cause pH-dependent membrane disruption and solubilization. Poly(acrylic acid) derivatives were found to associate with phosphatidylcholine membranes and modify phase-transition behavior in a pH-dependent manner.[30] The most hydrophobic of the soluble poly(acrylic acid) derivatives, poly(2-ethylacrylic acid) (PEAA) (8), was found not only to adsorb strongly to membranes at low pH but also to permeabilize and, at high enough concentrations, to fully solubilize them when the membranes were in the liquid crystalline (L_α) phase. Figure 2 shows the



8

fluorescence from a preparation of sonicated vesicles of egg phosphatidylcholine (egg PC) that have entrapped 6-carboxyfluorescein, a self-quenching, membrane impermeant fluorescent dye. The aqueous medium contains 0.03% w/v PEAA. At pH 7.4, the fluorescence is stable for many hours, which shows that the dye remains trapped and self-quenched under these conditions. When the pH is lowered to 6.5, the fluorescence increases dramatically. Essentially all of the dye is released: the addition of detergent results in no significant further increase in fluorescence.[31]

PEAA appears to permeabilize PC membranes by disrupting their lamellar structure and reorganizing the membrane into a micellar aggregate of mixed (polymer and lipid) composition. By negative stain electron microscopy, these mixed micelles are discoidal, with a thickness of about 5.5 nm and a diameter of about 16 nm[32] (Figure 3).

The stacking of the disks observed in Figure 3 is apparently an artifact of the preparation for electron

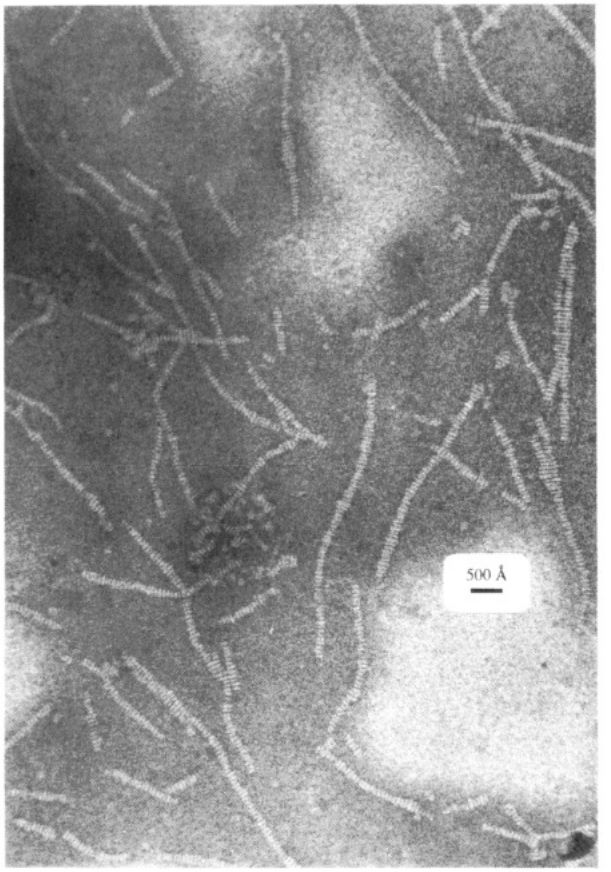


Figure 3. Negative stain (2% phosphotungstic acid) electron micrograph of DPPC/PEAA mixed micelles at pH 5.5. Reprinted with permission from ref 32. Copyright 1988 American Chemical Society.

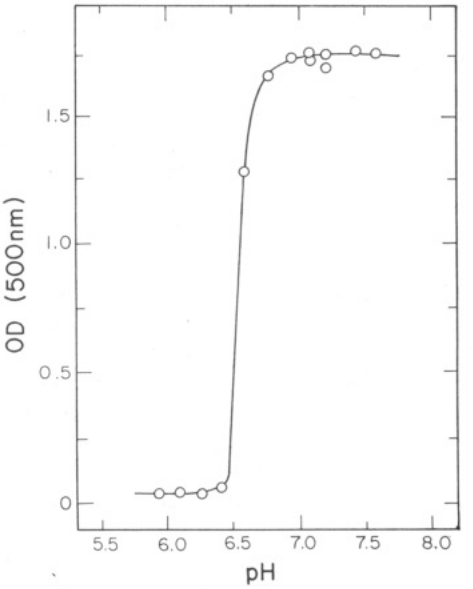


Figure 4. Optical density (500 nm) of a 1 mg/mL suspension of DPPC in phosphate-buffered aqueous PEAA (1 mg/mL). Reprinted with permission from ref 31. Copyright 1985 New York Academy of Sciences.

