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ARTICLE

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Permeability and the hidden area of lipid bilayers

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Abstract The passive water permeability of a lipid vesicle membrane was studied, related to the hydrostatic (not osmotic) pressure difference between the inner and the outer side of the vesicle in a water environment without additives. Each pressure difference was created by sucking a vesicle into a micropipette at a given sucking pressure. The part of the membrane sucked into the micropipette (the projection length) was measured as a function of time. The time dependence can be divided into two intervals. We put forward the idea that smoothing of membrane defects, accompanied by an increase of the membrane area, takes place during the initial time interval, which results in a faster increase of the projection length. In the second time interval the volume of the vesicle decreases due to the permeability of its membrane and the increase of the projection length is slower. The hidden area and the water permeability of a typical lipid bilayer were estimated. The measured permeability, conjugated to the hydrostatic pressure difference, is an order of magnitude higher than the known value of the permeability, conjugated to the osmotic pressure difference. A hypothesis, based on pore formation, is proposed as an explanation of this experimental result.

Keywords Membranes · Permeability · Surface energy · Surface tension · Vesicles

Introduction

The basic function of a living cell biomembrane is its barrier function. However, a membrane functions not only as a barrier. It is also a medium of connection between the interior of the cell and the exterior environment (Alberts et al. 1989; Lipowsky and Sackmann 1995). This is effected via the permeability of the membrane (often anisotropic) for different molecules and ions. The membrane permeability is strongly affected by the presence of proteins, transporting various molecules across the membrane via different mechanisms such as facilitated diffusion, primary and secondary active transport, etc. (Alberts et al. 1989). The rapid exchange of molecules such as water across a cell membrane is essential, so that the intrinsic water permeability (so-called passive water permeability) of the membrane and the presence of water channel proteins (aquaporins) (Preston et al. 1992; Borgnia et al. 1999) are of great importance. Thus, the permeability is one of the factors determining the way in which a membrane functions.

According to the fluid mosaic model (Singer and Nicolson 1972), a biomembrane can be considered as a two-dimensional sea of lipid molecules, forming a lipid bilayer with integral proteins floating in it. Consequently, the barrier function of a biomembrane is determined, to a great extent, by the permeability of its lipid bilayer.

The passive water permeability of a lipid bilayer has been extensively investigated (Lawaczeck 1979; Finkelstein 1987; Gennis 1989; Paula et al. 1996; Huster et al. 1997; Smith and Gardiner 1999; Olbrich et al. 2000; Verkman 2000). Usually, the permeability is studied by creating an osmotic pressure difference of water molecules on both sides of the membrane. This pressure difference is induced by the presence of admixture molecules, dissolved in the water with different concentrations, to which the membrane is considered as impermeable (Paula et al. 1996). Recently, a modification of this method, involving micromanipulation of giant vesicles, was reported (Olbrich et al. 2000).

The present work is also devoted to the investigation of the passive water permeability of lipid bilayers. Contrary to the preceding studies, its aim is to measure the permeability related to the hydrostatic (not osmotic) pressure difference on both sides of the membrane. Our

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experiments consisted of sucking giant vesicles into micropipettes. The obtained results permitted us to determine the water permeability of a typical lipid bilayer due to the purely hydrostatic pressure difference created between its two sides and to estimate the hidden area of typical giant lipid vesicles.

Materials and methods

Liposomes were made from 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC, Avanti Polar Lipids, USA). The membrane was observed with an inverted Axiovert 100 microscope (Zeiss, Germany) in a fluorescent regime (a detailed description is given in Bivas et al. 2000). In order to make membranes visible under fluorescence, 1.5 mol% of a fluorescent dye [lyssamin rhodamine B, 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (rhodamine DHPE, L-1392) (Molecular Probes, USA)] was added together with the SOPC lipid. Giant vesicles formed spontaneously in excess water conditions (gentle hydration method: Reeves and Dowben 1969). In each sample, ~2 mg of SOPC lipid were dissolved in 1 mL of 2:1 (v/v) chloroform/methanol solvent. The organic solution was placed in a 30 mL flask and the solvent was removed under vacuum for about 5 h. Then, the lipid film was hydrated with 25 mL of deionized water and the sample was kept at room temperature for 72 h. Prior to micromanipulation, samples were taken out of the flask, taking care not to disturb the solution. The selected vesicles were unilamellar objects without any visible defects on the bilayer and with diameters in the range of 12.5–25 μm.

Let a membrane divide two solutions of a solute dissolved in one and the same solvent (water in our considerations). The hydrostatic pressure, the partial pressure of the solute, and the volume concentration of the solute on one side of the membrane (denoted "first") are P_1, π_1, and c_1, respectively. These quantities on the other side of the membrane (denoted "second") are P_2, π_2, and c_2, respectively. Evidently, $\pi_1 = RTc_1$ and $\pi_2 = RTc_2$, where R is the gas constant and T is the absolute temperature. We define Δc, ΔP, and $\Delta\pi$ via the relations $\Delta c = c_2 - c_1$, $\Delta P = P_2 - P_1$, and $\Delta\pi = \pi_2 - \pi_1$. Let J_0^v be the volume flow through the membrane from its "first" to its "second" side. As is shown in Appendix A:

$$J_0^v = -D_{\Delta P}\Delta P + D_{\Delta\pi}\Delta\pi \quad (1)$$

where $D_{\Delta P}$ and $D_{\Delta\pi}$ are the permeability coefficients related to the hydrostatic pressure difference and osmotic pressure difference, respectively. Let v_0 and j_0 be the molar volume and the molar flow of the solvent molecules through the membrane. As is shown in Appendix A, if $\Delta P = 0$ then:

$$j_0 = D_{\Delta c}\Delta c \quad (2)$$

where:

$$D_{\Delta c} = D_{\Delta\pi} v_0 RT \quad (3)$$

The values of $D_{\Delta c}$ are usually obtained in experiments on membrane permeability, including most of the works cited above. For one and the same membrane and solvent, this coefficient also depends on the kind of the solute. One of the aims of the present paper is to measure the permeability coefficient $D_{\Delta P}$ for a SOPC membrane.

