Role of Proton Gradients in the Mechanism of Osmosis

ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · SEPTEMBER 2009

Impact Factor: 3.3 · DOI: 10.1021/jp9021568 · Source: PubMed

6 AUTHORS, INCLUDING:

Jeff Magula
University of Washington Seattle
7 PUBLICATIONS **147** CITATIONS

SEE PROFILE

Role of Proton Gradients in the Mechanism of Osmosis

Qing Zhao, Kate Ovchinnikova, Binghua Chai, Hyok Yoo, Jeff Magula, and Gerald H. Pollack*

Department of Bioengineering, Box 355061, University of Washington, Seattle, Washington 98195

Received: March 10, 2009; Revised Manuscript Received: May 1, 2009

Experiments were carried out to determine whether the newly identified "exclusion zone" found adjacent to hydrophilic surfaces might play a role in osmosis. Two chambers were juxtaposed face to face, separated by a membrane made of cellulose acetate or Nafion. One chamber contained water, the other 100 mM sodium sulfate solution. Osmotically driven transmembrane fluid flow from low to high salt was observed using both membranes, in agreement with previous reports. Characteristic pH differences and potential differences between chambers were also noted. Visual examination with microsphere markers revealed extensive exclusion zones adjacent to both types of membrane. As these zones routinely generate protons in the water regions beyond, unequal proton concentrations in the respective chambers may be responsible for creating both the pH and potential gradients, which may be ultimately responsible for the osmotic drive.

Introduction

Osmosis refers to the movement of fluid, typically water, through a semipermeable membrane, from a region of lower to higher solute concentration. It is a physical process that apparently requires no input of energy.

Although the classical van't Hoff equation is considered a fundamental expression underlying osmotic theory, acceptance of this theoretical basis is by no means universal. Alternative formulations have been proposed, stemming from concern over discrepancies between theory and experiment. These formulations include the so-called solute-bombardment theory,[1] solute-attraction theory,[2] water-concentration theory,[3−9] and water-tension theory.[10−17] Notwithstanding some 300 years of research, it is fair to say that the underlying mechanism of this fundamental process remains debated.

Of potential relevance to the osmotic mechanism is a newly discovered phenomenon involving water and solutes. Colloidal and molecular solutes suspended in aqueous solution are profoundly excluded from the regions next to a broad range of hydrophilic surfaces.[18,19] Termed the "exclusion zone" (EZ for short), the solute-free zone is typically several hundred micrometers wide. Electrodes placed between the EZ and the aqueous zone beyond the EZ have revealed potential differences of 100−200 mV, with the EZs being negatively charged.[18,19]

The presence of such charged exclusion zones adjacent to the hydrophilic membranes commonly used in classical osmosis experiments raises the possibility that these zones might play some role in the osmotic process. This led us to the experiments reported here, whose results are indeed suggestive of such a possibility.

Experimental Methods

The chamber used for studying osmosis is schematized in Figure 1. It was fabricated out of two 16 mm long cylindrical glass tubes (inner diameter 10 mm), referred to, respectively, as chambers I and II, butted together through an 80 μm thick round cellulose acetate membrane (Nest Group, Inc., 100 Da, 12 mm diameter). Pressure could be exerted on the membrane by two threaded screws, which connected the two plastic-block "frameworks" situated at the outer ends of the cylindrical chambers. Seals were created by O-rings placed between the cylinders' outer ends and the respective framework. Turning the screws exerted pressure on both the O-rings and the membrane, preventing leaks. Any remaining membrane extending beyond the butted cylinders was carefully trimmed and removed.

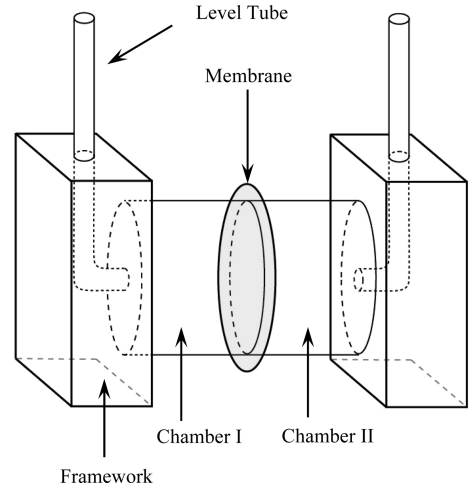

Figure 1. Experimental chamber. Drawing not to scale.

The respective chambers were contiguous with vertically oriented "level tubes" (see Figure 1), which were used to assess volume changes. The tubes had an inner diameter of 2 mm and a length of 80 mm. These tubes projected upward and were left open to the air. At their bottoms, they were each connected to the respective chamber through a hole. By tracking the time course of water-meniscus heights in each tube, water flow across the membrane could be inferred.

Prior to each experiment, all surfaces were cleaned thoroughly with ethanol and deionized water.

For carrying out experiments, the membrane which came in solution was first soaked in deionized water for 30 min. It was

* To whom correspondence should be addressed. E-mail: ghp@u.washington.edu. Phone: (206) 685-1880. Fax (shared): (206) 685-3300.

10.1021/jp9021568 CCC: $40.75
Published on Web 07/13/2009

then placed between the two glass chambers which were carefully pressed between the end-framework blocks by turning the two screws. Then, chambers I and II were filled, respectively, with water and salt solution through the holes in the respective framework, using a syringe needle. Then, the two level tubes were carefully placed into the two holes located on the top of the plastic frameworks. A 0.9 mm i.d., 139 mm long biopsy needle (BD Westcott, BD Medical) was used to add additional water and salt solution into two respective chambers without creating air bubbles. The tubes were filled to achieve meniscus positions convenient for tracking.

