brief communication

Effect of solute permeability in determination of elastic modulus using the vesicular swelling method

Rickey L. Rivers and James C. Williams, Jr.
Department of Anatomy and Cell Biology, Medical University of South Carolina, Charleston, South Carolina 29425-2204

ABSTRACT The modulus of elasticity of artificial and biological membranes can be determined in membrane vesicles by monitoring the limitation of vesicular swelling during a slow decrease in medium tonicity. The higher the elastic modulus of the membrane, the more effectively the vesicles will resist swelling. This method assumes that the solutes in the system are impermeant, so that the final volume of the vesicles is determined solely by a balance of osmotic and hydrostatic forces. In this paper, we present the results of computer simulation of vesicular swelling in which the solute permeability of the membrane was varied. We find that even a small permeability will lead to a loss of solute from the vesicle that will retard the increase in vesicular volume during dilution of the medium, and thereby cause the apparent modulus of elasticity to be much greater than the true value. For example, if one takes the mannitol permeability in brush border membrane vesicles from small intestine to be 0.004 μm/s (a reasonable estimate), one finds that a vesicular swelling study using mannitol as the principal solute will show the apparent elastic modulus of the vesicles to be >10 times larger than the true value. With higher permeabilities, the effect is even more dramatic. We conclude that determination of impermeance of solutes is a critical prerequisite for making valid determinations of membrane elastic modulus using the vesicular swelling method.

INTRODUCTION

Cells must change their shape in order to function. From erythrocytes that are squeezed through the microvasculature to cells extending processes for motility and communication, cell function requires stressing the lipid bilayer. The resistance of the cell membrane to the deformation can be subdivided into three types of modulus of elasticity: area expansion, shear, and bending (Hochmuth and Waugh, 1987). The area expansion modulus, with its components of force/length, refers to the amount of isotropic force in the plane of the membrane that is required to deform the membrane one unit of area. Several studies have sought to quantify this elastic modulus by various means such as micropipette aspiration (Evans et al., 1976), lateral pressure (Rand et al., 1980), and osmotic swelling (Miyamoto et al., 1988; Li et al., 1986). When the bilayer being studied is in the form of membrane vesicles, osmotic swelling allows a "hands-off" approach to studying this force because stress is induced in the membrane by a decrease in the osmotic pressure of the surrounding medium. By measuring the resulting increase in the diameter of the vesicle via dynamic light scattering, the strain on the membrane can be calculated.

Measurements of elastic moduli using this method were first conducted with synthetic membrane vesicles composed of one or more types of phospholipid. Li et al. (1986) prepared vesicles from dioleoylphosphatidic acid and found the elastic modulus to be between 15 and 200 dyn/cm (assuming a membrane thickness of 5 nm). The extremes of this range are attributed to the different solutes used in the medium; KCl effected the highest modulus while sucrose brought about the lowest. The elastic modulus of vesicles derived from biological membranes has been studied by Miyamoto et al. using brush border vesicles (1988) and chromaffin granules (Miyamoto and Fujime, 1988). By using mannitol as their solute, Miyamoto et al. found the elastic modulus of intestinal brush border vesicles to be 150 dyn/cm. This value lessened when glucose was in the media but remained constant when phlorizin was used to block glucose transport (Miyamoto et al., 1988). In chromaffin granules the elastic modulus was measured to be much smaller: ~20 dyn/cm (Miyamoto and Fujime, 1988). This value dropped to almost zero when the vesicles were treated with calcium. Sucrose was the principle solute used in the chromaffin granule experiments.

In the above examples and in osmotic swelling in general, one of the primary assumptions is the impermeance of the solutes in the medium. Whereas no solute can be absolutely impermeant, solutes such as mannitol are considered to be virtually just that. In biological membranes in particular, such assumptions may not be valid. For example, the data of Stevens et al. (1982, 1984) suggest that mannitol permeability in intestinal brush

0006-3495/90/03/627/05 $2.00

border vesicles is between 0.001 and 0.01 μm/s (converting data from Stevens et al. [1982, 1984] with an estimate of brush border surface area from Parsons [1983]).