The scheme used in the experimental set-up is shown in Fig. 1. In fact, this is the experimental set-up proposed for determination of the mechanical properties of lipid vesicle bilayers (Evans and Needham 1987; Evans and Rawicz 1990).

The experimentally created pressure difference of the water molecules on both sides of the membrane was of purely hydrostatic origin. Each pressure difference was created by sucking a vesicle into a micropipette at a given sucking pressure. The length of the cylindrical part of the membrane sucked into the micropipette (the projection length) was measured as a function of time.

Let a giant vesicle with a radius R_v be sucked in a (micro)pipette with a radius R_p, with $R_v > R_p$. The projection length is L. The hydrostatic pressures in the experimental cell, the micropipette, and inside the vesicle

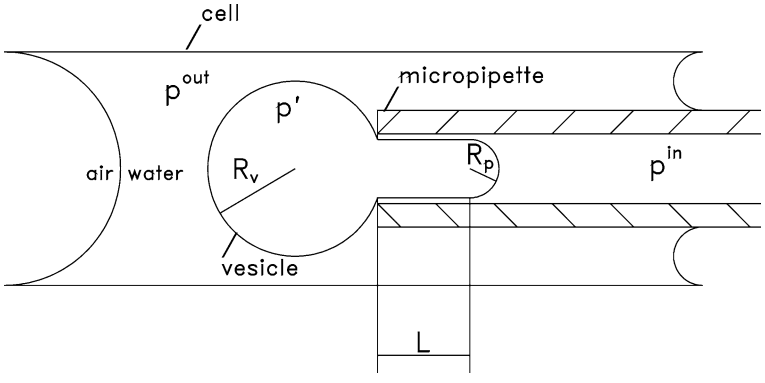

Fig. 1 Scheme of the experimental set-up for permeability measurements. A vesicle with a radius R_v is sucked into a micropipette with a radius $R_p < R_v$. The hydrostatic pressures inside and outside the micropipette and inside the vesicle are p^{in}, p^{out}, and p', respectively. The length of the membrane sucked into the micropipette is L. The permeability is determined from the dependence of L on the time t, keeping the pressure difference $\Delta p = p^{out} - p^{in}$ constant

are p^{out}, p^{in}, and p', respectively. The experimentally controlled quantity is the hydrostatic pressure difference: $\Delta p = p^{out} - p^{in}$. If we denote the tension of the vesicle membrane by σ, then, according to the Laplace law (Kwok and Evans 1981):

$$\sigma = \Delta p \frac{R_v R_p}{2(R_v - R_p)} \tag{4}$$

and:

$$\begin{aligned} p' - p^{out} &= \Delta p \frac{R_p}{R_v - R_p} \\ p' - p^{in} &= \Delta p \frac{R_v}{R_v - R_p} \end{aligned} \tag{5}$$

The stability of the system requires the inequality $p' > p^{out} > p^{in}$ to be fulfilled. When deducing Eq. (5), it was implicitly assumed that the membrane does not fluctuate. Let us show that the corrections due to fluctuations are negligible.

The area S_0 of the tension-free state of a patch of a bilayer is the area of the flat state of this patch with tension $\sigma = 0$ (Helfrich 1973). When the bilayer is deformed (stretched and/or bent) and is presented by its neutral surface, its energy of deformation is a simple sum of its bending and stretching energies (Seifert 1997). Then the tension σ of a symmetric bilayer with a spontaneous curvature equal to zero (we consider namely such bilayers) can be presented in the form (Bivas 2002):

$$\sigma = k_s \frac{S^{def} - S_0}{S_0} \tag{6}$$

where S^{def} is the area of the deformed patch and k_s is the stretching elasticity modulus of the membrane. If the membrane fluctuates, the quantity S^{def} must be substituted by its time average $\langle S^{def} \rangle$ in the above equation. Let S be the area of the average surface around which the membrane fluctuates. Both the parts of the aspired vesicle membrane out of and into the pipette fluctuate and their average surfaces are parts of spheres with radii R_v and R_p. For a fluctuating quasi-spherical vesicle with a radius R (this is the radius of a sphere with the same volume as the one for the vesicle), the following relation between $\langle S^{def} \rangle$ and $S = 4\pi R^2$ exists (Milner and Safran 1987):

$$\frac{\langle S^{def} \rangle - S}{S} = \frac{kT}{8\pi k_c} \sum_{n=2}^{n_{max}} \frac{2n+1}{(n-1)(n+2)[n(n+1) + \bar{\sigma}]} \tag{7}$$

where kT is the Botzmann factor, k_c is the bending elasticity of the membrane, $\bar{\sigma} = \sigma R^2 / k_c$ is the normalized value of the tension, $n_{max} \approx \sqrt{4\pi R^2 / s_0}$, and s_0 is the mean area per lipid molecule in the bilayer (Milner and Safran 1987). For a SOPC bilayer, $k_c \approx 10^{-12}$ erg, $k_s \approx 200$ erg/cm^2 (Evans and Rawicz 1990), and $s_0 = 61.4$ Å (Koenig et al. 1997). For $R \approx 5$ μm (of the order of the radii of the vesicle and the micropipette) the calculated value of n_{max} is 1.8×10^4.

