ELSEVIER

Biophysical Chemistry 65 (1997) 101–108

Biophysical Chemistry

A theory on the instability of tubular membranes to the periodic conformation by laser tweezers

Yukio Suezaki *

Physics Laboratory, Department of General Education, Saga Medical School, Saga 849 Japan

Received 30 April 1996; revised 17 August 1996; accepted 1 November 1996

Abstract

A mechanical theory to analyze the stability of tubular membranes perturbed by optical tweezers is proposed. I assume that the optical tweezers cause the temporal elevation of hydrostatic pressure inside tubular membranes due to the thermal expansion of solvent water, and I relaxed the conservation of volume per unit length which was strictly maintained in old theories based on the well-known Rayleigh instability. The mechanical energy composed of bending rigidity, interfacial tension, and hydrostatic pressure terms can explain the condition of the previously observed peristaltic and pearling instability. The spontaneous curvature of the membrane is postulated in the theory. The enhanced pressure causes the tubules to enter a peristaltic state with a spinodal line in the phase diagram. © 1997 Elsevier Science B.V.

Keywords: Tubular membrane; Bending moduli; Peristaltic state; Pearling instability

1. Introduction

Recently tubular membranes have been formed under the induced flow of lipid solutions [1]. Tubular radii are stable after ceasing the flow with the values from 0.3 to 5 μm depending upon the initial preparations. In a previous report, the authors applied optical tweezers to the tubules and obtained peristaltic states with periodic structures similar to Rayleigh's instability [2]. When the power of laser tweezers is large, the peristaltic structure deforms into the shape resembling a necklace of pearls; when the tweezers are turned off, the original tubules are eventually recovered. The authors tried to analyze the observed structures by employing the bending elasticity term with zero spontaneous curvature and the interfacial tension term. Several authors also analyzed the dynamic aspect of the instability by an extension of Rayleigh's instability where the volume is conserved upon deformation [3–6]. In this report, the author will analyze the static aspect of the same problem in a similar way by explicitly including the hydrostatic pressure term.

In the following paragraphs, the experimental situation is carefully reviewed to develop the physical insight into the phenomenon. Tubular membranes are formed under an induced flow of the solvent and the tubules are anchored at both ends by massive lipid globules. The tubules are stable for hours experimentally after stopping the flow. As we are not con-

* Tel.: +81-952-342194. Fax: +81-952-342022. E-mail: suezaki@post.saga-med.ac.jp.

PII S0301-4622(96)02255-7

cerned with the absolute thermal equilibrium of lipid membranes, but rather are going to study the thermodynamically metastable tubular membranes, the spontaneous curvature can exist in this case [7,8]. Now, I will explain the assumption of the spontaneous curvature because it is crucial in my theory. Helfrich first showed the possible existence of the spontaneous curvature of the bilayer when the deformation goes on faster than the exchange of lipids between the two constituent monolayers [7]. By putting the bending elastic energy to zero and assuming the non-zero spontaneous curvature, Deuling and Helfrich showed that the tubules can possess the shape of the strings of beads, the shape of which is similar to that we concern [8]. I will mention further on this point in the last section.

As is well known, an hour or more is needed for the flip–flop exchange of lipids between both monolayers of the bilayer membrane. On the other hand, the time scale of the observation of the tubular system after the laser tweezing is of the order of 10 min or less. Thus, let us suppose the following story on the formation of tubules; the initial drug by induced flow might have caused a non-spherical lipid globule to be prolonged by developing the tubule. The driving force of the drug could be a difference of Stokes' viscous forces of the different part of a single massive globule. Among many candidates of the lipid globules, limited number satisfy the growing condition with a spontaneous curvature that grow into the tubules with massive globules at both ends. Anyway, the time scale is much faster than the time to attain the thermal equilibrium. In this sense, it is reasonable to represent the initial condition of the system by the assumption of the spontaneous curvature.

The assumption of the spontaneous curvature that depends on the initial condition could explain the variety of the distribution of the initial radius of tubules from 0.3 to 5 μm. The further deformation of the tubule by the laser irradiation cannot attain the thermal equilibrium of lipid molecules because the bilayer integrity is not destroyed and the time of irradiation is short (flip–flop exchange of molecules does not occur). The enclosed initial volume of water inside tubules also determines the size of tubules that would be correlated with the spontaneous curvature. The partition of the water volume into the tubule and the into the massive globules should occur at the formation of the total system after stopping the induced flow. Thus, the radius should not be taken as an initial value but is determined by the mechanical balance. Because we do not possess enough and quantitatively reliable information on the lipid globules, I stop my expectation at this point. As far as we have no physically quantitative information on the formation of the tubules with globules, it is necessary to assume a reasonable model system as a first step. If the initial radii are determined only from the bending modulus and the interfacial tension, the radii would be unconditionally determined because both the bending modulus and the interfacial tension are material constants that do not change by the initial condition of the preparation.

