Migration of Poly-L-lysine through a Lipid Bilayer

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Insinuating ourselves into poly-L-lysine/membrane research, and devoting our energies and imaginations to it, was preceded by a determined crawl through the appropriate literature. This literature, recounted briefly in the remainder of the paragraph, persuaded us of the topic's general interest. Thus, we learned that poly-L-lysine adsorbs onto[1] and penetrates[2] negatively charged lipid bilayers. Binding of poly-L-lysine to such bilayers protects the peptide from pressure-induced conformational changes[3] while increasing the interfacial tension of the outer bilayer leaflet, thereby forming concavities in the membrane.[4] Poly-L-lysine induces a pH-dependent fusion of negative vesicles,[5] transiently blocks certain ion channels,[6] modifies bilayers' electrical properties (conductivity and permittivity),[7] and causes phase separations into microdomains.[8] Rabbit leukocytes experience a time- and concentration-dependent rupture in the presence of poly-L-lysine.[9] Also the peptide has been studied on several occasions in connection with its ability to enhance DNA transfection efficiency.[10,11] Our own work with poly-L-lysine began with the discoveries that it serves as a bilayer adhesive[12] and that it mediates the permeation of a cancer drug, doxorubicine, into the vesicle interior.[13]

The present investigation was initiated when we happened to expose a negatively charged giant vesicle to poly-L-lysine. Dark spots developed on the vesicle walls which grew, after many minutes, into internal linear structures visible by light microscopy (Figure 1). These structures, henceforth referred to as "ropes", differed in their thickness from experiment to experiment. No ropes formed with vesicles lacking an anionic lipid. Cationic peptide poly-L-arginine (MW 10 800), but not neutral poly(vinylpyrrolidone), also induced ropes. The question arose, quite naturally, as to the composition of the ropes. Initially we had a "reasonable doubt" that poly-L-lysine could be a component because this would demand a fairly rapid migration of the polycationic peptide across an apolar lipid barrier. Reasonable doubt, however, was our ticket to a further study that, as will be seen, ultimately verified the presence of poly-L-lysine in the ropes. Before giving details of our evidence, however, we must present a few experimental details.

Giant vesicles (30–90 µm in diameter), composed of 90 mol % of the neutral 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) plus 10 mol % the anionic 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG), were prepared by electroformation[14] in 2 mL glass chambers containing purified water at room temperature. Poly-L-lysine (0.12 mM) was then added in three portions (to give 1.2 µM in the cell and a pH = 7.5) over ca. 30 min at which time ropes within the vesicles became visible by phase-contrast microscopy. Production of ropes could be intensified, and their visibility by fluorescence microscopy could be accentuated, by adding a total of 9 µM poly-L-lysine to the electroformation cell (Figure 1). Growth continued for another half hour

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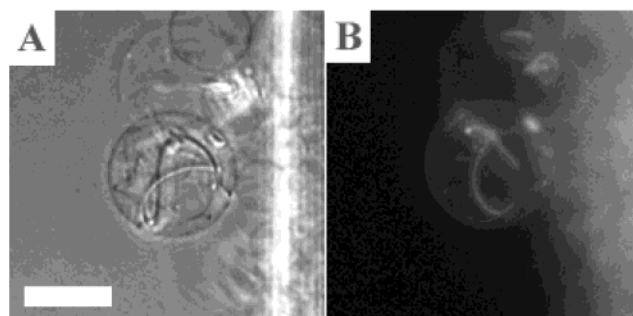
Figure 1. (A) Phase-contrast photomicrograph of an anionic giant vesicle containing ropes after external addition of poly-L-lysine, MW 500–2000. (B) Epi-fluorescence photomicrograph of a poly-L-lysine rope formed in a giant vesicle composed of POPC and a fluorescent anionic lipid (see text for details). Bar = 20 µm.
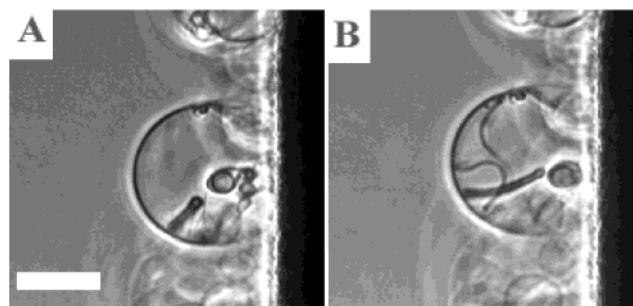
Figure 2. Rope formation in an anionic giant vesicle at (A) 30 min and (B) 60 min following addition of poly-L-lysine, MW 29 300, to a giant vesicle composed of POPC and POPG (90:10 mol %). Bar = 20 µm.
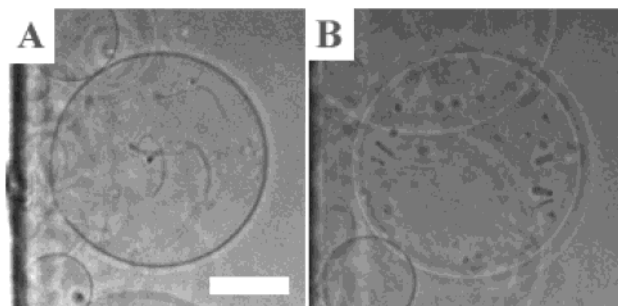
(Figure 2). No discontinuity, distortion, or size change of the vesicles themselves was seen during rope formation. This fact alone suggested, but did not prove, that poly-L-lysine might be a major component of the ropes. At the beginning, we could be certain only that poly-L-lysines of molecular weight 500–29 300 (but not 228 000) promote the creation of ropes inside anionic giant vesicles.