In our experiments the applied tension σ was in the interval (0.015–0.4 dyn/cm), and therefore the condition $\sigma / k_s << 1$ was always fulfilled. Then, from Eq. (6) it follows that $\langle S^{def} \rangle \approx S_0$. The maximal numerical value of the right-hand side of Eq. (7), calculated for the minimal value of σ in the above-mentioned interval, is 6×10^{-5}. Consequently, $\langle S^{def} \rangle \approx S$. The above considerations lead to the conclusion that in the interval of the tensions used by us, the relation $\langle S^{def} \rangle \approx S \approx S_0$ is valid. In the calculations presented below we use the area S of the sphere (or the part of the sphere) around which the vesicle membrane fluctuates.

The membrane fluctuations will also affect the Laplace law, deduced for non-fluctuating membranes. For the fluctuating quasi-spherical vesicle introduced above we denote the normalized value of the hydrostatic pressure difference Δp inside and outside the vesicle with $\overline{\Delta p}$: $\overline{\Delta p} = \Delta p R^3 / k_c$. The classical Laplace law, used in the deduction of Eq. (4), is $\overline{\Delta p} = 2\bar{\sigma}$. We have calculated the modification of the Laplace law in the case of fluctuating vesicles. For vesicles with a symmetric bilayer it can be presented in the following form (Bivas 2000):

$$\overline{\Delta p} = 2(\bar{\sigma} - 1) \left\{ 1 + \frac{kT}{8\pi k_c} \sum_{n=2}^{n_{max}} \frac{2n+1}{(n-1)(n+2)[n(n+1) + \bar{\sigma}]} \right\} \tag{8}$$

The numerical estimations presented above show that the difference between the values of the tension, calculated with the help of the classical and corrected Laplace law, is less than 0.2%. This permits us to use the projection area of the vesicle membrane (it depends on the radii R_v and R_p) and to calculate the tension σ from Eq. (4).

Because of the non-zero water permeability of the vesicle membrane there will be a leakage of liquid from the interior of the vesicle to the experimental cell and the micropipette. As it is shown in Appendix B, the permeability coefficient $D_{\Delta P}$ is expressed by the quantities introduced above in the following way:

$$D_{\Delta P} = \frac{1}{\Delta p} \frac{(R_v - R_p)^2}{\left[4(R_v)^2 + 2R_v R_p - (R_p)^2 \right]} \left[\frac{dL}{dt} - \frac{1}{2\pi(R_v - R_p)} \frac{dS_0}{dt} \right] \tag{9}$$

Consequently, the permeability $D_{\Delta P}$ can be calculated if the derivatives d L/dt and d S_0/dt are known. On the other hand, the quantity d S_0/dt can be obtained if the permeability $D_{\Delta P}$ of the membrane is known and the derivative d L/dt is measured. Later on, we will apply these theoretical results to process our experimental data.

As d L/dt is the quantity participating in Eq. (9) for its determination, it is sufficient to measure the difference $L(t) - L(0)$. As is explained elsewhere (Bivas et al. 2000), this difference can be measured by registration of the image of the vesicle and the micropipette at time 0 and at time t. The second image (the one taken at time t) is translated until the two images of the hemispherical

parts of the membrane inside the micropipette overlap to their best, and then the number of pixels necessary for this translation is registered. The method permits us to determine $L(t)-L(0)$ with a precision of ~0.5 pixel. In our set-up, 1 pixel = 72 nm. This precision is considerably higher than the precision of the measurement of $L(t)$ itself, which is of the order of the wavelength of visible light, i.e. ~500 nm.

Results and discussion

For each of the studied vesicles the sucking pressure was kept constant with time. The pressure differences Δp were different for different vesicles, all in the range of 25–1040 dyn/cm^2. The length L of the membrane sucked in the micropipette (see Fig. 1) was measured as a function of the time t. For this purpose an image was captured every first or second minute for $t < 10$ min, and every fifth minute for $t > 10$ min. After appropriate image processing, the length $L(t)-L(0)$ was determined. Usually, at pressures less than 200 dyn/cm^2, the vesicles remain intact for a longer period of time (> 10 min). Two experimentally measured evolutions of $L(t)-L(0)$ for different values of Δp are shown in Fig. 2a ($\Delta p = 1040$ dyn/cm^2) and Fig. 2b ($\Delta p = 200$ dyn/cm^2).

The membrane permeability D can be determined if the number of molecules constituting the bilayer (or, equivalently, its area S_0 in a flat tension-free state), is kept constant, i.e. $dS_0/dt = 0$. As was noted before (Olbrich et al. 2000), the membrane contains a hidden area. This reservoir of hidden area possibly arises from invisible defects in the lipid bilayer, containing lipid molecules. The hidden area is different from the excess area, which is due to the membrane thermal fluctuations (Helfrich and Servuss 1984; Milner and Safran 1987). The main difference is that after increasing the tension of the membrane followed by a subsequent decrease, the excess area is restored, while the hidden area disappears irreversibly. To avoid the undesirable influence of the hidden area on the measurements, the vesicles can be prestressed (Olbrich et al. 2000) by subjecting them first to a suction pressure Δp, corresponding to a tension of ~0.5 dyn/cm. The time evolution of the length L at pressures creating tensions of this order is shown in Fig. 2a. After the second minute, L changes linearly with time, while in the time interval between the initial moment and the first minute the change of L is faster. A typical behaviour of L with the time t for low sucking pressure Δp, creating a low enough membrane tension σ, is presented in Fig. 2b (the vesicle is not prestressed). In this case the time dependence can be divided into two linear regions, the first one at $t < t_0$ and the second one at $t > t_0$. In the initial interval the derivative d L/dt has a higher value than its value in the second one.

