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ELECTRO-OSMOSIS AT THE SURFACE OF PHOSPHOLIPID BILAYER MEMBRANES

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The electro-osmotic velocity is the velocity of a fluid near an interface produced by an electric field parallel to a surface. The velocity adjacent to fixed phospholipid bilayer membranes was measured by observing the velocity of small vesicles suspended in the fluid. The charge densities of the bilayers ranged from 0 to 1 electronic charge per lipid and experiments were performed at temperatures above and below the transition temperature of the phospholipid bilayer in 1, 10 and 100 mM NaCl solutions. The Helmholtz-Smoluchowski equation correctly predicted the electro-osmotic velocity from the known value of zeta potential of the phospholipid bilayer.

Introduction

The imposition of an electric field parallel to the surface of a muscle cell membrane redistributes macromolecules on the membrane [1,2] and it has been suggested that electro-osmosis, the field-induced movement of fluid adjacent to a surface [3], plays a role in this redistribution [4]. Earlier work showed that the electro-osmotic velocity adjacent to many surfaces can be described by the Helmholtz-Smoluchowski equation [5,6]. We investigated whether this equation can describe electro-osmotic flow adjacent to a phospholipid bilayer, an important component of all biological membranes.

The inner surface of a cylindrical glass microelectrophoresis tube was coated with a thin layer of lipids. Upon addition of an aqueous solution, the lipids adjacent to the water should assume a bilayer configuration. Electro-osmotic fluid flow adjacent to the bilayer was produced by applying an electric field along the axis of the tube. The velocity of the fluid at various distances from the surface of the tube was estimated by observing the velocity of small vesicles of known electrokinetic mobility. The fluid velocity adjacent to the interface, the electro-osmotic velocity, was obtained by extrapolation *.

Abbreviations: DMPC, L-α-dimyristoylphosphatidylcholine; DMPG, L-α-dimyristoylphosphatidylglycerol; Mops, 3-(*N*-morpholino)propanesulfonic acid.

* Strictly speaking, the electro-osmotic fluid velocity at a distance x from the membrane surface, $v(x)$, is zero both at the surface $x=0$, and at the hydrodynamic plane of shear, which is about 2 Å from the surface of a phospholipid bilayer [14]. $v(x)$ increases monotonically with x across the diffuse double layer and is predicted to be proportional to the difference between the zeta potential, the electrostatic potential at the hydrodynamic plane of shear, and the electrostatic potential at x [6]. Thus, $v(x)$ is more than 90% of its maximum value when x is larger than a few Debye lengths. As the Debye lengths in the 0.1, 0.01, and 0.001 M NaCl solutions used in these experiments are about 10, 30 and 100 Å, respectively, and the vesicles cannot approach closer than a few microns to the surface, the fall in the electro-osmotic velocity within the diffuse double layer immediately adjacent to the surface is ignored.

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Materials and Methods

L-α-Dimyristoylphosphatidylcholine (DMPC) and L-α-dimyristoylphosphatidylglycerol (DMPG) were obtained from Avanti (Birmingham, AL), 3-(*N*-morpholino)propanesulfonic acid (Mops) from P-L Biochemicals (Milwaukee, WI), NaCl from Fisher (Fairlawn, NJ), EDTA from Baker (Phillipsburg, NJ) and spectrophotometric grade $CHCl_3$ from Aldrich (Milwaukee, WI). All solutions were prepared using 18 MΩ-cm water obtained from a Super Q™ System, Millipore Corporation (Bedford, MA). The concentrations of the NaCl solutions were verified by measuring the conductivity at 20°C. The N_2 used for drying the lipid was of the O_2-free grade (99.99% pure) obtained from Linde (Union Carbide Corp., NY). The electrophoresis apparatus was obtained from Rank Bros. (Cambridge, U.K.) and is based on the design of Bangham et al. [7].