Chamber I was filled with water, and chamber II, with water that contained salt. All water was deionized, obtained from a Barnstead D3750 Nanopure Diamond purification system (Type-I HPLC grade (18.2 MΩ cm) 2 μm, polished). For the salt solution, 100 mM sodium sulfate (Fisher Scientific) was used. Its molecular weight (142 Da) is slightly larger than the nominal membrane cutoff (100 Da).

The volumes of water and salt solutions introduced into the respective chambers were sufficient to position the vertical tube menisci at convenient heights for tracking. After the respective chambers were filled, videos of the meniscus were taken with a Logitech Quick Camera and Vidcap software over a period of 2 h, one frame per minute.

Standard 3 M KCl-filled tapered glass microelectrodes were used to measure the potential difference between water and salt chambers. The glass microelectrodes were made using a model P-87 Flaming Brown Micropipet Puller, and the size of the microelectrode tips was approximately 1 μm in diameter. To accommodate the electrodes, holes were drilled in the tops of each chamber, symmetrically about the central membrane. Two such electrodes were used for each experiment. The reference electrode was inserted carefully into a hole on the water side, while the measuring electrode was placed into the corresponding hole on the salt side. Electrode tips were thus positioned at the same relative distance (3 mm) from the membrane. A WPI Electro 705 electrometer and Fluke 99B Scopemeter were used to record electrical data points at a rate of one point per second. The recording process extended over a period of 2 h.

To measure the pH in both water and salt chambers, an Orion 350 Thermo pH meter was used. The pH probe (2 mm in diameter) was inserted into one of the holes described above, to obtain a continuous record for a period of 1 h. Then, in a duplicate experiment, it was inserted into the hole on the other side. This alternating procedure was used to avoid any disturbance that might be caused by repeatedly moving the pH electrode from one chamber to the next, although, by this procedure, small errors might arise out of experiment-to-experiment pH variation. Data points were recorded manually, after the meter reading had stabilized, to yield an effectively continuous record.

In order to observe visually the nature of the transmembrane water flow, 200 μL of microsphere solution were added to both solution-filled chambers. The microspheres were surfactant-free sulfate, white, polystyrene-latex microspheres, 2 m in diameter (product number 1-2000, Interfacial Dynamics Corporation, Portland, OR). The microsphere concentration in each chamber was the same, at a final volume fraction of 0.08%. Particles of this size undergo vigorous Brownian motion, and are sufficiently large to be imaged with a conventional light microscope. To observe and record their movements, we used an inverted Zeiss Axiovert-35 optical microscope, in the bright-field mode with a 5× objective lens and an attached color digital camera (Scion Corporation, CFW-1310C).

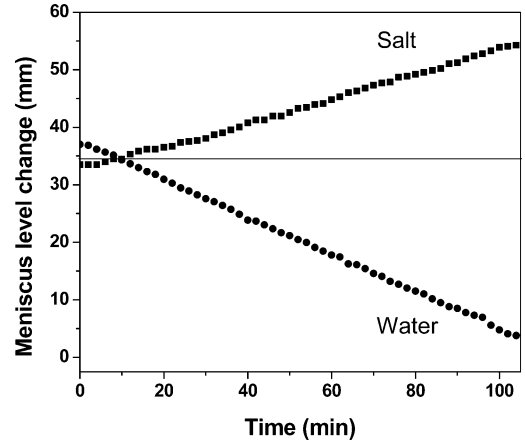

Figure 2. Chamber volume as a function of time. "Salt" refers to the chamber containing salt solution. Zero point on the ordinate corresponds to the point of insertion of the vertical tube into the plastic block.

Experiments similar to those carried out with cellulose acetate were carried out with Nafion. Nafion has been studied extensively in this laboratory because of its prominent and well-characterized EZ.[18,19] The Nafion 117 perfluorinated membrane was purchased from Sigma-Aldrich (274674-1EA). It is composed of a carbon−fluorine backbone with perfluoro side chains containing sulfonic acid groups, fabricated from a copolymer of tetrafluoroethylene and perfluorinated monomers. A 0.007 in. thick Nafion membrane was positioned in the same manner as the cellulose acetate membrane so as to separate water and salt compartments.

Results

The initial set of experiments was carried out using a cellulose acetate membrane. The time course of meniscus-level change in both water and salt chambers is shown in Figure 2. In the experiment shown, the menisci were initially situated at 37 mm on the water side and 33 mm on the salt side. Following an initial latent period on the salt side, the levels changed roughly linearly, dropping on the water side and rising on the salt side as water moved through the membrane from water toward salt.

A curious feature was that the volume loss and gain were not the same. Consistently, the loss of volume on the water side exceeded the increase of volume on the salt side, yielding an overall "volume loss". This volume loss during this 2 h period was approximately 1.5% of the chamber volume, implying either a genuine loss through leakage or evaporation or an apparent loss arising from a water-density increase or membrane-water absorption. Six repeats of these experiments were carried out, and the results were consistent. The mean volume loss rate on the water side was 1.0 ± 0.1 mm^3/min, while the mean volume gain rate on the salt side was 0.6 ± 0.1 mm^3/min.