The experimental results can be explained within the framework of the following model. For low enough pressures Δp, the predominant factor determining the increase of the length L in the initial time interval is the membrane smoothing (incorporation of molecules comprising the membrane defects into the observable surface of the vesicle), which is equivalent to an increase of S_0 (see Eq. 9). At the same time, the vesicle volume V decreases due to the membrane permeability, but this effect has less influence on L than the first one. At a time $t \approx t_0$, all the defects are exhausted as a source of matter and for $t > t_0$ only the decrease of V determines the time evolution of L. When the pressure difference is high enough, the value of t_0 decreases and is somewhere between the first and the second minute (see Fig. 2a). The permeability $D_{\Delta P}$ should be determined from the derivative d L/dt in the time interval $t > t_0$. The data satisfying these conditions were fitted with a straight line and its slope was considered to be equal to the derivative d L/dt. The mean relative error in the calculation of d L/dt was between 15% and 55%, the higher values referring to lower membrane tensions. Both the radii of the sucked

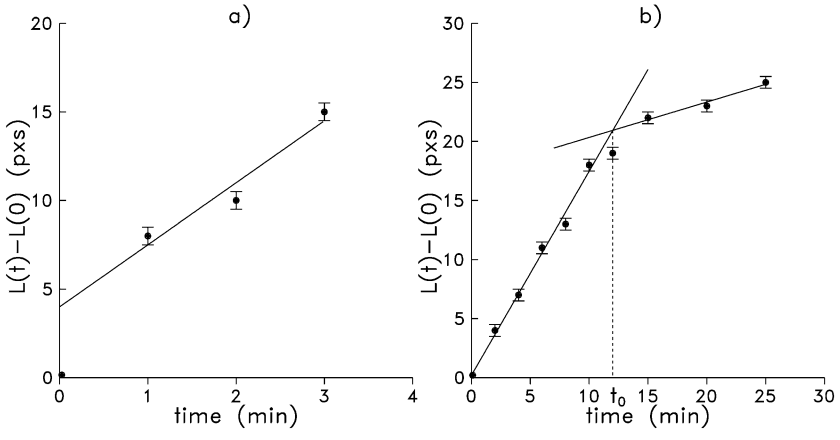

Fig. 2a, b Typical time dependence of the length $\Delta L = L(t)-L(0)$ (in pixels) of a membrane sucked up into a micropipette. 1 pixel = 72 nm. *Solid points*, experimental data; *lines*, linear fits. (**a**) The radii of the vesicle and the micropipette are ~9.3 μm and ~4.1 μm, respectively, and $\Delta p = 1040$ dyn/cm^2. Except for the first data point ($t = 0$), the experimental data are fitted well with a straight line. (**b**) The radii of the vesicle and the micropipette are ~6.3 μm and ~3.5 μm, respectively, and $\Delta p = 200$ dyn/cm^2. The experimental data in both the initial and the second time intervals are fitted well with straight lines with different slopes; t_0 is the value of the abscissa at the cross-point of the two lines

vesicle and the micropipette were measured and the quantities σ and $D_{\Delta P}$ were calculated from Eq. (4) and Eq. (9). The tensions σ were in the range 0.018–0.315 dyn/cm. The experimental error for each radius was assumed to be 0.5 μm (of the order of the wavelength of visible light). The mean relative errors in the calculation of σ and the factor $(R_v-R_p)^2/[4(R_v)^2+2R_vR_p-(R_p)^2]$ from Eq. (9) were estimated to be 15%, originating from the uncertainty of R_v and R_p.

The permeabilities of the SOPC membrane, determined by us for different values of the tension σ of the membrane, are presented in Fig. 3, together with their errors. They are in the range of $(1.4–3.5)\times10^{-11}$ cm^3/(dyn s).

The mean water permeability of SOPC membranes, determined from our experimental data under the assumption that it does not depend on the tension σ, is $D_{\Delta P}=(2.6\pm0.26)\times10^{-11}$ cm^3/(dyn s). Its value is about 2.5 times higher than that of $D_{\Delta\pi}$, calculated by means of Eq. (3) from the $D_{\Delta c}$ reported by Paula et al. (1996), and about 12 times higher than $D_{\Delta\pi}$ calculated from the results for $D_{\Delta c}$ of Olbrich et al. (2000). Both works refer to SOPC membranes with an osmotic pressure difference created by a gradient of a sugar solute concentration in the solvent on the two sides of the membrane. Strictly speaking, the influence of the hydrostatic pressure difference ΔP in the experiment of Paula et al. (1996) cannot be neglected and only a combination of the quantities $D_{\Delta P}$ and $D_{\Delta\pi}$ can be calculated from their data.

Our experimental results show an increase (although statistically insignificant) of the permeability with an increase of the membrane tension σ. A theoretical explanation of such dependence could be given on the basis of hydrophilic pore formation in the membrane and an increase of the pore number and mean radius with the increase of the membrane tension. Pore formation could also explain the high value of the permeability $D_{\Delta P}$ compared to $D_{\Delta\pi}$. When a hydrostatic pressure difference is applied on the two sides of the membrane, there is a hydrodynamic flow through the pore. The magnitude of the flow depends on the pore radius. Consequently, $D_{\Delta P}$ depends on the dimensions of the pores in the membrane as well as on their density per unit area of the membrane. Evidently, pores also exist in membranes embedded in water with additives (sugars in particular). When $\Delta P=0$ and $\Delta\pi\neq0$, there is only a diffuse flow of the additive through the pore, compensated by a water flow in the opposite direction, providing that there is no change of the volumes on both sides of the membrane. The diffuse water flow through the pore for $\Delta P=0$ and $\Delta\pi=C$ is expected to be much weaker than the hydrodynamic one for $\Delta P=C$ and $\Delta\pi=0$. Consequently, the presence of pores should increase the value of $D_{\Delta\pi}$ much less than that of $D_{\Delta P}$. This problem deserves a separate theoretical investigation.

The dependence of the length L on the time t in the initial time interval was fitted with a straight line. The time t_0 was determined as the abscissa value of the line cross-point, fitting the experimental data in the initial and the second time interval. The experimentally determined values of t_0 as a function of σ are presented in Fig. 4. Evidently, this quantity depends on the tension σ of the membrane.