microscopy, since measurements by quasi-elastic light scattering show particles with average hydrodynamic radii of ca. 5.5 nm, consistent with the individual disk sizes. The thickness (5.5 nm) of each disk is comparable with that of the original lipid membrane. The disk diameter seems to have no special relationship to the dimensions of the polymer or of the undisrupted mem-

(30) Seki, K.; Tirrell, D. *Macromolecules* **1984**, *17*, 1692–1698.
(31) Tirrell, D.; Takigawa, D.; Seki, K. *Ann. N.Y. Acad. Sci.* **1985**, *446*, 237–248.
(32) Borden, K.; Eum, K.; Langley, K.; Tan, J.; Tirrell, D.; Voycheck, C. *Macromolecules* **1988**, *21*, 2649–2651.

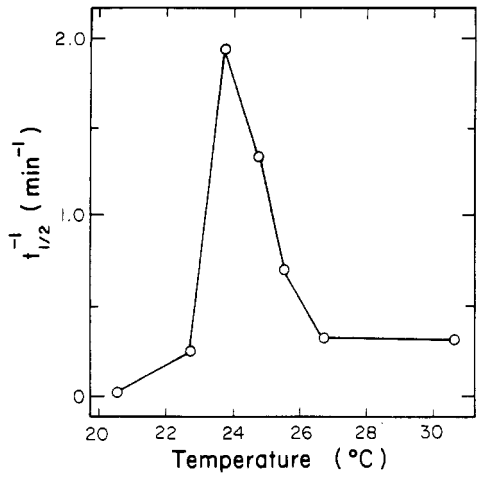


Figure 5. Effect of temperature on the rate of decrease in the optical density ($t_{1/2}^{-1}$) of dimyristoyl PC multilamellar vesicle suspensions upon addition of PEAA and acidification to pH 6.45. The quantity ($t_{1/2}^{-1}$) is the time required for a loss in optical density of one-half that observed at long ("infinite") time. Data from ref 44.

brane. Similar discoidal structures are seen when lipids are solubilized by cholate or other bile salts[33] (at equimolar ratios) or by combination with apolipoproteins.[34]

The solubilization of lipid membranes by PEAA can be easily monitored in multilamellar suspensions through optical density measurements[35] (Figure 4). When PC is dried from a chloroform solution and then rehydrated without sonication, vesicles up to microns in diameter with dozens or perhaps hundreds of lamellae are obtained. These suspensions scatter light effectively. In the presence of PEAA, lowering the pH to 6.5 clarifies the suspension within minutes.

PEAA appears to be quite generally capable of disrupting phosphatidylcholine bilayers. Distearoyl, dipalmitoyl, dimyristoyl, dilauroyl, and dioleoyl PC all are disrupted by PEAA at reduced pH. In each case, the temperature must be above the T_m (the gel to liquid crystal transition temperature) for the lipid used. For dimyristoyl PC, the rate of solubilization is maximal in the vicinity of T_m, vanishing at lower temperatures and diminishing somewhat at higher temperatures (Figure 5). Interestingly, the permeability of PC membranes to small molecules is also at a maximum near T_m.[36-40] This effect has been attributed to putative crystalline defects in the membrane structure at those temperatures[38,41] or to critical fluctuations in the lateral compressibility of the bilayer;[42,43] it may be that the kinetics of PEAA disruption is also determined by these factors.

