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Macromolecular Signaling Processes in Synthetic Bilayer Membranes

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MACROMOLECULAR SIGNALING PROCESSES IN SYNTHETIC BILAYER MEMBRANES

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ABSTRACT

Phospholipid bilayer membranes sensitive to changes in pH, in temperature, or in the concentrations of specific organic solutes can be prepared by exploiting the conformational transitions of hydrophobic polyelectrolytes in aqueous solution. This paper provides a review of the design and preparation of responsive bilayer membranes, as well as a brief discussion of the kinetics and mechanisms of the signaling processes that are operative in such systems.

The bilayer membranes that surround cells and subcellular organelles are involved in nearly every aspect of cellular function. The recognition of other cells, the biochemical response to drugs and hormones, the transport of mass and information, the conversion of energy into useful forms–all of these and many others are *membrane* processes in biology.

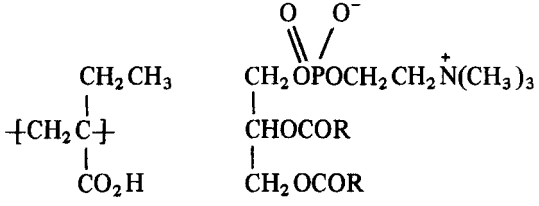
An essential characteristic of these versatile biological membranes is a capacity to respond to physical and chemical signals of various kinds. While the mechanisms of biomembrane signaling processes are not in general well understood, the shared structural features of biomembranes tell us in broad outline what kinds of mechanisms these must be. Biological membranes may be described most simply and usefully as consisting of a lipid bilayer membrane, to which is attached a variety of proteins and glycoproteins. The "Fluid Mosaic

Model" of Singer and Nicholson [1], as well as a great deal of experimental evidence [2], suggest that the macromolecular components of the membrane are characterized by relatively rapid diffusional motions within the bilayer. Two kinds of signaling mechanisms then appear to be viable, the first an intramolecular reorganization (i.e., a conformational transition) within a protein or glycoprotein receptor, and the second an intermolecular reorganization, e.g., a "clustering" of membrane proteins modulated by changes in packing within the lipid phase.

Within the last several years we have undertaken the preparation of *synthetic* bilayer membranes that share this essential capacity to respond to signals. We have retained the central strategy of biomembrane design, in that we have constructed our membranes from mixtures of amphiphilic and polymeric substances, and we have exploited the conformational properties of our membrane-bound macromolecules to achieve the required signal-sensitivity. This strategy has allowed us to prepare synthetic bilayer membranes that respond to changes in pH, in temperature, or in the concentrations of certain specific organic solutes. We provide here an overview of the design and preparation of these membranes, as well as a brief discussion of the kinetics and mechanisms of the signaling processes operative in such systems.

THE POLY(2-ETHYLACRYLIC ACID)-PHOSPHATIDYLCHOLINE SYSTEM

Most of our work to date has concerned the use of poly(2-ethylacrylic acid) (PEAA, **1**) to effect molecular switching in membranes prepared from natural or synthetic phosphatidylcholines (**2**).



1

2 a: R = $C_{15}H_{31}$
b: R = mixed hydrocarbon chains
c: R = $C_{13}H_{27}$
d: R = $C_{11}H_{23}$

We anticipated that the marginal water solubility of PEAA would allow the polymer to bind strongly to phospholipid surfaces suspended in aqueous media, and we have subsequently found, as will be described here, that the consequences of surface binding are exquisitely sensitive to the conformational state of the bound polyelectrolyte chain. The fact that PEAA undergoes a highly cooperative conformational transition [3-6] from an expanded coil at high pH to a globular structure upon very mild acidification (to ~pH 6) makes this conformational switching a process of some interest in biology and medicine.

The phosphatidylcholines (**2**) serve as a convenient source of pure, structurally variable surfactants capable of forming stable bilayer membranes in aqueous suspensions. Their structural and thermodynamic properties have been thoroughly studied (though they are by no means thoroughly understood), and they can be formulated into single- or multiple-walled vesicles through a variety of established procedures [7, 8].

Our approach has been to prepare vesicular membranes from mixtures of phosphatidylcholines and poly(2-ethylacrylic acid) or its derivatives, and to exploit the conformational transition of the polyelectrolyte to effect membrane reorganization. In our initial experiments [6, 9-11], we relied upon polyelectrolyte adsorption to provide the needed concentration of interfacial chains, but more recently we have prepared modified PEAAs that appear to constitute an integral part of the bilayer membrane [12]. We discuss briefly the behavior of each of these kinds of signal-sensitive synthetic membranes.

pH SIGNALING

Recent research in cell biology has revealed the critical role of local variations in pH in controlling the intracellular processing of ligands and receptors [13], and in determining the infectious nature of certain pathogenic microorganisms [14]. In many cases the critical events appear to be mediated by pH-sensitive macromolecules bound to the relevant membranes. The proton-driven conformational transition of PEAA, and its occurrence in the physiologically relevant range of pH, have allowed us to accomplish analogous molecular switching in synthetic bilayer membranes [6, 9-12].