The derivative d L/dt for $t<t_0$ was assumed to be equal to the slope of the fitting line in this time interval. Using the membrane permeability $D_{\Delta P}$, determined by us, we calculated the slope d S_0/dt for each of the studied vesicles (Vitkova et al. 2002). Let $\Delta S_0/S_0$ be the relative

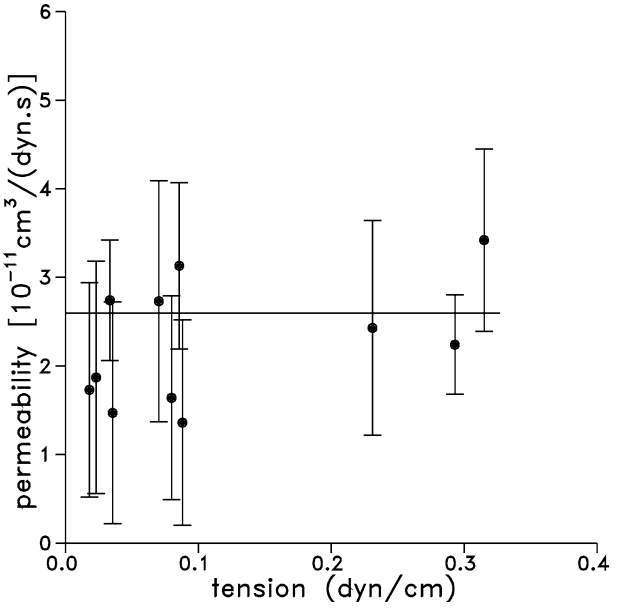

Fig. 3 Measured SOPC bilayer water permeability. *Solid points*, experimental data and the corresponding precision; *lines*, the weighted mean value of all the measurements

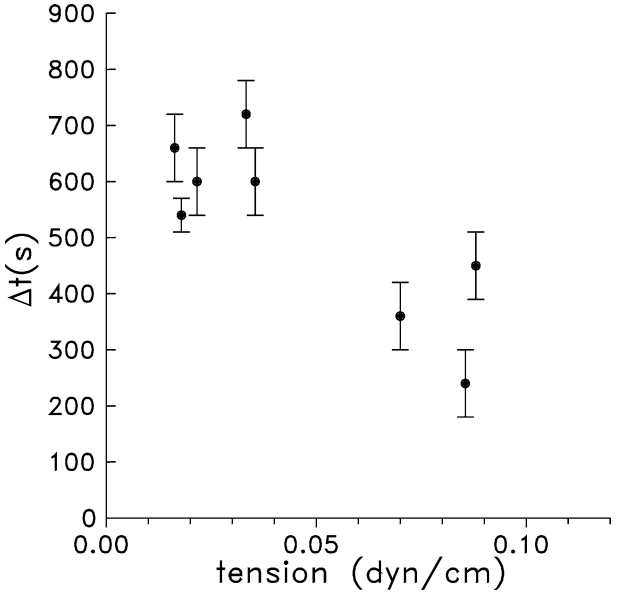

Fig. 4 Dependence of the time t_0, necessary for smoothing of an SOPC membrane, on the membrane tension σ, together with the experimental errors

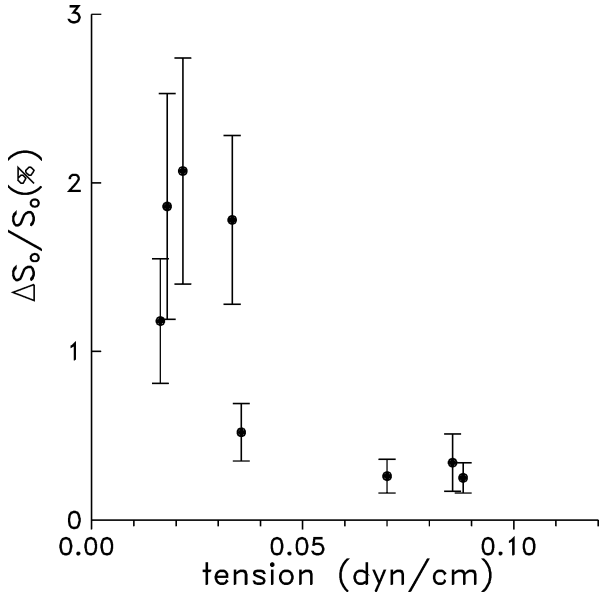

Fig. 5 Dependence of the relative area increase $\Delta S_0/S_0$ (in %), after smoothing of a SOPC membrane, on the membrane tension σ, together with the experimental errors

part of the hidden area of the vesicle membrane, which passes into the visible area during the suction. This quantity can be expressed via t_0 and (d S_0/dt)/S_0 as follows:

$$\frac{\Delta S_0}{S_0} = \frac{1}{S_0}\frac{\mathrm{d}S_0}{\mathrm{d}t}t_0 \tag{10}$$

The values of $\Delta S_0/S_0$ calculated from our experimental data according to the above equation are presented in Fig. 5 (for convenience, the data for $\Delta S_0/S_0$ in the figure are given as a percentage). The dependence of this quantity on the tension σ is a consequence of the fact that some part of the hidden area disappears during the pressure increase after the initial sucking of the vesicle into the micropipette, due to the non-zero time necessary to increase the pressure to the desired value. The error bars of the two quantities account for the errors in the measurement of the dimensions R_v, R_p, and L and the finite length of the time interval between two readings of L. The errors due to the scattering of the time for attaining the stationary value of the tension σ (between 30 s and 40 s in our experiment), and the inherent scattering of the hidden area in the ensemble of vesicles in the suspension, are not included in the error bars in Figs. 4 and 5. The vesicles prepared by spontaneous swelling (see Materials and methods) have a tension practically equal to zero. Consequently, the area hidden in them can be obtained by extrapolating our measurements to $\sigma = 0$. Our data show that the expected value of $\Delta S_0(0)/S_0$ is about 2%. We present here the first experimental proof for the existence of this quantity, introduced by Olbrich et al. (2000), and its first numerical estimation.

