Vesicles as Osmotic Motors

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We report on a quantitative study of the motion of model osmotic motors—lipid vesicles in a solute concentration gradient. The vesicles move through recoil produced by osmotic pumping, but the transformation of entropic osmotic energy into mechanical motion is found to be unexpectedly efficient: The drift velocity is *more than 3 orders of magnitude faster* than predicted by linearized nonequilibrium thermodynamics. [S0031-9007(99)09445-4]

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The study of directed transport at the macromolecular level is undergoing a surge of interest in biophysics. Motor proteins, like myosin and dynein, capable of transforming the chemical energy of energetic compounds (like ATP or GTP) into mechanical work have been the focus of an intense experimental and theoretical effort [1]. Various scenarios have been proposed for the transformation of free energy stored in energetic compounds into mechanical work, with particular focus on the possible role of so-called "Brownian ratchet" mechanisms [2]. Motor proteins responsible for the rotation of bacterial flagellae [3] are particularly interesting since they are able to transform purely entropic free energy into "osmotic" work.

Osmotic work is obtained by transporting solvent molecules from a reservoir with a certain concentration of solute molecules to another reservoir with a higher solute concentration. The maximum osmotic work is equal to the change in chemical potential per molecule $k_B T \ln[c(+)/c(-)]$ with $c(+)$ and $c(-)$ the solute concentrations of the two reservoirs. It has in fact been long known [4] that an osmotic pressure difference across a *macroscopic* semipermeable membrane separating two reservoirs of different solute concentrations can perform mechanical work by pumping solvent into the reservoir with the higher solute concentration.

To explore the transformation of osmotic energy into mechanical work at the microscopic level under nonequilibrium conditions—as for the bacterial motor—we report in this Letter on a study of a simple "mesoscopic" osmotic motor namely lipid vesicles. Lipid bilayers permit passage of water molecules but not of solute molecules, like sugars or salts, so lipid vesicles are exposed to the effects of osmotic pressure. Lipid vesicles placed in a high osmotic pressure environment are known to steadily shrink through water permeation across the bilayer [5]. Osmotic shrinkage would seem to be a possible mechanism to form the basis for a model osmotic motor. However, unidirectional motion of a spherical lipid vesicle in a *uniform* solution in this manner is not possible because of rotational symmetry, so we imposed in our study a controlled solute *concentration gradient* on a vesicle solution in order to break the rotational symmetry.

We generated vesicles of both DMPC (dimyristoyl phosphatidycholine) and SOPC (stearoyl oleoyl phosphatidycholine) in water containing 5 mM NaCl and 40 mM sucrose using the standard method of electroswelling [6]. The DMPC was first dissolved in a mixture of chloroform and methanol (2:1 volume ratio). After preparation, a 1.5 μl latex bead solution was added to 1 ml of the vesicle solution. The solution was then placed inside a chamber containing two parallel dialysis tubes with a separation of 1 mm. By pumping sucrose solutions of varying concentration through the tubes, controlled concentration gradients could be produced inside the cell. The cell was mounted on the stage of an inverted Zeiss Axiomat Microscope, which allowed vesicle observation, either by bright field microscopy (BFM) or by reflection interference contrast microscopy [7] (RICM). For the evaluation of the vesicle motion, images of selected vesicles were taken with a CCD camera. The observed vesicles moved over the glass cover slip, which formed the bottom of the chamber (at a distance of 1 mm from the dialysis tubes). RICM was used to check that the vesicles did not adhere to the cover slip. As a check, the experiments were repeated with vesicles containing polyethylene glycol lipids (which should significantly reduce any adhesion) with no change in results.

Figure 1(a) shows a series of RICM images (taken every 10 sec) of a 10 μm radius DMPC vesicle in a uniform aqueous solution of 5 mM NaCl and 40 mM sucrose. A latex bead of radius 7.5 μm is visible as well. No systematic motion is observed: Both vesicle and bead performed random, isotropic Brownian motion. Next, a solution of 5 mM NaCl and 50 mM sucrose was pumped through dialysis tube 1 in Fig. 1(b) and, as before, a solution of 5 mM NaCl and 40 mM sucrose through tube 2 in Fig. 1(b). This generates a (time-dependent) concentration gradient of the order of 10 mM/mm. As shown in the series of RICM images in Fig. 1(b) (again every 10 sec), the vesicle started to move in the direction of low sucrose