The application of optical tweezers to tubular membranes results in a small but finite amplitude peristaltic state. Stronger application of the tweezers leads to the pearling state that is similar to pearls on a string. In the theoretical analyses [1,3–6], the conservation of volume in the tubule was strictly postulated during the deformation based on the analogy of the Rayleigh instability. For closed tubules, for instance, the conservation of the interior volume should be strictly maintained [9], however, the tubular system about which are concerned is different from the closed system; instead, there exist reservoirs of lipid globules of the aqueous solvent and membrane molecules at both ends of the tubules. Even in the closed tubules, the periodic deformation can take places by changing the total length of the tubule. Thus, conservation of the volume per unit length is not necessarily based on the physical and geometrical consideration. The initial radius (R) has been given arbitrary by previous authors, however, the radius should be determined to minimize the mechanical energy because the straight tubules are stable initially for 1 h. Our case is different from the dynamical jet flow from the orifice in which it makes sense to define the initial radius of the jet depending on the initial condition.

Next, previous workers postulated that the energy of the laser tweezers generates a sucking interfacial tension to cause the deformation [3–6]. Off course, the electric field does affect the membrane shape and movement [10,11]. However, there is no reliable physical reason to propagate the locally enhanced

interfacial tension of the liquid membrane by laser tweezers to the whole tubules, which fact has been expected and postulated by previous authors. Such a tension can be maintained only in a very short time scale (within order of ms) as the workers realized, while the pearling state remains stable at least more than several 10 s. As far as we postulate massive globules of lipids as the reservoir of lipids, the interfacial tension of the tubular membrane should recover the initial value even if the laser tweezers enhance the tension locally in a short time scale. Mechanically, the localized area of the enhanced interfacial tension of the tubule (focused region of the applied laser tweezers defines the area) might suck lipids to form the lipid lens with the Plateau–Gibbs border. Eventually, the mechanical balance between the oil lens (lipid lens) and the tubule would be settled in a moment with recovering the initial interfacial tension of the tubule at the outside of the laser spot. Thus, there is no physically reliable driving force to enhance the interfacial tension outside the laser spot for a time scale of the experiment [1]. Thus, I think that the analysis of non-linear dynamics without sound physical background does not make sense.

Lastly, the previous authors mainly concern the dynamical aspect of the propagation of beads of the pearling state. In many cases, however, the transition from the tubules to periodical state occurs suddenly in many periods of beads around the laser spot, and the propagation of beads begins in a visible speed. We can easily show that the time to settle the stationary state of the thermal conduction after the onset of the laser tweezers is very quick, the value of which is of the order of several 10 ms. This is much faster than the velocity of beads visually seen by videotape.

In the following section, the effect of the local heating up of the interior of the tubules by laser tweezers will be considered and will be analyzed theoretically.

2. Rayleigh instability caused by heating up of the tubules

Instead of considering the effect of the energy of laser tweezers only on the membrane, I simply assume that the optical energy enhances the temperature of the interior of the tubule and eventually enhances the hydrostatic pressure inside the membrane compared with that outside the tubule. In reality, there is no reliable measurement of the enhancement of the local temperature by laser tweezers. Bar-Ziv et al. estimated the approximate maximum enhanced temperature, ΔT_0, to be 0.5°C at the tweezing point of the membrane [12]. From the equation of the stationary thermal conduction, the enhanced temperature $\Delta T(r)$ at the distance r from the tweezing point becomes [12]

$$\Delta T(r) = \frac{I}{4\pi\lambda r} \tag{1}$$

where I and λ are the absorbed laser power at the tweezing point and the thermal conductivity of water ($\lambda = 0.56\ \mathrm{W^{-1}\ K^{-1}}$), respectively. We can easily show by elementary calculus that the thermal expansion inside the tubule is larger than that of whole spherical regions centered from the tweezing point even if the lipid globules are attached at both ends of the tubule when the volume of the globules is not too large. For instance, by a simple numerical calculation, the relative volume expansion of the tubules (with the lipid globules at both ends of the tubules) is shown to be larger than that of surrounding water region when the volume of the globules is less than that of the tubule by approximately less than 10 times. The above estimation was made by the assumptions that the length of the tubule is 200 μm, the radius of the tubules is 1 μm, the region of the localized laser spot spreads over the spherical region of the radius of the tubule, and the laser spot is located at the point with same lengths from both globules. From this estimation, we propose the following experimental check to assure the validity of my theory of the effect of the thermal expansion. The pearling instability may not occur if the volume of the globules at both ends of the tubule is larger than around ten times of the volume of the tubule.