In all considerations so far it was assumed that the water phase does not contain admixtures. Evidently, this is an idealization of the real situation. It is important to consider the possible influence of the admixtures in the water environment on the experimental results.

In order to accomplish the micromanipulation, the experimental cell shown in Fig. 1 is opened on one side, and there is evaporation of water from it. As a result, the concentration of the admixtures in the cell outside the vesicle increases with the time. It is accompanied by the appearance of an osmotic pressure difference $\Delta\pi^{adm}$ between the outside and inside of the vesicle, creating an additional water flow j^{adm} from the vesicle interior to the cell. The maximal observation time of the vesicle in our experiments was about 30 min for the lower hydrostatic pressure differences Δp and it decreased to 4–5 min for the higher ones. This time was determined by the lifetime of the vesicle sucked into the micropipette. It can be assumed that the rate of evaporation is constant. Then the osmotic pressure difference $\Delta\pi^{adm}(t)$ and the flow j^{adm} related to it are linear functions of the time t, measured from the moment of filling the experimental cell with the vesicle suspension. If j^{adm} were comparable with the flows due to the applied hydrostatic pressure difference Δp, the function $L(t)$ would be a polynomial of second degree. We tried to fit the measured dependencies $L(t)$, containing four or more points, by such a polynomial, both in the case in which the effective area of the vesicle increased and the one in which the changes of L were due to the squeezing of water through the membrane. All the obtained values of the polynomial coefficient, multiplying the second order of t, were statistically insignificant, with errors greater than the values themselves. Consequently, in the range of the pressure differences Δp used by us, the effect of the water evaporation from the experimental cell was lower than the experimental errors and had no influence on the final results. There is one more argument supporting this conclusion. If the effect of the evaporation is not negligible, it must decrease when the tension σ increases, because for higher tensions Δp increases, while $\Delta\pi^{adm}$ decreases due to the decrease of the lifetime of the vesicle (equal to the time of water evaporation). Then the uncorrected values of the permeability $D_{\Delta P}$ must be higher for the lower tensions σ. As was mentioned above, the linear fit of the data in Fig. 3 showed a statistically insignificant increase (and not decrease) of $D_{\Delta P}$ with the increase of σ.

On the basis of the data and the considerations presented above, we conclude that our experimental procedure allowed us to measure directly the water permeability of the SOPC membrane in pure water. In addition, we were able to estimate the hidden area of the vesicle membrane, due to the accumulation of lipid in membrane defects.

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Appendix

Appendix A: thermodynamics and definition of the passive permeability

Let us consider the system described in Materials and methods and consisting of a membrane dividing two solutions with different solute concentrations. In addition to the quantities, defined in Materials and methods, we introduce the following ones. The chemical potentials of the solvent and the solute on the first side of the membrane are μ_0^1 and μ_1^1, respectively, and μ_0^2 and μ_1^2 are the same potentials on the second side of the membrane. The molar volume of the solute is denoted by v_1. The quantity c is defined by the relation $c=(c_1+c_2)/2$. When the condition $\Delta c << c$ is fulfilled, the chemical potential differences $\Delta\mu_0=\mu_0^2-\mu_0^1$ and $\Delta\mu_1=\mu_1^2-\mu_1^1$ can be expressed by means of the quantities introduced above and in Materials and methods as follows (Kedem and Katchalsky 1963):

$$\begin{aligned}\Delta\mu_0 &= (\Delta P-\Delta\pi)v_0\\ \Delta\mu_1 &= (\Delta P-\Delta\pi)v_1+\tfrac{1}{c}\Delta\pi\end{aligned} \tag{A1}$$

Let j_0 be the molar flow of solvent molecules and j_1 be the molar flow of solute molecules through the membrane. The volume flow J_0^v through the membrane (the volume flow of both the solvent and the solute) is $J_0^v=j_0v_0+j_1v_1$.

Expressing $\Delta\mu_0$ and $\Delta\mu_1$ via ΔP and $\Delta\pi$, and J_0^v and j_1 as linear combinations of ΔP and $\Delta\pi$, one obtains (Kedem and Katchalsky 1963):

$$\begin{aligned}J_0^v &= -\left[L_{11}(\Delta P-\Delta\pi)+L_{12}\tfrac{\Delta\pi}{c}\right]\\ j_1 &= -\left[L_{21}(\Delta P-\Delta\pi)+L_{22}\tfrac{\Delta\pi}{c}\right]\end{aligned} \tag{A2}$$

where $L_{12}=L_{21}$ according to Onsager's relations.

When $\Delta\pi=0$ (i.e. $\Delta c=0$), the flow j_1 must be proportional to c and ΔP, $j_1\propto c\Delta P$. This relation imposes the following dependence of L_{12} on c: $L_{12}=L_{21}=cL^0$. When $\Delta P=0$, j_1 must depend linearly on c_1 and c_2, and consequently $L_{22}=cL_{22}^0$. Keeping the first powers with respect to c_1 and c_2, we obtain the well-known practical Kedem–Katchalsky equations in the following form:

$$\begin{aligned}J_0^v &= -\left[L_{11}(\Delta P-\Delta\pi)+L^0\Delta\pi\right]\\ j_1 &= -\left(cL^0\Delta P+L_{22}^0\Delta\pi\right)\end{aligned} \tag{A3}$$

Denoting $D_{\Delta P}=L_{11}$ and $D_{\Delta\pi}=L_{11}=L_0$, from the first of Eq. (A3) we obtain Eq. (1).