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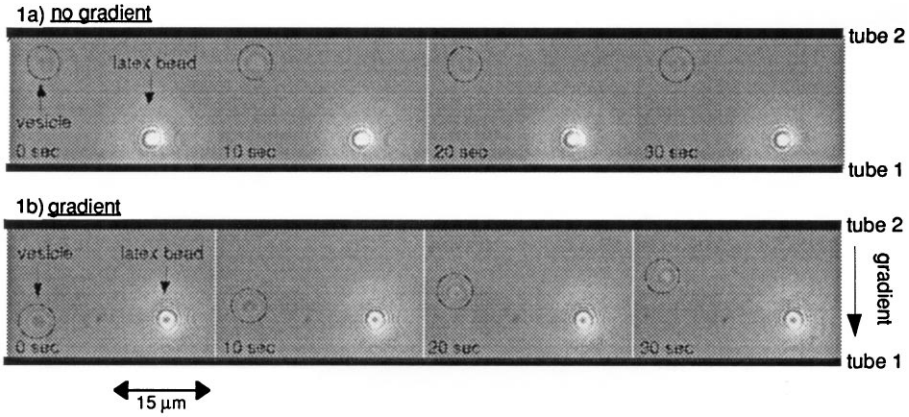

FIG. 1. RICM time series showing a DMPC vesicle and a latex bead (a) without and (b) with a concentration difference of 10 mM between the dialysis tubes (indicated schematically). The vesicle position is indicated by a circle. RICM shows the parts of the vesicle and bead close to the supported surface.

concentration, i.e., in the direction of the sucrose diffusion current, about 10^3 sec after the concentration gradient had been switched on. *The latex bead still showed a purely diffusive motion.* This demonstrates that hydrodynamic flow was not responsible for the vesicle motion.

Using RICM and BFM we determined the trajectory of the moving vesicle, as well as the vesicle radius. A typical result is shown in Fig. 2. The vesicle drift velocity is of the order of a few microns per second. Surprisingly, *no osmotic shrinkage was observed during vesicle drift*, even though the outside osmotic pressure must have been rising. To be certain that the vesicle motion was determined by intrinsic physical properties of the lipid bilayer, we repeated the velocity measurements for a range of temperatures. Pure DMPC lipid bilayers undergo a transition from a fluid, high-permeability phase to a gel-like low-permeability phase at a "freezing" temperature of about 24 °C [8]. The drift velocity should be greatly reduced in the gel state if membrane osmosis is indeed responsible for the motion since the permeability is reduced in the gel state. In Fig. 3, the ratio $v = V/a$ of vesicle velocity V and vesicle radius a is shown for a single vesicle with the chamber temperature T varying from an initial temperature $T = 25.4$ °C to a final temperature of $T = 19.2$ °C in intervals of 0.2 °C, followed by a final increase back to 22.5 °C. The concentration difference between the two dialysis tubes was kept fixed at $\Delta c = 10$ mM sucrose during the measurement. In the temperature interval from $T = 25.4$ °C to $T = 20$ °C, v drops gradually from about 0.15/sec to 0.11/sec. Around 20 °C, there is a dramatic drop in the drift velocity to about 0.017/sec at $T = 19.2$ °C. Below this temperature, steady drift no longer can be distinguished from random diffusive motion. When the temperature is restored to 22.5 °C, the vesicle moved again with a rate of about 0.11/sec. The expected breakdown of osmotic drift in the gel phase is consistent with our observations, provided we are allowed to assume that the freezing temperature is somewhat reduced by the combined effects on the DMPC bilayer of the sucrose solution and of the chloroform and/or methanol absorbed during preparation.