In the present paper, we report the effect of solute permeability on the validity of measurements of an elastic modulus as determined by vesicular swelling such as carried out by Miyamoto et al. in small intestine brush border membrane vesicles. When solute permeabilities greater than zero were introduced into the model the resulting apparent modulus of elasticity was greatly affected, even when modest permeabilities were tested. After testing a range of values in the model, we conclude that reasonable solute permeabilities in vesicular membranes can lead to overestimation of the membrane elastic modulus when using the vesicular swelling method.

MATERIALS AND METHODS

In the procedure being modeled, Miyamoto et al. used brush border vesicles (rat small intestine) that were prepared in a 200 mM buffered mannitol solution. Vesicular swelling was brought about by slowly adding mannitol-free buffer to the external solution. The time course of their experiment is diagrammed in Fig. 1. Modeling this procedure was carried out by calculating the fluxes of water and solute through the membrane of a spherical vesicle as the osmotic pressure of the external solution was reduced. The change in volume (V) and solute (Q) at time t was calculated by integrating the following using the Euler method:

$$\frac{dV}{dt} = A_t L_p \left(RT\Delta C + \frac{2M\Delta A}{A_o r} \right)$$

$$\frac{dQ}{dt} = PA_t\Delta C$$

$$\text{where } \Delta C = C_{in} - C_{out} = \frac{Q}{V} - C_{out}.$$

Surface area of the vesicle (A) has subscripts denoting time, while L_p is the hydraulic conductivity. The volume flux is driven by two different forces. The first component is the osmotic pressure which arises from the difference in internal and external solute concentrations ($C_{in} - C_{out}$); R and T are the gas constant and temperature. The second component, the hydrostatic pressure, arises from the tension developed from stretching the membrane. This pressure depends on the change in area of the membrane ($\Delta A = A_t - A_o$, with r the radius of the vesicle) and the membrane's modulus of elasticity (M). When the solute is permeant, C_{in} depends on both the flux of water into the vesicle and the flux of solute out. The rate of change in the amount of intravesicular solute depends on the magnitude of the permeability of the membrane to solute (P). It is implicit in these equations that the passage of solute and water through the membrane is not coupled; that is, the reflection coefficient of the solute is assumed to be 1.0. This assumption is consistent with recent work on the osmotic permeability of membrane vesicles (Pearce and Verkman, 1989). C_{out}, the external concentration, was decremented to simulate dilution of buffer surrounding vesicles (as shown in Fig. 1). Computations were completed on a Macintosh II (Apple Computer Inc., Cupertino, CA) using Turbo Pascal (Borland International, Scottsvalley, CA). The results obtained were confirmed by fourth-order Runge-Kutta using the modeling program Stella (High Performance Systems, Lyme, NH).

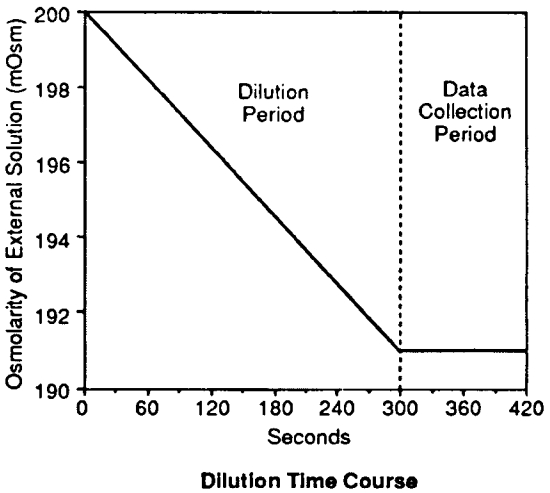

FIGURE 1 This figure illustrates the time course of vesicular swelling as carried out by Miyamoto et al. (1988). The osmolarity of the external solution drops due to addition of diluting medium. The diameter measurement is taken after the dilution has been stopped and the osmolarity levels off. Such a time course of change in C_{out} was modeled in the vesicular swelling curves shown in Fig. 2.