To test for the role of evaporation, two same-sized (2 mm i.d.) vertically oriented tubes filled either with water or with 100 mM salt solution were sealed at their bottoms with parafilm and left to evaporate. The evaporation rate for water was 0.006 ± 0.001 mm^3/min, and that for salt was 0.007 ± 0.001 mm^3/min. These rates are 2 orders of magnitude lower than the overall volume-loss rate; hence, evaporation effects are negligible. To test for inadvertent leakage from the chamber, a second chamber of the same design was built; the results were indistinguishable. Hence, it appears that the volume loss was not an artifact of leakage or evaporation.

As a control to test for any transport of salt into the water compartment, water-compartment osmolarity was checked after

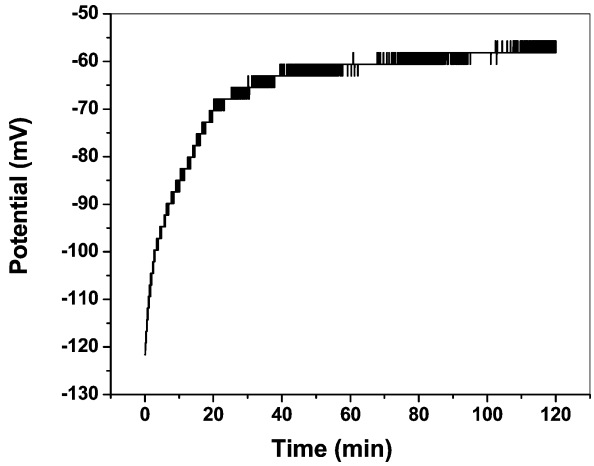

Figure 3. Transmembrane electrical potential difference as a function of time.

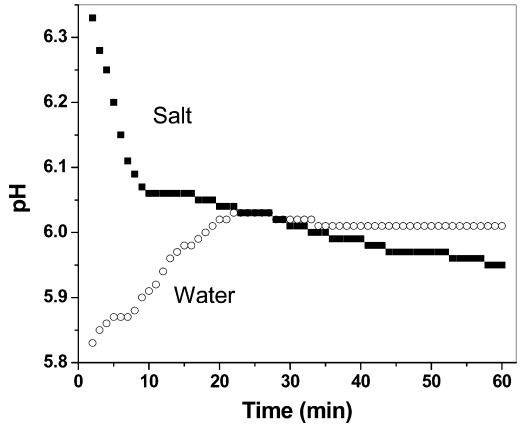

Figure 4. Time course of pH in respective chambers.

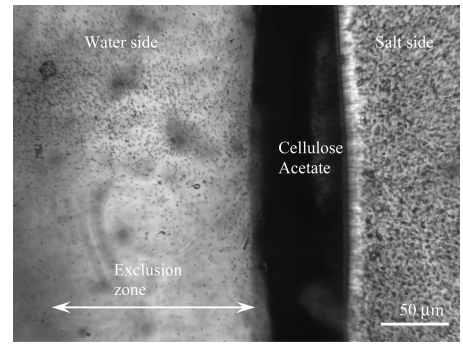

Figure 5. Optical microscopic image of the region surrounding the cellulose acetate membrane, with microsphere markers added to the water side and the salt side. On the water side (left), some near-membrane regions exclude microspheres ("exclusion zone"), while other regions do not. On the salt side, no exclusion zone is detectable. (Note that the bright band next to the membrane on the salt side is not an exclusion zone, as similar bands were sometimes seen on the water side as well, and may arise as an optical artifact of slight membrane tilt.)

the volume-change experiments had been completed. Left for 12 h postexperiment prior to measurement, the contents of chamber I had an osmolarity that was indistinguishable from that of deionized water.

A plot of the electrical potential difference between two chambers as a function of time is shown in Figure 3. The salt side shows an initial negative potential of ~120 mV, which rises up to −60 mV in about 20 min, and then rises more slowly. Six such experiments were carried out, with consistent results. The potential change was fit with an exponential, and the time constant was 14.2 ± 3.9 min. In several experiments carried out at lower salt concentration (10 mM), the negative potential went all the way to zero, in approximately 40 min.

Representative measurements of the time course of pH change are shown in Figure 4. On the water side, the pH increased from an initial value of ~5.8 to a plateau value of ~6.05. On the salt side, the pH dropped from 6.3 to a plateau at ~6.0. Thus, the water became more basic, while the salt solution became more acidic, indicating proton or hydronium-ion transfer across the membrane from water toward salt. It is worth pointing out that the plateau values attained on either side were almost the same. Repeats of this experiment showed mean pH changes on the salt side of 0.6 ± 0.1 and on the water side of 0.2 ± 0.1 ($n = 6$). Final pH values on both sides differed by 0.2 ± 0.1.

To visually observe the trans-membrane water-flow pattern, microspheres were employed. The microspheres were added to both fluids and mixed thoroughly before injection into the respective chambers. Figure 5 is a representative image taken near the membrane 30 min after the microsphere-containing solutions had been put into the chambers. On the water side, microspheres are distributed unevenly. In some regions along the membrane, microspheres penetrated all the way to the membrane, while, in other regions, the near-membrane microspheres are absent. Such microsphere-free zones correspond to the EZs commonly seen earlier next to hydrophilic surfaces.[18,19] These EZs grew with time, requiring tens of minutes to develop fully. Hence, they were not an artifact of poor mixing, which would imply progressive disappearance instead of progressive appearance. EZs on the water side appeared consistently.