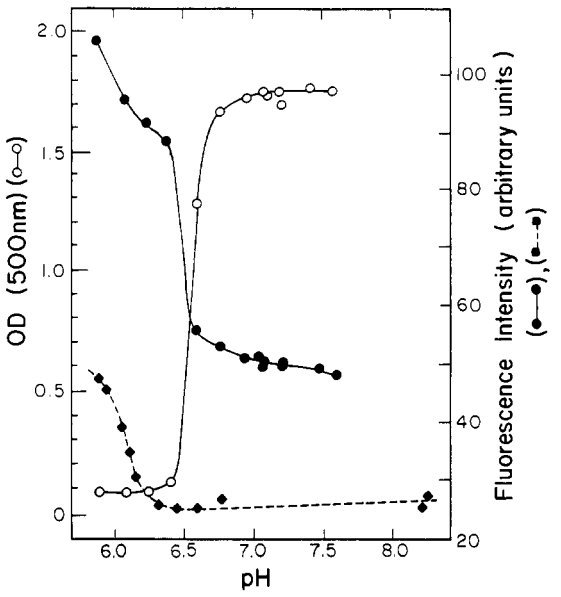


Figure 6. Optical density decrease in DPPC/PEAA suspensions on acidification (○) increase in fluorescence from PEAA copolymerized with 1-pyreneacrylic acid (1.5×10^{-3} mol %) (in the presence of DPPC vesicles) (●), and fluorescence from solution of pyrene (5 μM) in PEAA (in the absence of DPPC vesicles) (◆). The conformational transition of the polymer, as indicated by the appearance of hydrophobic domains that give rise to an increase in pyrene fluorescence, occurs about 0.4 pH unit below the pH for membrane disruption. Data from ref 35.

Oddly, the disruption of the longer chain distearoyl PC shows an onset near T_m but no maximal rate.[44]

The reorganization of vesicles of the unsaturated lipid dioleoyl PC (DOPC) by PEAA yields a product that is profoundly different from the small, apparently discoidal mixed micelles formed from the saturated dipalmitoyl PC (DPPC). Quasi-elastic light scattering studies show that multilamellar DOPC liposomes, with average hydrodynamic radii of ca. 500 nm, are transformed into 40–50 nm radius particles by adsorption of PEAA.[45] Particles of this size cannot be micellar; presumably they are vesicular. The reorganization occurs only above a threshold PEAA/DOPC weight ratio of between 3 and 10%. This is less PEAA than is required to solubilize DPPC liposomes: complete solubilization of DPPC requires at least 50% weight ratio of PEAA.[46] In addition, the disruption of DOPC is significantly slower than the disruption of DPPC, when both lipids are in the L_α phase. DOPC reorganization takes about an hour, while DPPC restructures in minutes.

A remarkable similarity between DOPC and DPPC disruptions is that in both cases, no intermediate-sized structures have ever been observed. Lamellar reorganization appears to proceed spontaneously, perhaps even catastrophically (on the molecular scale), to the final state. Most membrane solubilization processes, either by detergents[47] or by lipoproteins,[34] result in mixed micelles whose sizes diminish as the concentration of the solubilizing agent is increased. The bimodal size distributions obtained with DOPC and PEAA

(33) Mazer, N. A.; Benedek, G. B.; Carey, M. C. *Biochemistry* **1980**, *19*, 601–615.
(34) Tall, A. R.; Small, D. M.; Deckelbaum, R. J.; Shipley, G. *J. Biol. Chem.* **1977**, *252*, 4701–4711.
(35) Borden, K.; Eum, K.; Langley, K.; Tirrell, D. *Macromolecules* **1987**, *20*, 454–456.
(36) Blok, M. C.; Van der Neut-Kok, E. C. M.; Van Deenen, L. L. M.; deGier, J. *Biochim. Biophys. Acta* **1975**, *406*, 187–196.
(37) Jacobson, K.; Papahadjopoulos, D. *Biochemistry* **1975**, *14*, 152–161.
(38) Marsh, D.; Watts, A.; Knowles, P. F. *Biochemistry* **1976**, *15*, 3570–3578.
(39) Lee, A. G. *Biochim. Biophys. Acta* **1977**, *472*, 237–281.
(40) Bramhall, J. *Biochim. Biophys. Acta* **1984**, *778*, 393–399.
(41) Tsong, T. Y.; Kanehisa, M. I. *Biochemistry* **1977**, *16*, 2674–2680.
(42) Doniach, S. *J. Chem. Phys.* **1978**, *68*, 4912–4916.
(43) Nagle, J. F.; Scott, H. L., Jr. *Biochim. Biophys. Acta* **1978**, *513*, 236–243.
(44) Devlin, B. Ph.D. Dissertation, University of Massachusetts, Amherst, MA, 1990.
(45) Eum, K. M.; Langley, K. H.; Tirrell, D. A. *Macromolecules* **1989**, *22*, 2755–2760.
(46) Borden, K. Ph.D. Dissertation, University of Massachusetts, Amherst, MA, 1989.
(47) Helenius, A.; Simons, K. *Biochim. Biophys. Acta* **1975**, *415*, 29–79.