It is usually considered in the scientific literature that the volume flow through the membrane is created by the partial pressure difference $\Delta\pi$ of the solute, which is due to the solute concentration difference Δc between the two sides of the membrane. This approach implicitly assumes that $\Delta P=0$. Hence:

$$J_0^v=\left(L_{11}-L^0\right)\Delta\pi=D_{\Delta\pi}\Delta\pi \tag{A4}$$

For low enough c, $j_0\approx J_0^v/v_0$. Taking into account the relation between Δc and $\Delta\pi$, we obtain:

$$j_0=D_{\Delta\pi}v_0RT\Delta c \tag{A5}$$

The permeability $D_{\Delta c}$ is frequently defined as:

$$j_0=D_{\Delta c}\Delta c \tag{A6}$$

Substituting Eq. (A6) in Eq. (A5), Eq. (3) is derived.

Appendix B: deduction of Eq. (9)

Let S and V be the area and the volume of the vesicle sucked into the micropipette. They are expressed by the radii R_v and R_p of the vesicle and of the micropipette in the following way:

$$S=2\pi(R_v)^2\left[1+\sqrt{1-\frac{(R_p)^2}{(R_v)^2}}\right]+2\pi R_pL+2\pi(R_p)^2 \tag{B1}$$

and:

$$V=\frac{2}{3}\pi(R_v)^3+\frac{2}{3}\pi(R_v)^2(R_v+R_p)\sqrt{1-\frac{(R_p)^2}{(R_v)^2}}+\pi(R_p)^2L+\frac{2}{3}\pi(R_p)^3 \tag{B2}$$

Differentiating the above expressions we obtain:

$$dS=4\pi R_v\left[1+\sqrt{1-\frac{(R_p)^2}{(R_v)^2}}-\frac{1}{2}\frac{(R_p)^2}{(R_v)^2}\frac{1}{\sqrt{1-\frac{(R_p)^2}{(R_v)^2}}}\right]dR_v+2\pi R_p\,dL \tag{B3}$$

and:

$$dV=2\pi(R_v)^2\left\{1+\left[1+\frac{1}{6}\frac{(R_p)^2}{(R_v)^2}\right]\sqrt{1-\frac{(R_p)^2}{(R_v)^2}}-\frac{\frac{1}{3}\frac{(R_p)^2}{(R_v)^2}\left[1+\frac{1}{2}\frac{(R_p)^2}{(R_v)^2}\right]}{\sqrt{1-\frac{(R_p)^2}{(R_v)^2}}}\right\}dR_v+\pi(R_p)^2dL \tag{B4}$$

The differential d R_v can be eliminated from the above two equations and the result is:

$$\frac{dS-2\pi R_p\,dL}{dV-\pi(R_p)^2dL}=\frac{1}{R_v}\phi\left(\frac{R_p}{R_v}\right) \tag{B5}$$

where the function $\phi\left(R_p/R_v\right)$ is defined by the expression:

$$\phi\left(\frac{R_p}{R_v}\right)=\frac{1+\sqrt{1-\frac{\left(R_p\right)^2}{\left(R_v\right)^2}}-\frac{1}{2}\frac{\left(R_p\right)^2}{\left(R_v\right)^2}\frac{1}{\sqrt{1-\frac{\left(R_p\right)^2}{\left(R_v\right)^2}}}}{\frac{1}{2}+\frac{1}{2}\left[1+\frac{1}{6}\frac{\left(R_p\right)^2}{\left(R_v\right)^2}\right]\sqrt{1-\frac{\left(R_p\right)^2}{\left(R_v\right)^2}}-\frac{1}{6}\frac{\left(R_p\right)^2}{\left(R_v\right)^2}\left[1+\frac{1}{2}\frac{\left(R_p\right)^2}{\left(R_v\right)^2}\right]\frac{1}{\sqrt{1-\frac{\left(R_p\right)^2}{\left(R_v\right)^2}}}} \tag{B6}$$

The Taylor series of the function $\phi(x)$ is:

$$\phi(x)=2-\frac{1}{3}x^2+\frac{1}{18}x^4+O\left(x^6\right) \tag{B7}$$

From Eqs. (B5) and (B6) the following relation between the time derivatives $\partial L/\partial t$, $\partial S/\partial t$, and $\partial V/\partial t$ is deduced:

$$\frac{\partial L}{\partial t}=\frac{1}{2\pi R_p\left[1-\frac{1}{2}\frac{R_p}{R_v}\phi\left(\frac{R_p}{R_v}\right)\right]}\frac{\partial S}{\partial t}-\frac{\phi\left(\frac{R_p}{R_v}\right)}{2\pi R_v R_p\left[1-\frac{1}{2}\frac{R_p}{R_v}\phi\left(\frac{R_p}{R_v}\right)\right]}\frac{\partial V}{\partial t} \tag{B8}$$

The time derivative $\partial V/\partial t$ is related to the volume flow through the vesicle membrane. We denote with $j^{out}=D_{\Delta P}(p'-p^{out})$ the flow from the interior of the vesicle to the experimental cell through that part of the membrane which is out of the micropipette, and with S^{out} the area of the same part of the membrane. Let $j^{in}=D_{\Delta P}(p'-p^{in})$ be the volume flow from the vesicle to the micropipette through that part of the membrane inside the micropipette, which is not in contact with the internal surface of the micropipette, and let S^{in} be the area of that part of the membrane. Let $Q^{out}=j^{out}S^{out}$ and $Q^{in}=j^{in}S^{in}$ be the respective total volume flows. We will show first that the total volume flow through the cylindrical part of the membrane, which is in close contact with the micropipette, is negligible. For this aim, we will assume that the water layer between the membrane and the micropipette has a constant thickness d, determined by the interactions between the membrane and the inner surface of the micropipette. The film is enclosed between two cylindrical surfaces with radii much larger than the film thickness and we will consider it as flat in the calculations. Let x be the distance between a point in the water layer and the plane perpendicular to the axis of the micropipette and situated at its end (the distance L in Fig. 1 is measured exactly towards this plane). Evidently, $0 \le x \le L$. Let $p(x)$ be the distribution of the hydrostatic pressure in the water film. Let $q(x)$ be the volume flow of the water at a distance x per unit length of the perimeter of the cylindrical part of the membrane (the flow is parallel to the axis of the micropipette). Let $Q^{cyl}(x)=2\pi R_p q(x)$ be the total volume flow of the water in the film at this distance x. To facilitate the calculations, first we consider the case when all the flow is directed to the interior of the micropipette. From the laminar viscous flow of a liquid between two planes it is known that the relation between $q(x)$ and $p(x)$ is:

$$q(x)=-\frac{d^3}{12\eta}\frac{\partial p(x)}{\partial x} \tag{B9}$$

On the other side, the change d $q(x)$ is related to the permeability of the membrane:

$$\mathrm{d}[q(x)]=D_{\Delta P}[p'-p(x)]\mathrm{d}x \tag{B10}$$

where p' is the hydrostatic pressure inside the vesicle. From Eqs. (B9) and (B10) the following differential equation is obtained for $p(x)$:

$$\frac{\partial^2 p(x)}{\partial x^2}-\frac{12\eta D_{\Delta P}}{d^3}[p(x)-p']=0 \tag{B11}$$

The boundary conditions of the above equation are: $p(L)=p^{in}$, and $[\partial p(x)/\partial x]_{x=0}=0$. The former is evident, and the latter is a consequence of the fact that $q(0)=0$. We denote with α the length: $\alpha=\sqrt{d^3/(12\eta D_{\Delta P})}$. Then the solution of the differential equation of Eq. (B11) satisfying these boundary conditions is:

$$p(x)=p'-\frac{\exp(-x/\alpha)+\exp(x/\alpha)}{\exp(-L/\alpha)+\exp(L/\alpha)}\left(p'-p^{in}\right) \tag{B12}$$

From this solution it follows that:

$$-[\partial p(x)/\partial x]_{x=L}=\tanh(L/\alpha)\left(p'-p^{in}\right)/\alpha<\left(p'-p^{in}\right)/\alpha \tag{B13}$$

Evidently, $Q^{cyl}(L)=2\pi R_p q(L)$ is the total volume flow through the cylindrical part of the membrane. From Eqs. (B9) and (B13) it follows that:

$$Q^{cyl}(L)<\frac{d^3}{12\eta D_{\Delta P}\alpha R_p}2\pi\left(R_p\right)^2\left(p'-p^{in}\right)=\frac{\alpha}{R_p}Q^{in} \tag{B14}$$

From the expressions for j^{out} and j^{in} and from Eqs. (5) it follows that $Q^{out}/Q^{in}\approx 2R_v/R_p$. Then the relative part of Q^{cyl} (with respect to the total volume flow through the parts of the membrane not in contact with the micropipette $Q^{out}+Q^{in}$) is:

$$\frac{Q^{cyl}(L)}{Q^{out}+Q^{in}}<\frac{\alpha}{2R_v+R_p} \tag{B15}$$

The analysis of our experimental data for the permeability due to the hydrostatic pressure difference gave

the value $D_{\Delta P} \approx 3\times10^{-11}\mathrm{cm}^3/(\mathrm{dyn\ s})$. Substituting $\eta = 1$ cP (the viscosity of the water in the film is assumed the same as that of bulk water) and $d = 24$ Å [this is the thickness of the water layer in the lamellar L_α phase in the system SOPC–water (Rand and Parsegian 1989)], we obtain $\alpha = 0.62$ μm. The radii of the vesicles and the pipettes used in our experiments satisfy the inequalities $R_v \geq 6.3$ μm and $R_p \geq 3.5$ μm. Using these minimal values, we obtain that $Q^{cyl}(L)/(Q^{out} + Q^{in}) < 0.05$. In the same way it can be shown that in the case when the total volume flow through the cylindrical part of the membrane is directed to the interior of the experimental cell, it obeys an inequality of the kind in Eq. (B15), but with right-hand side multiplied by R_p/R_v. The real volume flow through the cylindrical part of the membrane cannot exceed the sum of these two particular cases. Consequently, it is less than 8% of the total volume flow through the whole membrane of the vesicle, considerably less than the experimental precision of the measurements. Later on, this contribution will be neglected. Then the time derivative $\partial V/\partial t$ can be written as:

$$\frac{\partial V}{\partial t} = -D_{\Delta P}\left[S^{out}\left(p' - p^{out}\right) + S^{in}\left(p' - p^{in}\right)\right] \tag{B16}$$

where p^{out}, p', and p^{in} are the hydrostatic pressures in the experimental cell, inside the vesicle, and inside the micropipette. Evidently:

$$S^{out} = 2\pi(R_v)^2\left[1 + \sqrt{1 - \frac{(R_p)^2}{(R_v)^2}}\right] \tag{B17}$$

and:

$$S^{in} = 2\pi(R_p)^2 \tag{B18}$$

The pressure differences $(p'-p^{out})$ and $(p'-p^{in})$ are determined by Eqs. (5).

The permeability $D_{\Delta P}$ can be expressed from Eqs. (5), (B8), (B16), (B17), and (B18) as a function of R_p, R_v, d L/dt, and d S/dt. Taking into account that $\phi(R_p/R_v) \approx 2$ (see Eq. B7; this approximation has been used by Olbrich et al. 2000) and replacing $\sqrt{1 - (R_p)^2/(R_v)^2}$ with $\left[1 - (R_p)^2/2(R_v)^2\right]$, we obtain Eq. (9). The error due to these two approximations is of the order of $1/6 \times (R_p)^2/(R_v)^2 \approx 0.02$. It is negligible in comparison to the experimental errors of the measurements.

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