On the salt side, by contrast, such EZs are either too small to resolve or absent. This is consistent with earlier work, which has shown that EZ size diminishes with salt.[18] The type of salt seems to play only a minor role: when the standard salt was replaced by NaCl or by $CaCl_2$ in several experiments, diminished EZ size was again confirmed. The fact that EZs were of different size on water and salt sides raised the question whether this asymmetry might play a role in osmotic drive, for EZs are ordinarily charged.[19] A difference of charge on the two sides could perhaps play some role in generating the water movement.

To pursue this line of approach, osmotic experiments were undertaken using Nafion membranes instead of cellulose acetate membranes. Nafion-based exclusion phenomena have been well studied.[18,19] Such membranes produce large EZs with highly charged regions in and around them. The strategy was to determine whether Nafion-based osmotic phenomena were similar to those obtained with the more common cellulose acetate membranes, and, if so, to determine the extent to which they might be connected with exclusion zones.

Figure 6a is a control experiment showing a Nafion membrane with the two straddling chambers containing water. Microsphere markers were included as well. Clear EZs approximately 150 μm wide were seen equally on both sides of the membrane, although only one side is shown for clarity. This image is similar to many others previously obtained.[18,19] EZs are present along the membrane's entirety.

Unlike the image of Figure 6a, which contained water on both sides, in Figure 6b, chamber II contained salt. The water side still shows sizable exclusion of microspheres but only regionally; EZs are interrupted in several places by regions where microspheres penetrate all the way to the membrane surface. Such interrupted EZs are similar to those seen above with cellulose acetate (Figure 5). Under the conditions examined

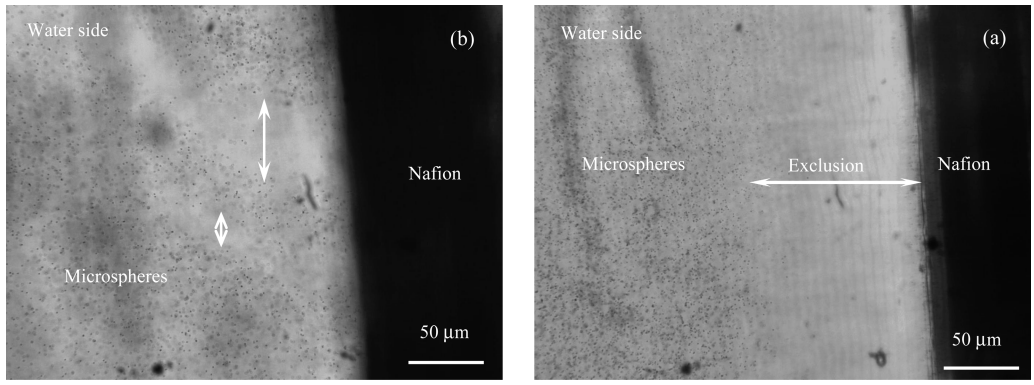

Figure 6. (a) Optical microscopic image of the region next to the Nafion membrane with both chambers filled with water and microsphere markers. Note the large, uniform exclusion zone. (b) Similar to part a except that chamber II (not shown) contained salt. Note the exclusion-zone regions (arrows) and other regions in which microspheres penetrate all the way to the membrane.