The inner surface of a cylindrical electrophoresis tube was coated with the zwitterionic lipid DMPC, the anionic lipid DMPG or mixtures of these two lipids. DMPC and DMPG were chosen because they have the same transition temperature (23°C) and, therefore, do not undergo phase separation [8,9] and because they are less susceptible to oxidation than lipids with unsaturated hydrocarbon chains. The capillary portion of the electrophoresis tube (2 mm inside diameter, 150 mm length) was filled with 0.5 ml of a 3 mg/ml solution of the lipids in $CHCl_3$. A gentle stream of N_2 was passed through the tube and the excess $CHCl_3$ solution that flowed into the electrode chamber was removed with a syringe. N_2 was passed through the chamber for an additional 2 h: during this period the chamber was maintained at 30°C. For most experiments the chamber was cooled to 20°C and the appropriate aqueous solution was added to the tube. Both DMPG and DMPC are in the gel state at this temperature. The thickness of the lipid coat was negligible (< 30 μm) in comparison to the radius of the tube (1000 μm).

We initially attempted to use multilamellar vesicles formed from the zwitterionic lipid DMPC to measure the fluid velocity. However, we observed that the mobility of these vesicles changed with time when they were placed in a tube coated with DMPG. We concluded that the initially neutral DMPC vesicles had become contaminated in some way with the anionic DMPG molecules*. For this reason we estimated the fluid velocity by measuring the mobility of multilamellar vesicles formed from the same lipid mixture as was used to coat the tube. Chloroform solutions of DMPC and DMPG were combined to yield a mixture (3 mg lipid/ml chloroform) with the required composition. A fraction of this mixture (0.5 ml) was used to coat the tube and the remainder was used to form multilamellar vesicles by the method of Bangham et al. [10]. The solution containing the vesicles was placed in the lipid-coated tube and a potential difference was applied. The mobilities of the vesicles were determined at 8–10 points varying in distance from 25 to 1000 μm from the lipid-coated surface of the tube.

The electrokinetic mobility of vesicles from the same preparation was measured at the stationary layer of an uncoated electrophoresis cell in a separate experiment. The zeta potential of the vesicles, ζ, the electrostatic potential at the hydrodynamic plane of shear, was determined from the observed values of the electrokinetic mobility, u, using the Helmholtz-Smoluchowski equation:

$$\zeta = \frac{u\eta}{\epsilon_0 \epsilon_r} \tag{1}$$

where ϵ_r is the dielectric constant of the medium, ϵ_0, is the permittivity of free space and η is the viscosity of the medium [11–13]. EDTA was added to the solutions containing vesicles prepared at low NaCl concentrations (1 and 10 mM) to remove any divalent cation contaminants; EDTA did not produce any significant change in the zeta potential of these vesicles.

* When DMPC multilamellar vesicles that had been in a DMPG-coated tube for 45 min were transferred to an uncoated tube their zeta potential was −35 mV, which corresponds to a DMPG content of over 20% [14]. However, when DMPC multilamellar vesicles were dialyzed against DMPG multilamellar vesicles for 6 h at 20°C the electrokinetic mobilities of both sets of vesicles remained unchanged, i.e. no transfer of lipid occurred across the dialysis membrane. Thus, it seems unlikely that the acquisition of DMPG molecules by DMPC vesicles occurs by a solubility-diffusion transfer of monomers through the aqueous phase [26]. When vesicles were formed from the same lipid mixture used to coat the tube, their mobilities did not change with time.

Results

The observed mobility of DMPC vesicles was zero at all distances from the surface of a tube coated with this lipid (Fig. 1). That is, as predicted theoretically, there was no electro-osmotic flow when the tube was coated with the zwitterionic lipid. Electro-osmotic flow was observed when the tube was coated with the anionic lipid DMPG (Fig. 1). At any distance x from the surface of the tube the observed velocity of a vesicle, $v(x)$, is the sum of the fluid velocity, $v_f(x)$, and the electrokinetic velocity v_{ek}:

$$v(x) = v_{ek} + v_f(x) \tag{2}$$

The fluid velocity can be described by Eqn. 3:

$$v_f(x) = v_{eo}\left[2(a-x)^2/a^2 - 1\right] \tag{3}$$

where v_{eo} is the electro-osmotic velocity, the fluid velocity adjacent to the wall, and a is the radius of the tube [3]. The mobility of the vesicles, their observed velocity divided by the electric field strength, has been plotted as a function of the distance from the surface of the tube in Fig. 1. It is apparent that the observed mobility of DMPG vesicles is zero, within experimental error, at the surface of a tube coated with the same lipid. Similar results were obtained with DMPC:DMPG mixtures in 0.1 M NaCl. The results of experiments performed at different salt concentrations in tubes coated with a 10:1 DMPC/DMPG mixture are illustrated in Fig. 2. It is apparent that the observed velocities of these vesicles are also approximately zero at the surface. Note, from Eqn. 2, that if the observed velocity of the vesicles at the surface of the tube, $v(0)$, is zero, then the fluid velocity at the surface, $v_f(0)$, must be equal in magnitude and opposite in direction to the electrokinetic velocity of the vesicles, v_{ek}, i.e., $v_f(0) = -v_{ek}$. The fluid velocity at the surface, $v_f(0)$, is by definition, the electro-osmotic velocity, v_{eo} (Eqn. 3). Thus, our experiments confirm the theoretical prediction that $v_{eo} = -v_{ek}$.

The data described above were analyzed quantitatively. The electro-osmotic velocity was determined from each experiment by a least squares best fit of the data to Eqns. 2 and 3,

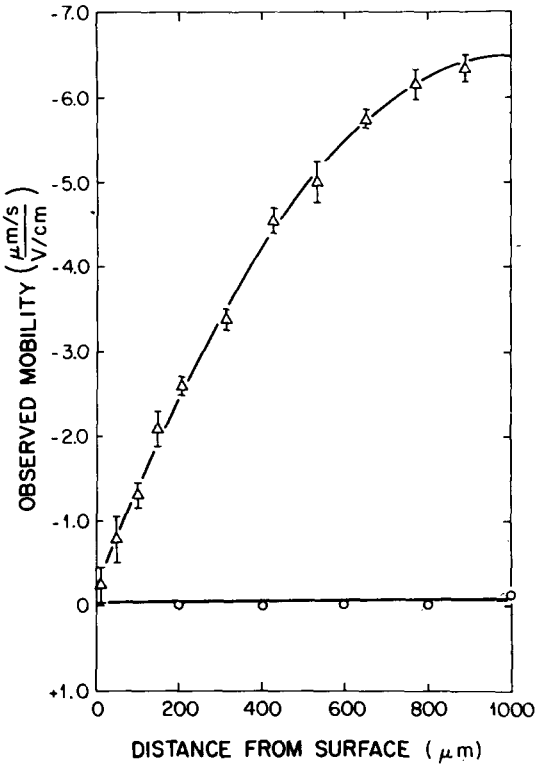

Fig. 1. The observed mobility of DMPC(○) and DMPG(△) vesicles plotted as a function of the distance from the surface of the tube coated with the same lipid. The vesicles were prepared in 0.1 M NaCl buffered with 0.01 M Mops to pH 7.4. Both experiments were performed at 20°C. The vertical bars have lengths equal to twice the standard deviations for the DMPG measurements and the circles have diameters equal to or greater than twice the standard deviations for the DMPC measurements.

treating both v_{eo} and v_{ek} as variables (Table I, column 4). The electro-osmotic velocity was also calculated from Eqn. 1 using the value of zeta potential, which was determined in a separate experiment. These values are listed in Column 5 of Table I. The agreement is considered satisfactory, in view of the uncertainty about the uniformity of the lipid coat, which was always thinner than 30 μm.

It was also possible to obtain stable liquid coats above the phase transition temperature, where the lipids are in the lipid crystalline rather than in the gel state. We determined electro-osmotic velocities in tubes coated with either DMPC or DMPG as well as 10:1 and 2.5:1 mixtures of the two lipids