suggest that intermediate-sized structures are not realized with this system.

Polymer Adsorption. PEAA, like many other polyelectrolytes, undergoes a pH-dependent coil-to-globule conformational transition. This intramolecular micellization can be detected by an increase in fluorescence from pyrene probes (either in solution or bound to the polymer) as the pH is lowered through the conformational transition. The pyrene fluorescence yield increases dramatically in hydrophobic environments. The conformational transition has also been observed with quasi-elastic light scattering. The hydrodynamic radius of PEAA of number-averaged molecular weight 20 000 decreases from ca. 6 nm at high pH to ca. 4.4 nm below pH 5.9, in 50 mM Tris-HCl buffer.[45]

This conformational transition is centered at about 0.4 pH units *lower* than the critical pH for membrane disruption, as determined by optical density measurements (for PEAA/DPPC at 1 mg/mL each in 400 mM phosphate buffer; Figure 6).[35] The simplest interpretation of this result is that adsorption and membrane disruption occur without conformational change: as the polymer becomes more hydrophobic, membrane association is preferred over intramolecular micellization. A similar effect is seen with detergents, which usually solubilize membranes below the critical micelle concentration of the pure surfactant. In addition, the critical pH for membrane reorganization is concentration dependent. With DOPC vesicles in Tris-HCl buffer, the critical pH is raised from 6.7 to 7.1 when the PEAA concentration is increased 5-fold.[45] This is consistent with the hypothesis that membrane adsorption is sufficient for reorganization.

Potentiometric titration of PEAA shows that, even though the conformational collapse is centered at a pH well below the critical pH for membrane lysis, some small fraction of the polymer (ca. 5%) has undergone a conformational collapse at the point of membrane lysis.[48] It is possible that this small percentage of PEAA is the "active" agent in driving membrane solubilization. However, the fluorescence from PEAA-bound pyrene increases sharply at membrane lysis (in the presence of DPPC). If only the collapsed fraction of PEAA drives membrane solubilization, the solubilized product must have a much higher affinity for PEAA than does the intact membrane in order to account for this fluorescence increase. Thus while we cannot rule out the possibility that a conformational change in the polymer is required for membrane adsorption, our observations are consistent with the simpler hypothesis that no conformational change is required. It is possible that, once adsorbed to the membrane, PEAA undergoes a structural rearrangement that mediates membrane lysis.

Ionization and pH. Factors, including but not limited to pH, which affect the ionization state of PEAA also influence its ability to reorganize membrane vesicles. For example, lowering the ionic strength of the buffer from 500 to 50 mM raises the critical pH for DOPC reorganization from 6.5 to 7.1.[45] The explanation for this phenomenon is that the ions screen repulsive interactions between ionized carboxylic acid groups, allowing those groups to deprotonate at lower pH's. In low ionic strength buffer, the charges are poorly