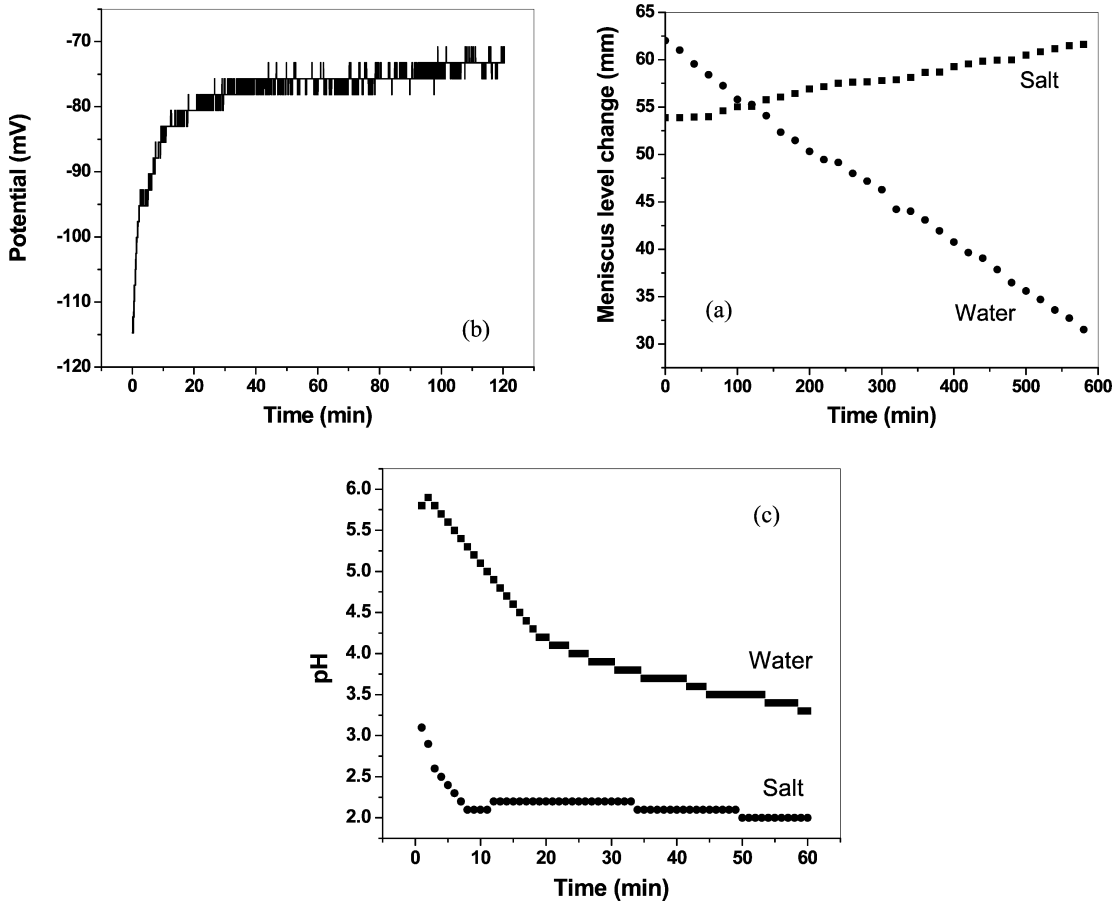

Figure 7. Osmotic phenomena observed as a function of time with the Nafion membrane: (a) volume changes; (b) electrical potential difference changes; (c) pH changes.

with Nafion membranes, these non-EZ regions covered as much as ~80% of the water-side membrane. If EZs do not permit bulk water to pass (see the Discussion), then the EZ breaches—where microsphere markers can penetrate all the way to the membrane—constitute regions through which water could access the membrane and pass through, if properly driven.

Experiments were thus designed to test whether Nafion membranes sustain osmotic flow, and representative results are shown in Figure 7. Figure 7a indicates that water moved across the membrane toward the salt side, albeit more slowly than with the cellulose acetate membrane, possibly because of the smaller pore size. The early latent period on the salt side seen with cellulose acetate is also seen here. Overall water-volume loss is also apparent: the mean volume loss rate on the water side was 0.16 ± 0.01 mm^3/min, while the gain rate on the salt side was 0.04 ± 0.01 mm^3/min.

Electrical potential measurements also showed behavior similar to cellulose acetate (Figure 7b). The initially highly negative value diminished progressively with time. The pH behavior, on the other hand, differed slightly (Figure 7c) in that values on both sides of the membrane diminished with time—possibly because of Nafion's well-recognized proclivity to release protons. However, the pH difference between chambers behaved similarly to cellulose acetate in that the difference diminished progressively with time. Hence, while experiments carried out with Nafion membranes were less extensive than those with cellulose acetate membranes, in most

aspects, the osmotic behaviors of the two were similar, as were the EZ behaviors.

Discussion

We found that water movement during osmosis may be associated with recently discovered exclusion zones (EZs). Such near-surface zones, containing more-ordered water, have been found next to hydrophilic surfaces of varied composition[18,19] and were found here as well next to osmosis membranes (Figure 5 and 6).

EZ buildup releases protons to bulk water, which then associate with the water to form hydronium ions.[18−20] Hydronium-ion concentration is appreciable, and the attendant pH changes are unexpectedly large.[21] Any inequality of hydronium-ion concentration in salt vs water compartments would then create a potential gradient across the membrane, which could propel water flow—at least in places where access to the membrane is not blocked. This is the hypothesis under consideration.

Evidence for such a mechanism was found in the experimental results. We first explored osmotic flow under standard conditions: two chambers separated by a cellulose acetate membrane, one containing water alone and the other containing 100 mM sodium sulfate solution. Water flowed steadily from the water compartment toward the salt-containing compartment, as is routinely reported.[22]

Parenthetically, it was notable that, during osmotic flow toward the salt side, an overall volume loss was inferred in both the cellulose acetate and Nafion experiments. This loss was determined not due to evaporation or leakage (see the Results section). Although relatively minor, the loss was nevertheless consistent. One possible explanation is electrostriction, a water-density increase that occurs when water organizes in the presence of salt.[23] Another possibility is that the membranes might absorb some amount of passing water. In support of the latter hypothesis, during the time that the water side was losing water, we observed an early period of no volume increase on the salt side (Figures 2 and 7a). We found the same in the several experiments carried out with lower salt concentration (10 mM), supporting the generality of this presumption. Further, in a number of experiments carried out at higher salt concentration (1 M), we found that the drop rate on the water side was practically the same as the rise rate on the salt side. If electrostriction had been the correct explanation, then the elevated concentration of salt should have exaggerated the effect rather than diminish it toward zero. Hence, the other explanation seems more likely—that the loss of volume occurred as water passing through the membrane adsorbed onto its internal surfaces. The fact that the volume loss was greater with Nafion, a known superabsorber,[24] than with cellulose acetate is also consistent with this hypothesis.

We also observed a substantial potential difference between compartments, the salt side being more negative than the water side. This was measured using pairs of microelectrodes, one situated on the water side and the other on the salt side. The magnitude of this potential difference diminished with time, as water flowed from water toward salt. Potential differences between osmotic compartments were first noted many years ago by Loeb,[22] and subsequently confirmed by others.[25] Hence, they are quite typical although not commonly acknowledged.