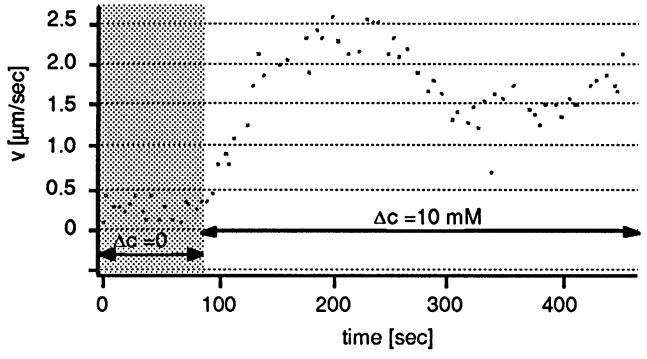

FIG. 2. Velocity profile of a vesicle in a concentration gradient. Initially, the aqueous solution had a uniform sucrose concentration ($\Delta c = 0$) when the vesicle exhibits random diffusive motion with velocities ranging from 0.1 to 0.4 μm/sec. Directed motion started about 10 min after the concentration gradient ($\Delta c = 10$ mM) was switched on (around $t = 100$ sec in the graph).

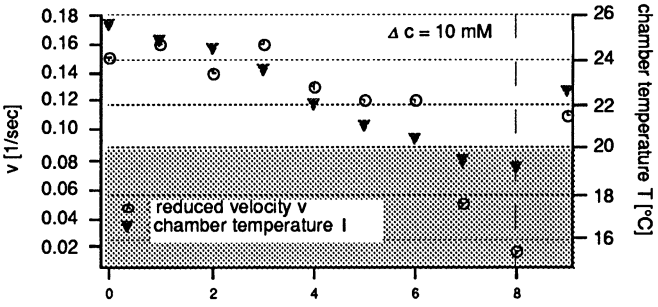

FIG. 3. Reduced velocity $v = V/a$ of a DMPC vesicle moving under a $\Delta c = 10$ mM concentration difference between the dialysis tubes as the temperature is reduced in steps of 0.2 °C. After the eighth step (dashed vertical line), the temperature was again increased.

If not osmotic shrinkage, then what was the origin of the mechanical force propelling the vesicles? According to the basic principles of nonequilibrium thermodynamics [9], a solute chemical potential gradient cannot produce a momentum flux (i.e., a net hydrodynamic flow) in the solvent fluid. However, as shown in Fig. 4, osmotic permeation of solvent across a spherical semipermeable membrane in a concentration gradient does lead to a pumping action: Solvent is pumped from the low to the high concentration side of the sphere. Since the concentration gradient cannot impart a net momentum to the system of solvent plus vesicle, momentum conservation requires that this "micropump" drifts by *recoil* towards the low concentration direction, consistent with our observations.

To compute the recoil drift velocity, we assumed that the vesicles are sufficiently close to thermal equilibrium to allow us to use Onsager theory. In Onsager theory [10], the osmotic volume current density per unit area J (units of velocity) across a semipermeable membrane is proportional to the difference of the osmotic and hydrodynamic pressure drops across the membrane:

$$J = L_p[\Delta\Pi - \Delta P]. \tag{1}$$

The Onsager transport coefficient L_p in Eq. (1) is known as the "filtration coefficient." For lipid membranes, L_p is of the order of 10^{-5} (cm/sec)/atm [11]. Equation (1) can be used with good results to predict the shrinkage rate of vesicles under osmotic pressure [12]. We used Eq. (1) as a boundary condition for a simultaneous solution of the Navier-Stokes and diffusion equations both inside and outside a rigid, semipermeable sphere of radius a. The other boundary conditions were (i) zero solute diffusion current across the membrane and (ii) zero tangential flow velocity. Momentum conservation then imposes the following drift velocity on a semipermeable shell of radius a moving at low Reynolds and Peclet numbers:

$$V = -\frac{1}{2}\,\frac{L_p N_A k_B T(\frac{dc}{dz})a}{1 + \frac{3\eta L_p}{a}}\,, \tag{2}$$

with η the solvent viscosity. The drift is along the (negative) gradient direction.

To test Eq. (2), we measured the dependence of drift velocity on the concentration difference Δc between the dialysis tubes (see Fig. 5). The drift velocity was indeed approximately proportional to the concentration difference. Solution of the diffusion equation for our dialysis cell geometry showed that the concentration gradient at the vesicle position should start to rise following switch-on after a lag time of the order of L^2/D (with D the sucrose diffusion constant), which is about 10^3 sec. Inserting the solution of the diffusion equation into the right-hand side of Eq. (2) produces a vesicle velocity that reaches a maximum value shortly after the lag time, followed by a slow drop. Both predictions are consistent with Fig. 2.