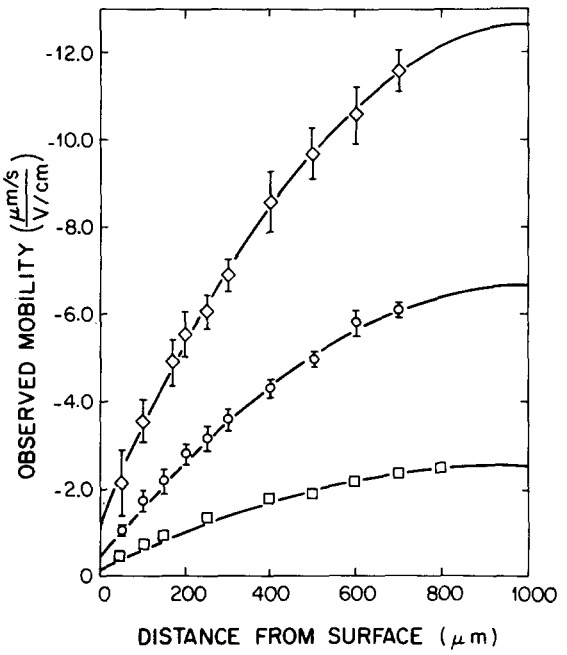

Fig. 2. The observed mobility of DMPC/DMPG(10:1) vesicles as a function of the distance from the surface of the tube coated with the same lipid mixture, at 20°C. Vesicles were prepared in 0.1 M(□), 0.01 M(○) and 0.001 M(◇) NaCl solutions buffered with Mops to a pH of 7.4. The vertical bars represent twice the standard deviations.

at 30°C. The particle velocity vs. distance plots obtained at 30°C were similar to those at 20°C. The calculated electro-osmotic velocity agrees, within experimental limits, with the observed values (Table I).

Discussion

The electrokinetic velocity of an insulating particle and the electro-osmotic velocity of the fluid adjacent to a fixed surface formed from the same material as the particle are predicted theoretically to be identical in magnitude if the radius of curvature of the particle is large compared with the thickness of the diffuse double layer; this prediction has been confirmed for several systems [5,6]. Our experimental observations extend this conclusion to phospholipid bilayers. As the electrokinetic mobility of a phospholipid bilayer vesicle can be predicted from the Gouy-Chapman-Stern theory [14–16], the electro-osmotic flow adjacent to a phospholipid bilayer can also be predicted from the Gouy-Chapman-Stern theory of the diffuse double layer.

Can our conclusions about electro-osmotic flow adjacent to lipid bilayers be extrapolated to biological membranes? We consider this question to be important because endogenous electric fields are present in some biological systems and the possible effect of both endogenous and exogenous electric fields on growth and regulation is a matter of current interest [17–22]. We anticipate that the Helmholtz-Smoluchowski equation can describe

TABLE I

Temp. (°C)	[NaCl] (M)	Lipid composition DMPC/DPMG	Electro-osmotic velocity (field strength = 3.33 V/cm)	
			Observed (μm/s)	Calculated (μm/s)
20	0.1	0 :1	−10.5	−11.9±0.6
		2.5:1	−7.8	−9.2±0.4
		4 :1	−7.0	−7.6±0.3
		5 :1	−6.3	−6.7±0.4
		10 :1	−4.0	−4.3±0.5
		1 :0	−0.2	−0.3±0.1
30	0.1	0 :1	−15.0	−13.7±1.1
		2.5:1	−11.5	−10.0±0.6
		10 :1	−5.5	−5.4±0.4
		1 :0	0	−0.1±0.1
20	0.01	10 :1	−10.4	−12.6±0.6
20	0.001	10 :1	−18.9	−22.0±2.6

both the electrokinetic mobility of a cell that is free in solution and the electro-osmotic fluid velocity adjacent to the surface of a cell that is immobilized [23], but that the Gouy-Chapman-Stern theory can only describe the relationship between charge density and zeta potential if the charges are located in the plane of the membrane. In the erythrocyte, for example, many of the negative charges on the extracellular surface of the membrane are on sialic acid residues [24], which are probably located more than a Debye length (about 10 Å in a physiological solution) from the lipid bilayer surface of the membrane. Thus, the assumption that the charges are located in the plane of the membrane, which is implicit in the Gouy-Chapman theory, may not be valid for most biological membranes. Additional theoretical treatments are required to describe the relationship between the charge density and the electrostatic potential [25] and additional measurements are required on well defined systems to investigate the relationship between these two parameters when the charges are not located at the interface. Such experimental measurements are in progress in this laboratory.

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