RESULTS

The solute permeability assumed for the membrane had a very significant effect on the modulus of elasticity calculated from changes in vesicle volume. When the solute was considered to be impermeant, and the elastic modulus imposed to be zero, vesicle diameter reached the value expected by the osmotic difference, as this combination produced the greatest volume flux with no tension forming in the membrane. This is shown by the top curve in Fig. 2 *A*. With higher elastic moduli, stress developing in the membrane restricted vesicle swelling, thus depressing the predicted maximum diameter as shown in Fig. 2 *A*. Under the conditions being modeled, Miyamoto et al. (1988, p. 507) showed in a typical experiment that intestinal brush border vesicles of 327.5 nm swelled to 329.1 nm when glucose was not being transported. Using Fig. 2 *A*, it can be seen that the elastic modulus is between 100 and 150 dyn/cm to account for this observed restriction in swelling. This is the apparent modulus of elasticity for the experiment.

Fig. 2, *B*–*D*, show increasing values for solute permeability. Fig. 2 *B* demonstrates that even a minute solute permeability (0.0001 μm/s) can significantly affect the apparent value of the elastic modulus. For the "observed"

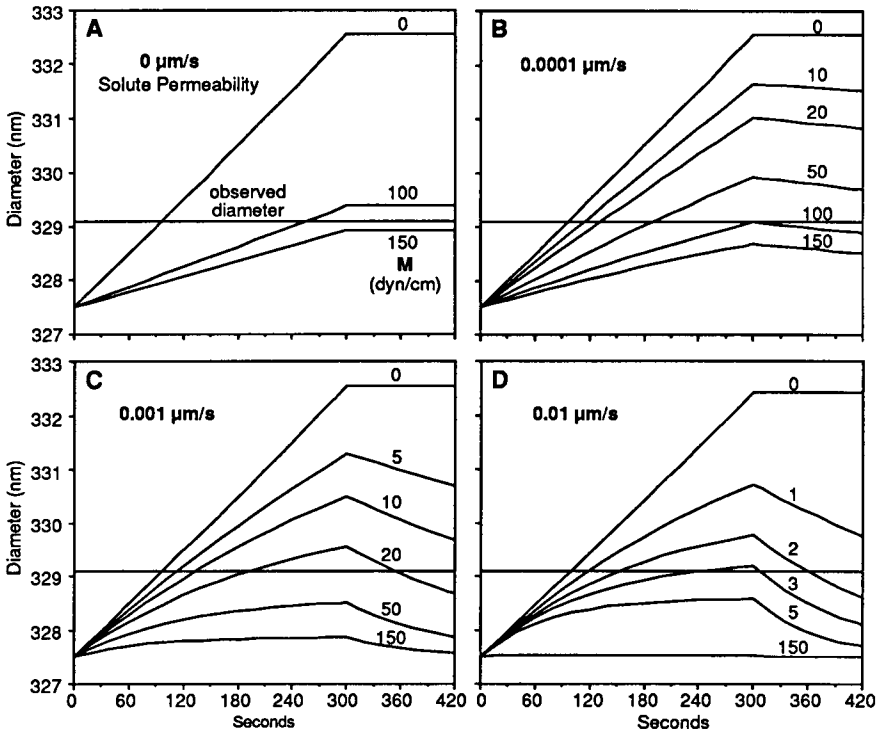

FIGURE 2 Effect of varying solute permeability and elastic modulus on the change in vesicle diameter during the sequence of medium dilution shown in Fig. 1. (*A*) Behavior assumed for experimental measurement (membrane solute permeability is equal to zero). The starting vesicle diameter is 327.5 nm; in a typical experiment by Miyamoto et al. (1988) using intestinal brush border membrane vesicles, such vesicles swelled to a diameter of 329.1 nm, indicated in figure as "observed diameter." Each curve shows the model predictions for a given value of the membrane elastic modulus (*M*). Note that the swelling observed by Miyamoto et al. is consistent with a modulus of elasticity close to 150 dyn/cm. (*B–D*) Increasing values for membrane solute permeability. As the solute permeability increases, the degree of swelling observed by Miyamoto et al. is consistent with increasingly smaller values of membrane elastic modulus. Note that the permeability of mannitol in intestinal brush border membrane vesicles is between 0.001 and 0.01 μm/s (see Introduction), suggesting that the degree of swelling observed is consistent with an elastic modulus of < 20 dyn/cm.

maximum diameter, the true modulus of elasticity is now below 100 dyn/cm. Note that the diameter begins to drop slightly after the peak at the beginning of the data collection period, as the pressure within the vesicle is slowly relieved by solute exit.