To test for compartmental differences of proton concentration, pH electrodes were used. On the water side, relatively low initial pH values rose with time, indicating progressive loss of protons or hydronium ions from the water compartment. Correspondingly, the salt-side pH moved from relatively higher values toward lower values, at least with cellulose acetate, indicating progressive gain of protons or hydronium ions. With Nafion, salt-side behavior was more complex; however, the difference between compartments nevertheless diminished with time, again indicating proton transfer across the membrane.

Thus, pH and electrical potential measurements show that, during transmembrane water flow, positive charges flow from the water compartment toward the salt compartment. This flow diminishes both the electrical gradient and the hydronium-ion gradient. Thus, it is positively charged water, i.e., hydronium ions, and not uncharged water, that must be at least a component of flow. This seems an inevitable consequence of the experimental findings.

If so, then some process apparently creates an excess of hydronium ions on the water side, which then flows down its concentration gradient toward the salt side. Although a charge-driven process of this sort resembles electro-osmosis,[26−28] in this case, the electrical gradient evidently arises not from any imposed potential difference but somehow from within the system itself.

From where might such a potential gradient arise?

Nature of Driving Force. A likely source of the electrical potential gradient is the EZ. Although EZs were first noted several decades ago,[29] and were implied in still older measurements,[30] they have been studied intensively only during the past several years.[18,19,21,31]

The most notable feature of the EZ is the extensive exclusion of particles and solutes, ranging in size from colloidal particles tens of micrometers in diameter down to solutes of molecular weight 100 or less.[19,21] The reason for such extensive exclusion appears to be a molecular organizational change: evidence obtained from a series of physical chemical approaches implies that this region is more stable and more ordered than bulk water, perhaps resembling a liquid crystal.[19,20,31] The more ordered structure excludes solutes in much the same way as does ice.

Of particular relevance here is the fact that these EZs bear negative charge.[19,31,32] In building this negative charge from neutral water, water must hydrolyze, and water-based charge separation has been confirmed: as negative charge of the EZ builds, positive charge builds correspondingly in the bulk-water region beyond.[21] Hence, the presence of an EZ adjacent to a surface implies the presence of positive charges in the bulk water beyond, and such positive charge is anticipated to appear in the form of hydronium ions, i.e., positively charged water molecules.

EZs are diminished by the presence of salts.[18,31] Hence, EZs that can be on the order of 100 μm or more in pure water are diminished in the presence of salt, and such diminution has been observed here as well. On the salt side, EZs were barely, if at all, discernible (Figure 5). As a consequence of the near-absent EZ, few hydronium ions are anticipated to be generated on the salt side.

The opposite was true of the water side. There, patches of EZ were observed both with cellulose acetate and Nafion membranes (Figures 5 and 6b). As the patches form and grow, hydronium ions are expected to accumulate in the water-containing chamber, creating both positive electrical potential and low pH. Both of these features were observed.

Hence, a clear difference was found between the membrane's water and salt sides. These differences should be sufficient for setting up a driving force to propel the positive hydronium ions

(charged water) through the membrane and toward the salt side. This driving force could well be responsible for the observed water flow.

Nature of the Barrier. An issue that complicates any such hypothesis is the resistance of the membrane barrier. Osmosis membranes are thought to have an effective "pore size", allowing passage of solutes up to a certain diameter while blocking the passage of larger ones.

Our experimental observations suggest that the pore size plays little if any role in excluding molecules and/or allowing the passage of water. The simplistic notion of membrane pore size is complicated and perhaps overshadowed by the presence of EZs adjacent to one or both membrane surfaces. The apparent barrier is not the membrane alone but a sandwich of two EZs surrounding the membrane in between. The fact that EZs exclude various solutes raises the question of what substances can actually penetrate and what substances cannot. Although many solutes are demonstrably excluded, this question is not yet fully answered because very small ionic solutes have not been systematically studied.

One observation, however, suggests that even water itself cannot penetrate into the EZ. Two chambers were set up, as in the present experiments, with cellulose acetate as the separating membrane, except that the salt solution was evacuated, initially through evaporation and later with a syringe, leaving chamber II empty. In no instance did the water from the water side flow through the membrane even after several days, and even when the chamber was turned vertically to allow gravitational forces to assist. Thus, in the absence of a suitable driving force, even water itself cannot penetrate through the EZ barrier.

The existence of a water barrier has made it difficult to envision a way in which water—either neutral or hydronium-charged—could easily pass through the semipermeable membrane, and thereby account for any osmotically driven flow. Thus, we were excited to see that, under the conditions of osmotic potential difference, as opposed to the conditions of still water mentioned just above, only part of the membrane surface was covered by EZs. Abundant regions were altogether devoid of EZs. This was true of both membrane types. Free of exclusion-zone barriers, these zones would not suffer any blockage; water could putatively flow into those blockage-free zones, and through the membrane. These microsphere-free zones, or channels, remained stable over time, even in presence of inevitable ambient thermal fluctuations, which might act to disrupt them.

A way that such channels could form is if EZs began building at slightly different rates in different membrane regions, resulting in local variations of hydronium-ion concentration (Figure 8). Hydronium ions would tend to flow toward the salt side where the electrical potential is more negative. Concentrated hydronium ions would inevitably find membrane areas where EZs had barely formed, initiating and maintaining the passage of water and thereby preventing any further EZ growth in those regions. Hence, regions sustaining passage of water would not permit EZ growth at all, while already growing EZs could grow with relative impunity. This process would yield the observed punctate pattern of EZs, as well as continued flow.