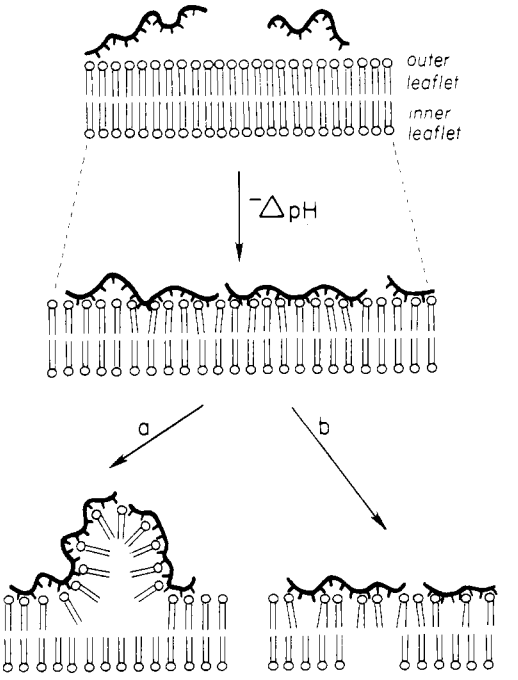


Figure 7. Possible mechanisms for membrane disruption by PEAA. Adsorption of PEAA to the outer leaflet leads to membrane expansion. The accumulated strain can be relieved by either (a) buckling of the outer leaflet or (b) rupture of the inner leaflet.

screened, so that carboxylic acids protonate more readily (at higher pH). Similar shifts are seen in the molecular weight dependence of the critical pH for the globule-to-coil transition and for membrane reorganization. Lower molecular weight preparations of PEAA respond at lower pH, closer to the pK_a of the monomer.[49]

Tentative Mechanism. These observations permit us to propose a tentative mechanism for liposome disruption by PEAA (Figure 7). As PEAA adsorbs to the outer leaflet of the bilayer, it will tend to increase the surface area of that leaflet. We assume that at least some part of the polymer chain (perhaps the ethyl group) intercalates into the bilayer. This expansion of the outer leaflet is resisted by the inner leaflet, which must cover as much area as the outer. Consequently, the inner leaflet is under tension, and the outer leaflet is under lateral compression. As additional PEAA is adsorbed, a point will be reached at which either (a) the outer leaflet buckles under the compressive stress or (b) the inner leaflet ruptures under the tension. At the point of failure, the membrane is free to relieve the accumulated stresses by bending, the curvature then being determined by the amount of adsorbed polymer. The expectation is that the size and even the shape of the resultant particles or vesicles will depend on a few physical parameters of each monolayer: the tensile strength, the compressive strength, and the elastic modulus.

Such a model might eventually explain some of the more puzzling aspects of PEAA reorganization. For example, the differing structure, kinetics, and threshold concentrations that are obtained with saturated and unsaturated lipids may be attributable to differences in their mechanical properties. The absence of intermediate-sized structures, as are seen with detergent-solubilized membranes, would be a natural consequence

(48) Schroeder, U.; Tirrell, D.; Langley, K. Manuscript in preparation.

(49) Schroeder, U.; Tirrell, D. *Marcomolecules* **1989**, *22*, 765–769.

of this mechanical breakdown model.

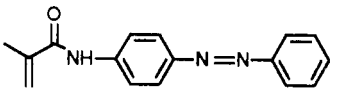
Nonetheless, the model is simplistic, and certain aspects of PEAA behavior are not explained. In particular, permeabilization of membranes seems to occur at PEAA concentrations that are below those necessary for solubilization. When PEAA is covalently anchored to small, unilamellar DPPC vesicles, only 5% PEAA by weight is needed to permeabilize the vesicles to calcein,[50] yet fully solubilized DPPC/PEAA mixed micelles are comprised of 50% PEAA by weight, as has been mentioned. It is not unreasonable that permeabilization can occur at much lower PEAA concentrations than solubilization; the same phenomenon is observed with many surfactants.[47,51-54] The mechanical model of PEAA disruption does not directly address this observation. However, it is possible that the highly stressed membrane has a surfeit of defects that permit calcein leakage. Alternatively, PEAA may have other modes of action once it is adsorbed to the bilayer, perhaps acting cooperatively to form aqueous channels by shielding the acyl chains of the lipids.

Permeabilization and solubilization by detergents seem to be two points along a continuum of increasing detergent concentration, with the same mechanism being operative in both states. It may be that, with PEAA, channel formation and membrane solubilization are mechanistically distinct processes. And, finally, we acknowledge the possibility that, like detergents, PEAA may solubilize membranes by an "edge-actant" mechanism.[53]

Modifications and Applications. These observations lay the groundwork for the modifications to the PEAA/PC system that are required to sensitize vesicular membranes to stimuli other than H^+ concentration. A stimulus can be coupled to a membrane response in the vesicle if it can be made to change either the ionization state or the hydrophobic/lipophobic balance of the PEAA "sensor". This hypothesis has been borne out by several experiments in our laboratory.