With a solute permeability of 0.001 μm/s, the "observed" vesicle diameter was best matched with an elastic modulus of ~20 dyn/cm (Fig. 2 *C*). Again the diameter shrinks after the 300-s mark but this time the rate of shrinkage has increased. This figure is important to consider because, as stated above, mannitol's membrane permeability is between 0.001 and 0.01 μm/s in small intestine brush border membrane vesicles.

The highest solute permeability modeled was 0.01 μm/s. With the curves being further depressed, Fig. 2 *D* depicts the true elastic modulus that would fit best at this permeability to be between 2 and 3 dyn/cm. The bottom curve on the last three figures shows what the diameter would be if the modulus of elasticity were 150 dyn/cm. As the solute permeability increases, this curve flattens until an almost straight line is predicted. In this case, the low osmotic pressure difference (low due to solute leakage) across the membrane is not enough to overcome the resistance of the membrane. Thus, the diameter does not change.

DISCUSSION

In the technique of determining the membrane elastic modulus using the vesicular swelling method, vesicles are allowed to slowly equilibrate with diluted medium, and the resultant vesicular swelling is measured. If the vesicle membrane has even a low permeability to a solute in the medium, the high ratio of surface area to volume in vesicles will allow substantial equilibration of the solute across the vesicular membrane. Thus, the vesicles will swell less and the elastic modulus will appear to be greater than it really is. Greater solute permeabilities allow faster equilibration, resulting in higher apparent elastic moduli.

Several factors can affect apparent membrane elasticity. Li et al. (1986) have shown that ionic and nonionic solutes bring about different elastic moduli. In their experiments, KCl produced a higher elastic modulus than did sucrose. Membrane permeability to KCl could account for this difference; that is, if KCl were more permeant than sucrose in these vesicles, it is conceivable that KCl permeation led to apparently higher elastic moduli in this preparation. However, it is difficult to estimate from the literature the KCl permeability of the phospholipid vesicles used by Li et al. Hantz et al. (1986) took salt permeability into account when estimating the elastic modulus for artificial vesicles but noted that values vary widely in the literature due to the large number of factors affecting solute permeability. It is also possible that stressing a membrane could affect solute permeability. Singer (1981) has shown that a bilayer's solute permeability is greatest when it reaches its phase transition temperature. This increase is thought to be due to flaws between organized (liquid crystal) and nonorganized (gel) areas of phospholipids. Similarly, it is also possible that stretching alone could lead to membrane phase discontinuities and thereby lead to an increase in membrane permeability when vesicles are swollen. Such a situation would complicate assessment of an elastic modulus using the vesicular swelling method.

Vesicles with proteins have been shown to have higher apparent elastic moduli than identical vesicles without them. Haines et al. (1987) demonstrated this when they compared vesicles composed of only *Escherichia coli* lipids with those made up of the same lipids and an integral protein (lactose carrier). If an integral protein is a nucleating center for phospholipid aggregations as proposed by Wendoloski et al. (1989), then solute permeability could be enhanced by the resulting flaws between the groupings which would allow greater solute permeation, and lead to a higher apparent elastic modulus.

The shape of the curves in Fig. 2 suggests a way for investigators to demonstrate impermeance of solute used during vesicular swelling. Nonzero permeabilities will allow the vesicles to shrink to original size after the dilution period. Lower solute permeabilities will result in lower shrinkage rates. Such shrinkage of vesicles after osmotic swelling has not been described in the literature, but neither has the stability of increased vesicular volume with time after swelling.

As mentioned in the introduction, Miyamoto et al. (1988) showed that the presence of glucose in the intra- and extravesicular media caused a decrease in the apparent elastic modulus of intestinal brush border membrane vesicles. They interpret this to indicate that activation of the glucose transporter in the membrane led to a lower elastic modulus. An alternative explanation might relate to the permeance of glucose in this system. If, as vesicular swelling were taking place, glucose flux from the outer medium into the vesicle were to lead to net addition of solute to the vesicle interior, then the vesicle would reach a larger volume even with no change in elastic modulus. This would be true regardless of whether the other major solute in the system (mannitol) were permeant or not. Thus, the change in vesicle diameter seen in the presence of glucose could be explained without assuming a change in membrane elastic modulus.