Osmosis without Membranes. Although osmosis *per se* is defined as requiring a membrane, osmotic drive can occur in the absence of a membrane, provided there are two phases. For example, dry polymer or protein can absorb huge volumes of water to become a gel, presumably as a result of osmotic drive. Such gels are surrounded by substantial EZs,[18,19] which are thus situated between gel and water phases. This in-between locus

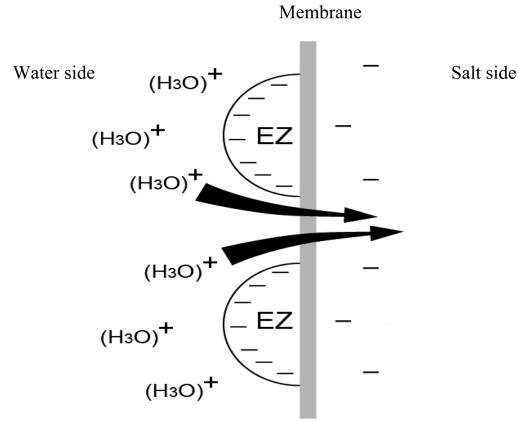

Figure 8. Model of the proposed osmotic mechanism. Hydronium ions situated in regions beyond exclusion zones are drawn toward the more negative electrical potential of the salt side, passing through membrane regions that are devoid of exclusion zones.

is analogous to that of the EZs reported here, which are in between water and salt compartments. Further, gel and water phases show an electrical potential difference,[18,19] similar to the potential differences measured here; even the magnitudes are similar. Such phase-dependent potential differences are more commonly known as "Nernst potentials" or "Donnan potentials",[33] and appear to be the equivalent of the electrical potentials measured across the EZ-separated compartments.

Osmotic drive, in other words, can result from potential differences arising as a consequence of EZs, whether membranes are, or are not, present. All that is required is two distinct phases.

Comparison with Prevailing Views. The exclusion-zone-based mechanism suggested here differs from other mechanisms that have been proposed. It relies centrally on the presence of an electrical potential gradient between membrane-separated compartments as the osmotic driving force. Charged hydronium ions on the water side are drawn to the more negative compartment on the other side of the membrane.

Except for electro-osmosis, which is likewise based on electrical gradients but relies on gradients imposed externally, existing theories of osmosis rest on different principles. Serious approaches to the osmotic mechanism began in the early 1800s when Dutrochet[34] studied osmosis in animal bladders and Pfeffer[35] and de Vries[36] studied water flow plants. From such early studies, van't Hoff (1885) derived the now-classical equation, expressing the osmotic pressure $\Pi = kT\Delta c$, where Δc is the difference of solute concentration between chambers, T is the temperature, and k is a constant. According to this widely used equation, osmotic drawing force, or pressure, at a given temperature should depend only on the difference of solute concentration. It is unrelated to charge.

While consistently invoking this now-standard expression, current views on the mechanism of osmosis run in two main streams, which are mutually exclusive. The dominant one is perhaps the "water-concentration theory", found in many textbooks.[3−9] Here, water has a chemical potential much like other substances, and water molecules move from regions of higher to lower water potential. Less commonly accepted though gaining attention is the "water tension" theory, developed more or less independently by several investigators.[10−17] This theory takes into account the interaction of solute and water molecules, presupposing a cohesion that creates a negative solvent pressure.

Less popular than those two streams are the solute-bombardment theory and the solute-attraction theory. The former is among the earliest explanations put forth by van't Hoff and

others.[1] Osmotic pressure is viewed as arising from molecular bombardment, a process that may be applicable in situations in which solutes interact weakly with solvent. The solute-attraction theory, on the other hand, supposes the solutes in the vicinity of a pore can suck water from the pore into the solution via solute−water attractive forces.[2,37]

None of these theories take account of the presence of EZs in the vicinity of a membrane, or the presence of potential differences on either side of the membrane. Hence, they are, at the very least, incomplete. Moreover, they suffer various conflicts with experimental data, which is what led to the abundance of alternative theories. For example, the most popular, water-concentration theory considers the osmotic flow to be diffusive, but generally, it is observed to be convective rather than diffusive.[38]

On the contrary, the mechanism proposed here is based on consistently observed phenomena—EZs and charge separation. Oddly, the charge separation observed here was reported in the century-old classical work of Jacques Loeb.[22] Loeb measured potential differences between salt and water compartments, noting their functional dependence on the initial pH of the chambers' solutions. He observed that the initial rate of water flow depended on hydrogen-ion concentration, and concluded that the driving force for osmosis was the potential difference across the membrane.

This line of thinking is similar to that proposed here. A difference is that we have given specificity to the hypothesis by identifying a source of charge leading to the transmembrane potential difference: the EZ. Although the presence of these charged zones is not yet broadly appreciated, they have been shown to generate positive charge,[21] and their asymmetry about the membrane implies that any such positive charge differs on the two sides, thereby giving rise to the difference of potential. This difference would drive hydronium ions through the membrane, accounting for the osmotic flow.