The simplest method of coupling is to convert a stimulus into protons directly. For example, the enzyme glucose oxidase has been used to couple vesicle reorganization to glucose concentration through the reaction product gluconic acid.[56] Vesicles that are light sensitive have been constructed by using a "caged proton" in the form of 3,3′-dicarboxydiphenyliodonium salts.[57] Irradiation at 254 nm lowers the pH, which in turn acts to drive PEAA to disrupt PC vesicles with release of contents.

Modification of PEAA has been used to avoid the necessity for elevated H^+ concentration as an intermediate step in photoinitiated vesicle disruption. Copolymerization with *N*-[4-(phenylazo)phenyl]methacrylamide (**9**) (24:1) renders the polymer directly photosensitive.[58]



9

In darkness, the azobenzene moiety assumes a trans conformation with a small dipole moment. Illumination at 350 nm yields the cis form, with a dipole moment of 3.0 D. This difference gives rise to a small change in chain ionization, as well as having a direct effect on hydrophobicity. The dark-adapted, trans form of the copolymer permeabilizes vesicles rapidly, while the irradiated cis form does not.

Finally, it should be noted that the critical pH for disruption of PC membranes can be lowered by copolymerization of 2-ethylacrylic acid with methylacrylic acid, presumably due to the reduced hydrophobicity of the latter monomer.[59] It appears that polymer–membrane interactions can be tailored with remarkable precision, provided that the balance of hydrophobic and hydrophilic properties is carefully adjusted.

Conclusions

Surface-active polyelectrolytes are capable of sensitizing phospholipid bilayer membranes to a variety of environmental stimuli. While other approaches have been used to achieve this goal, polymer sensitization may prove to have important advantages. First, the system can be tailored to different stimuli (e.g., light) by the addition of "sensor" moieties along the polymer chain. Second, polymers are capable of inducing different kinds of membrane structural and permeability changes that depend on the nature of the phospholipid and the extent of polymer adsorption. There is great potential for increasing the number of environmental cues that can be sensed, as well as for fine-tuning the membrane response to those cues. We are only beginning to explore this novel approach to membrane response and control.

We wish to acknowledge the assistance of Drs. Janos Fendler, Leaf Huang, Toyoki Kunitaki, David O'Brien, Demetrios Papahadjopoulos, Helmut Ringsdorf, Francis Szoka, and David Thompson, who provided reprints and preprints of their papers. Work in our own laboratories was supported by U. S. Army Research Office Grant DAAL03-88-K-0038 and by the 3M Corporation.

(50) Maeda, M.; Kumano, A.; Tirrell, D. *J. Am. Chem. Soc.* **1988**, *110*, 7455–7459.
(51) Schubert, R.; Beyer, K.; Wolburg, H.; Schmidt, K. *Biochemistry* **1986**, *25*, 5263–5269.
(52) Ollivon, M.; Eidelman, O.; Blumenthal, R.; Walter, A. *Biochemistry* **1988**, *27*, 1695–1703.
(53) Edwards, K.; Almgren, M.; Bellare, J.; Brown, W. *Langmuir* **1989**, *5*, 473–478.
(54) Vinson, P.; Talmon, Y.; Walter, A. *Biophys. J.* **1989**, *56*, 669–681.
(55) Fromherz, P.; Ruppel, D. *FEBS Lett.* **1985**, *179*, 155–159.
(56) Devlin, B.; Tirrell, D. *Macromolecules* **1986**, *19*, 2465–2466.
(57) You, H.; Tirrell, D. *J. Am. Chem. Soc.* **1991**, *113*, 4022–4023.
(58) Ferritto, M.; Tirrell, D. *Macromolecules* **1988**, *21*, 3117–3119.
(59) You, H. Ph.D. Dissertation, University of Massachusetts, Amherst, MA, 1991.