We then measured the filtration coefficient L_p of the vesicles, using the standard micropipette method, and found it to be consistent with published values for DMPC and SOPC [11]. However, using these values, Eq. (2) predicts drift velocities of about 10^{-3} μm/sec, far less than the observed drift velocities of about 1 μm/sec. Equation (2) thus appears to greatly underestimate the efficiency with which vesicles are able to transform osmotic gradients into mechanical motion. The *maximum* permitted drift velocity V_{therm} can be computed by simply assuming that the osmotic pressure difference across the vesicle directly acts as a standard mechanical pressure with the result $V_{\text{therm}} = -\frac{1}{6}\{[N_A k_B T(dc/dz)a^2]/\eta\}$. This is about 10^3 μm/sec for our case, i.e., much larger than the measured velocity so the measured velocities are not unphysical. It might be assumed that specific chemical interaction between solute and lipids is responsible for the discrepancy (e.g., through the Marangoni effect [13]). To test for this possibility we repeated our measurements with salt gradients instead of sucrose gradients, but we obtained very similar results.

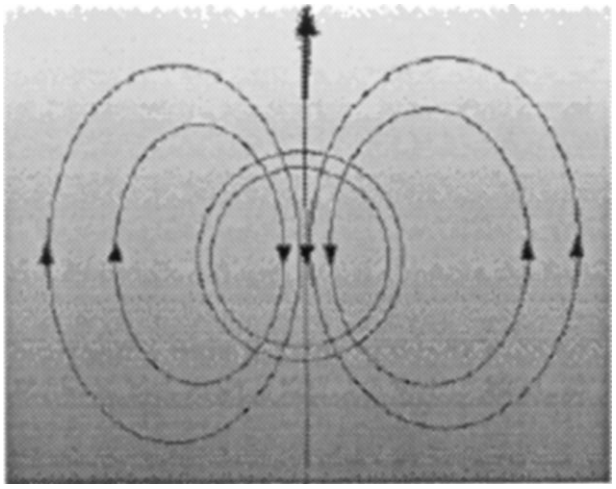

FIG. 4. Osmotic motion without shrinkage. A semipermeable vesicle placed in a concentration gradient will pump solvent in the direction of higher concentration by osmotic action. Momentum conservation requires net vesicle motion in the direction of low solute concentration.

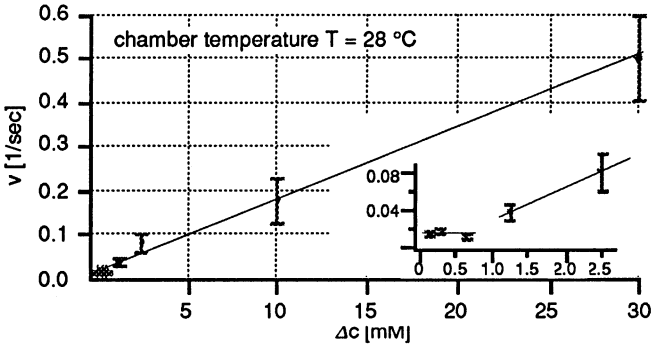

FIG. 5. Plot of vesicle velocity $v = V/a$ as a function of Δc, the concentration difference of the solutions pumped through the two dialysis tubes, at constant chamber temperature $T = 28\,^{\circ}\text{C}$. Inset: drift velocities for low concentration gradients.

Linearized Onsager theory certainly may break down for systems far from thermal equilibrium. An important symptom of failure of Onsager theory is asymmetry under reversal of the potential gradients. To test for such an asymmetry we inverted the sign of the concentration gradient produced by tube 1, i.e., the concentration in tube 1 was *lowered* with respect to tube 2 rather than raised. Hardly any vesicle drift was observed in this case. This indicates that nonlinear corrections may play an important role. A first-principles analysis of osmotic drift and nonlinear corrections encounters fundamental difficulties because, unlike the case of the very similar ideal gas law, there is as of yet no accepted *kinetic* interpretation of the van't Hoff law for osmotic pressure ($\Pi = k_B T c$) [14]. A Brownian ratchet mechanism may play a role [15]. However, we can speculate that a strong nonlinear coupling between osmosis and hydrodynamic flow (absent during shrinkage) may produce enhanced osmotic transport across the membrane. Alternatively, allowing for elastic deformation of the vesicle surface produces a more efficient transduction of osmotic into mechanical pressure.

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