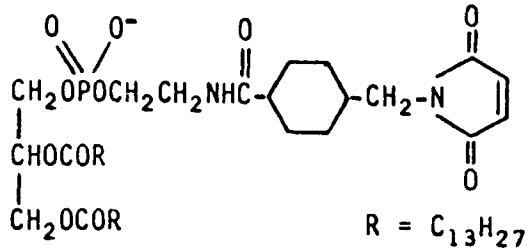
Hydration of pure synthetic phosphatidylcholines such as dipalmitoylphosphatidylcholine (DPPC, **2a**) in aqueous solutions of PEAA affords suspensions of closed vesicles. At pH 8 or above, the properties of such vesicle suspensions are little different from the properties of similar suspensions prepared in polymer-free buffer solutions: phase transitions, turbidity, particle

size (determined by quasi-elastic light scattering), and permeability (determined by the rate of efflux of entrapped dyes) are virtually unchanged by addition of the polymer at concentrations comparable on a weight basis to the concentration of the lipid. Upon acidification to pH 6, however, the situation changes markedly; the suspension modified by the addition of PEAA undergoes rapid clarification and loss of vesicle contents, while the properties of the polymer-free sample remain essentially constant. Modifications in the tacticity [9] or molecular weight [15] of the PEAA, or in the ionic strength of the medium [16], shift the "critical pH" for membrane reorganization in a manner to be expected from the effects of these variables on the titration behavior of poly(carboxylic acid)s.

The rapid release of vesicle contents and the controlled variation in the critical pH for release are features of some interest in medical diagnosis and therapeutics. One can imagine, for example, the use of vesicles of this kind to deliver therapeutic agents to tissues or body compartments of low ambient pH. There is then the further prospect that one can use the variation in the trigger pH to match rather precisely the prevailing pH at the target. Potential targets would include infected or inflamed tissues, certain tumors [17], regions of restricted blood flow, and certain intracellular compartments (e.g., lysosomes) involved in the degradation and recycling of ligands and receptors [13, 18].

But it is clearly impractical to implement this strategy as it has been described above. One cannot rely on adsorption equilibria to provide the needed concentration of chains at the vesicle surface. Instead, those chains must be anchored tightly to the membrane, so that the systemic concentration of soluble chains is effectively zero. The membrane itself must carry the polyelectrolyte to the target.

This can be accomplished by the use of hydrophobic reactive handles on the membrane surface. In particular, we have used the amphiphilic maleimide **(3)** to modify the surfaces of egg phosphatidylcholine **(2b)** vesicles [12]. Incorporation of 10 mol% of **3**, followed by incubation of the modified vesicles with a thiolated derivative of PEAA, results in the immobilization of ~60 mg PEAA/g lipid. Free and immobilized chains are readily separated by size-exclusion chromatography, and mild acidification of the vesicle suspension then causes rapid release of contents. It is worth noting that effective membrane reorganization in this system is triggered by a rather small concentration of surface-bound chains (~5 wt% of the membrane). We have not yet defined the lower limit of effective membrane loading.



R = $C_{13}H_{27}$

3

TEMPERATURE-DEPENDENT MEMBRANE REORGANIZATION

We have observed that the rate of membrane reorganization that follows acidification of phosphatidylcholine suspensions in aqueous PEAA solutions is highly dependent on temperature. To be more precise, the rate depends on the initial state of order in the membrane, in that reorganization occurs only slowly at temperatures below that of the main bilayer phase transition but quite rapidly at higher temperature [10, 19]. Similar temperature dependences have been observed in kinetic studies of the formation of lipoprotein models from apolipoproteins and phospholipids [20], and have been reasonably interpreted as suggesting a role for membrane defects in determining the reorganization rate.

This observation may be of some practical significance, as it suggests that vesicles might be formulated from mixtures of phosphatidylcholines and PEAA (surface-bound or not) in such a way that the system is below the critical pH for membrane reorganization, but is prevented from releasing its contents by kinetic restrictions on the reorganization. Gentle warming of such a system should be accompanied by rapid vesicle rupture with release of contents. Indeed, we have shown previously [10] that mixtures of DPPC and PEAA at pH 6.5 are apparently stable at room temperature, but are then rapidly clarified by warming through the DPPC melting transition at 41°C.

GLUCOSE AS THE SIGNAL

A particularly interesting kind of membrane signaling can be achieved by combining the chemistry just described with enzymatic generation of H^+. A number of enzymatic reactions are known that generate acidic products via hydrolytic or oxidative processes. In solutions of the appropriate enzymes,

acidification occurs subsequent to a rise in the concentration of an organic solute (the substrate for the enzyme), so that the net result is a release of vesicle contents triggered by that solute. One sees in such systems crude analogy to biochemical processes mediated by "second messengers," species that are generated subsequent to hormonal binding and that carry the message to an effector molecule. In our analogy the roles of hormone, messenger, and effector are played by the substrate, the proton, and the polyelectrolyte, respectively.

An example of a system of this kind is provided by our recent work on glucose-sensitive phosphatidylcholine vesicles [11]. Glucose is of interest in this context because it is converted at a reasonable rate to gluconic acid (which serves as a suitable source of H^+) and because of the potential utility of self-regulated insulin delivery in the treatment of diabetes.

Hydration of dilauroylphosphatidylcholine (**2d**) in a neutral aqueous solution that contains both glucose oxidase and PEAA gives a turbid suspension. Subsequent addition of glucose at a concentration comparable to that found in normal human plasma leads to clarification of the suspension over a period of 20-30 min, during which time the pH of the solution is depressed to ~6.1.

While dilauroylphosphatidylcholine is not the lipid of choice for the fabrication of robust vesicles for use in therapeutics, the proton-driven membrane reorganization that leads to clarification of this suspension is quite general, and there is no doubt that this approach to vesicle sensitization can be adapted to other vesicle formers and to other enzymatic reactions. The technical problems to be addressed in attempts to develop practical applications of this concept are substantial, but the fundamental problem of the control of membrane structure has been overcome.

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