Conclusion

The results reported here imply an osmotic mechanism quite different from any of the now-popular ones. The new mechanism is based on the prominent exclusion zones seen adjacent to osmotic membranes. These zones are negatively charged. As they build, they create equal and opposite positive charges in aqueous regions beyond them, which appear as hydronium ions. Being mobile, the hydronium ions constitute a source of potential energy. In situations in which they are more concentrated on one side of the membrane than on the other, they can move down their potential gradient and across the membrane. This trans-membrane hydronium-ion flow amounts to water flow, which is osmosis' most fundamental feature.

How general the proposed mechanism might be is not yet clear, and awaits the results of further experiments. If diverse osmotic systems are found to contain interfacial exclusion zones, then the proposed mechanism could well be generic.

Acknowledgment. We thank Albert Kalganov for providing software for measuring the meniscus-height change and Ivan Klyuzhin for his help with the figures. We also thank Adam Wexler, Xavier Figueroa, and Frank Borg for their useful discussions and detailed comments on the manuscript. This study was supported by NIH grants AT-002362 and AR-44813 and ONR grant N00014-05-1-0773.

References and Notes

(1) van't Hoff, J. H. *Z. Phys. Chem.* **1892**, *9*, 477.
(2) van't Hoff, J. H. *Z. Phys. Chem.* **1887**, *1*, 481.
(3) Mauro, A. *Science* **1957**, *126*.
(4) Ray, P. M. *Plant Physiol.* **1960**, *35*, 783.
(5) Dainty, J. *Adv. Bot. Res.* **1963**, *1*, 279.
(6) Slatyer, R. O. *Plant-water relationships*; Academic Press: London, 1967.
(7) Petrucci, R. H.; Harwood, W. S.; Herring, F. G. *General Chemistry*, 8th ed.; Prentice Hall: New York, 2002.
(8) Brescia, F. *Fundamentals of Chemisty*, 3rd ed.; Academic Press: London, 1975.
(9) Martini, F. H. *Fundamental of Anatomy and Physiology*, 5th ed.; Prentice Hall: New York, 2001.
(10) Hulett, G. A. *Z. Phys. Chem.* **1902**, *42*, 353.
(11) Herzfeld, K. F. *Phys. Z.* **1937**, *38*, 58.
(12) Mysels, K. *Introduction to Colloid Chemistry*; Interscience Publishers, Inc.: New York, 1959.
(13) Duclaux, J. *J. Chim. Phys.* **1965**, *65*, 435.
(14) Hammel, H. T. Scholander, P. F. *Osmosis and tensile solvent*; Springer-Verlag: Berlin, Heidelberg, New York, 1976.
(15) Hammel, H. T.; Scholander, P. F. *Proc. Natl. Acad. Sci.* **1973**, *70*, 124.
(16) Scholander, P. F. *Science* **1967**, *156*, 67.
(17) Scholander, P. F.; Hammel, H. T.; Bradstreet, E. D.; Hemmingsen, E. A. *Science* **1965**, *148*, 339.
(18) Zheng, J. M.; Pollack, G. H. *Phys. Rev. E* **2003**, *68*, 031408.
(19) Zheng, J. M.; Chin, W. C.; Khijniak, E., Jr.; Khujniak, E.; Pollack, G. H. *Adv. Colloid Interface Sci.* **2006**, *127*, 19.
(20) http://uwtv.org/programs/displayevent.aspx?rID=22222.
(21) Chai, B. H.; Hyok, Y.; Pollack, G. H. *Phys. Chem. Chem. Phys.* 2009.
(22) Loeb, J. *J. Gen. Physiol.* **1921**, *3*, 213.
(23) Botti, A.; Bruni, F.; Ricci, M. A.; Soper, A. K. *J. Chem. Phys.* **1998**, *109*, 3180.
(24) Affoune, A. M.; Yamada, A.; Umeda, M. *J. Power Sources* **2005**, *148*, 9.
(25) Elimelech, M.; Chen, W. H.; Waypa, J. J. *Desalination* **1994**, *95*, 269.
(26) Choe, Y. K.; Tsuchida, E.; Ikeshoji, T.; Yamakawa, S.; Hyodo, S. *J. Phys. Chem. B* **2008**, *112*, 11586.
(27) Pivovar, B. S. *Polymer* **2006**, *47*, 4194.
(28) Pivovar, B. S.; Smyrl, W. H.; Cussler, E. L. *J. Electrochem. Soc.* **2005**, *152*, A53.
(29) Green, K.; Otori, T. *J. Physiol.* **1970**, *207*, 93.
(30) Henniker, J. C. *Rev. Mod. Phys.* **1949**, *21*, 322.
(31) Pollack, G. H., Cameron, I. L., Wheatley, D. N., Eds. *Water and the Cell*; Springer: The Netherlands, 2006.
(32) Zheng, J. M.; Wexler, A.; Pollack, G. H. *J. Colloid Interface Sci.* **2009**, *332*, 511.
(33) Jacobson, K.; Sheets, E. D.; Simson, R. *Science* **1995**, *268*, 1441.
(34) Dutrochet, H. *Memoires dea vegetaux et des animaux. I. De L'endosmos*; Meline Cans et Co.: Brussels, Belgium, 1837.
(35) Pfeffer, W. *Osmotische Untersuchungen*; W. Engelman: Leipzig, Germany, 1921.
(36) DeVries, H. *Phys. Chem.* **1888**, *2*, 415.
(37) Kill, F. *Am. J. Physiol.* **1989**, *256*, 801.
(38) Borg, F. arXiv:physics/0305011.

JP9021568