Along these lines, it is instructive to note that Miyamoto and Fujime (1988) have reported much lower values for elastic modulus in chromaffin granules than they found in intestinal brush border membrane. In the experiments with chromaffin granules, they used sucrose, rather than mannitol, as the principle solute in the system. Perhaps the lower permeance of sucrose in biological membranes was part of the reason for the lower values of elastic moduli measured.

In conclusion, we find that determination of membrane elastic moduli by the vesicular swelling method is critically dependent on the membrane being impermeable to the solutes in the medium. This requirement is probably met in most experiments with aritificial membrane vesicles, but is more of a problem in biological membrane vesicles, where solutes such as sucrose or raffinose will have to be used to ensure that membrane permeabilities are low enough for the measurement of membrane elastic moduli to be valid. In addition, the question of whether strain in the membrane may itself lead to increased solute permeability, and thereby lead to errors in measurement of the elastic modulus, must be addressed.

This study was funded by National Institutes of Health grant DK 39023. J. C. Williams is an Established Investigator of the American Heart Association.

Received for publication 18 May 1989 and in final form 13 November 1989.

REFERENCES

Evans, E. A., R. Waugh, and L. Melnik. 1976. Elastic area compressibility modulus of red cell membrane. *Biophys. J.* 16:585–595.

Haines, T. H., W. Li, M. Green, and H. Z. Cummins. 1987. The elasticity of uniform, unilamellar vesicles of acidic phospholipids during osmotic swelling is dominated by the ionic strength of the media. *Biochemistry.* 26:5439–5447.

Hantz, E., A. Cao, J. Escaig, and E. Taillandier. 1986. The osmotic response of large unilamellar vesicles studied by quasielastic light scattering. *Biochim. Biophys. Acta.* 862:379–386.

Hochmuth, R. M., and R. E. Waugh. 1987. Erythrocyte membrane elasticity and viscosity. *Annu. Rev. Physiol.* 49:209–219.

Li, W., T. S. Aurora, T. H. Haines, and H. Z. Cummins. 1986. Elasticity of synthetic phospholipid vesicles and submitochondrial particles during osmotic swelling. *Biochemistry.* 25:8220–8229.

Miyamoto, S., and S. Fujime. 1988. Regulation by Ca^{2+} of membrane elasticity of bovine chromaffin granules. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 238:67–70.

Miyamoto, S., T. Maeda, and S. Fujime. 1988. Change in membrane elastic modulus on activation of glucose transport system of brush border membrane vesicles studied by osmotic swelling and dynamic light scattering. *Biophys. J.* 53:505–512.

Parsons, D. S. 1983. Introductory remarks on the brush border (abstract). *CIBA Found. Symp.* 95:3–11.

Pearce, D., and A. S. Verkman. 1989. NaCl reflection coefficients in proximal tubule apical and basolateral membrane vesicles. Measurement by induced osmosis and solvent drag. *Biophys. J.* 55:1251–1259.

Rand, R. P., V. A. Parsegian, J. A. C. Henry, L. J. Lis, and M. McAlister. 1980. The effect of cholesterol on measured interaction and compressibility of dipalmitoylphosphatidylcholine bilayers. *Can. J. Biochem.* 58:959–968.

Singer, M. 1981. Permeability of phosphatidylcholine and phosphatidylethanolamine bilayers. *Chem. Phys. Lipids.* 28:253–267.

Stevens, B. R., H. J. Ross, and E. M. Wright. 1982. Multiple transport pathways for neutral amino acids in rabbit jejunal brush border vesicles. *J. Membr. Biol.* 66:213–225.

Stevens, B. R., J. D. Kauntiz, and E. M. Wright. 1984. Intestinal transport of amino acids and sugars: advances using membrane vesicles. *Annu. Rev. Physiol.* 46:417–433.

Wendoloski, J. J., S. J. Kimatian, C. E. Schutt, and F. R. Salemme. 1989. Molecular dynamics simulation of a phospholipid micelle. *Science (Wash. DC).* 243:636–638.