It was not within our province to isolate and analyze a rope from a giant vesicle. The composition had to be determined indirectly, with fluorescence playing a major role in this task. Thus, we carried out epi-fluorescence microscopy using labeled lipids and a Nikon Diaphot-TMD inverted microscope, an OSRAM HBO 50 W lamp, a Nikon B-2A filter combination cube, and an Optronics DEI-750TD Peltier-cooled 3-CCD color camera in tandem with an Optronics color monitor.

In Figure 1, the 10 mol % anionic component of the bilayer (admixed with 90 mol % POPC) consisted of a fluorescent-labeled compound, shown below, with λ_{ex} 504/λ_{em} 511 nm. Because the rope, induced by poly-L-lysine (MW 500–2000), is fluorescent, we can conclude that the rope must contain anionic lipid as one of

Figure 3. Effect of injecting poly(acrylic acid), MW 5000, into a vesicle containing ropes. (A) Before injection; (B) after injection. Bar = 20 μm.

Figure 4. Effect of injecting fluorescent dye FITC into a giant vesicle containing ropes. (A) Before injection (phase-contrast); (B) after injection (epi-fluorescence). Bar = 20 μm.

its constituents. This might happen, of course, if the cationic poly-L-lysine forms a complex with the anionic lipid and transports it into the vesicle interior. Interestingly, ropes became fluorescent also when vesicles of POPC/POPG (80/10 mol %) contained 10 mol % of a *neutral* fluorescent-labeled phospholipid.[15] Although vesicle-derived anionic and neutral lipids are both rope constituents, the rope's surface area is too small, relative to the giant vesicle's area, for us to perceive an actual vesicle contraction.
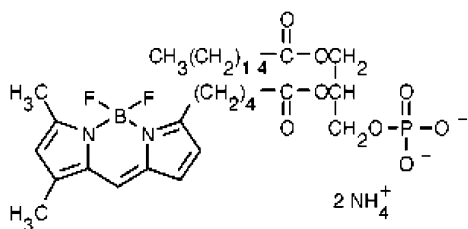
Further evidence of poly-L-lysine migration through the bilayer came from an experiment that involved direct injection into a giant vesicle by means of a micropipet with a 2 μm diameter tip. Thus, a poly(acrylic acid) solution (0.6 μM, 1.0 nL, MW 5000) was injected into a giant vesicle composed of POPC/POPG (90/10 mol %) containing ropes induced by externally added poly-L-lysine (1.2 μM, MW 15 700). As seen in Figure 3, the ropes immediately condensed into compact globules, as would be expected from the interaction between a polycation and polyanion. If a nonionic polymer such as poly(vinylpyrrolidone) is injected in place of poly-(acrylic acid), then the ropes remain in tact.

Perhaps the most definitive evidence for the presence of poly-L-lysine in the ropes came from experiments in which fluorescein isothiocyanate (FITC), a compound known to covalently label poly-L-lysine with a fluorescent moiety,[16] was injected into a rope-containing giant vesicle. In Figure 4A, we see a giant vesicle composed of POPC/POPG (90/10 mol %) in which a rope has been formed after the addition of poly-L-lysine (1.2 μM in the cell, MW 15 700). Upon injection of FITC (0.1 mM in pH = 9.0 buffer), the rope became fluorescent, a clear indication that the rope contains poly-L-lysine (Figure 4B). Residual poly-L-lysine also remains adhered to the outer vesicle surface.

We conclude that poly-L-lysine, despite its polycationic character, can pass through a lipid bilayer when complexed with an anionic lipid present in the vesicle wall. Entry of poly-L-lysine via endocytosis seems unlikely because no corresponding changes in morphology were observed with the giant vesicles. More likely,
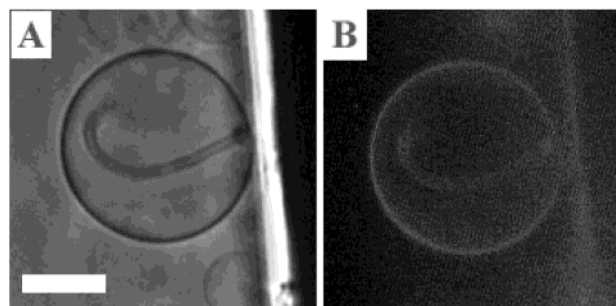
the permeability is related to domain formation which we[13] and others[17] have shown occurs when poly-L-lysine binds to vesicles bearing anionic lipid. Thus, the polycation gathers anionic lipid into domains (perhaps related to the dark spots that, as mentioned, precede our rope formation upon addition of poly-L-lysine). Defects may thereby be created at the domain boundaries. Evidence for the validity of this model stems from our observation that poly-L-lysine can mediate the permeation of sodium chloride from the inside to the outside of small anionic vesicles.[13] Likewise, doxorubicin will migrate inwardly when small anionic vesicles are exposed to poly-L-lysine.[13] Apparently, even a polycationic polymer (unless it is too large) can pass through a domain boundary or defect if the polymer first electrostatically adorns itself with lipid. This result may have a bearing on short oligomers of arginine that are known, by a currently undefined mechanism, to enhance the cellular uptake of drugs.[18]

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