The expected enhancement of the pressure inside the tubule Δp is estimated to be of the order of 0.1 Pa or more by assuming the thermal expansion constant ($4 \times 10^{-5}\ \mathrm{K^{-1}}$) and the bulk modulus ($2 \times 10^{9}$ Pa) of water at 40°C, which is of the same order of magnitude of the bending modulus divided by the

third power of the observed radius of tubules. In the above estimation, I used the value of the maximum enhancement of temperature to be 0.5°C at the laser spot [12]. As I stated, the estimated pressure is dependent on the geometry of the tubule and the globules. Then, the absorbed power I in Eq. (1) is estimated to be of the order of 0.001 mW, which might not be an over estimate if we consider the applied laser power of 50 mW [1]: the formation of the semi-macroscopic oil lens at the laser spot can possess the size of sub-microns (not of the bilayer thickness) which could enhance the absorbing capacity of the laser light energy than for the thin molecular membrane. The absorption by the aqueous solvent might not work in our case as the visible light is extremely transparent compared with lipids. Additionally, the permeation of inside water to the outside by this small pressure difference can be shown to be neglected if we assume the observed permeability of water through the membrane is of the order of 0.7 μm per s [13]. The changes of the osmotic pressure do cause the permeation of water [13,14] and result in the shape change, however, the magnitude of the pressure difference is very high compared with those in our case.

Previously, in closed membrane systems the hydrostatic pressure and the interfacial tension were treated as mathematical Lagrange's multipliers [15–17]. However, in the membrane system that is open to the lipid globules, I regard them as realistic physical parameters. In the following, we will analyze the mechanical stability of tubules with spontaneous curvature of the bilayer membranes.

Generally, the mechanical energy, F, of the membrane is given as [15–17]

$$F = \frac{1}{2}\kappa\int(c_x + c_y - c_0)^2\,\mathrm{d}A + \sigma\int\mathrm{d}A - p\int\mathrm{d}V \quad (2)$$

where κ, σ, p are the modulus of cylindrical bending, the interfacial tension, and the hydrostatic pressure inside the membrane when compared to the outside, respectively. The factors, c_x, c_y and c_0, are the principal curvatures and the spontaneous curvature, respectively. The integrals in the first and second terms of Eq. (2) are performed on the membrane interface and the last integral is performed inside the membrane volume. The term of Gaussian modulus has been omitted in this case because of the topology condition [18]. Although the membranes are formed spontaneously, the weak interfacial tension is assumed to exist. Otherwise, the system becomes unstable, as will be shown later.

For the axisymmetric tubular membranes about which we are concerned, Eq. (2) is rewritten as

$$F = \pi\kappa\int\left(\frac{R_{zz}}{X^3} - \frac{1}{RX} + \frac{1}{R_0}\right)^2 RX\,\mathrm{d}z + 2\pi\sigma\int RX\,\mathrm{d}z - \pi p\int R^2\,\mathrm{d}z \quad (3)$$

where

$$X = \sqrt{1 + R_z^2} \quad (4)$$

$$R_z = \frac{\partial R}{\partial z} \text{ and } R_{zz} = \frac{\partial^2 R}{\partial z^2} \quad (5)$$

and where R and R_0 are the radius of the tubule at the coordinate z in the direction of the tubular axis and the spontaneous radius of the tubule. The spontaneous curvature of the bilayer membrane is considered to be zero for its geometrical symmetry. However, as was discussed previous section, the formation of tubular membranes in a certain condition (under the induced flow) might have produced the mechanically balanced bilayer membranes (not in thermal equilibrium) which possess finite spontaneous curvatures.

First, I calculate the energy, F_0, of straight tubular membranes. In this case, F_0 becomes

$$F_0 = \pi L\left[\kappa R\left(\frac{1}{R_0} - \frac{1}{R}\right)^2 + 2\sigma R - pR^2\right] \quad (6)$$

where L is the arbitrary length of the tubule. The derivative of F_0 to obtain the minimum value of it turns out to be

$$\frac{\partial F_0}{\partial R} = \pi L\left[\kappa\left(\frac{1}{R_0^2} - \frac{1}{R^2}\right) + 2\sigma - 2pR\right] = 0 \quad (7)$$

The cubic equation to solve Eq. (7) becomes

$$2pR^3 - \left(\frac{\kappa}{R_0^2} + 2\sigma\right)R^2 + \kappa = 0 \quad (8)$$

When the pressure p is zero, the stable radius R from Eq. (8) becomes

$$R = \frac{R_0}{\sqrt{1 + \dfrac{2\sigma R_0^2}{\kappa}}} \tag{9}$$

In the presence of the pressure, p, the radius R is determined by elementary calculus to solve the cubic equation (Eq. (8)). The previous authors gave the initial radius R without minimizing the mechanical energy [1,3–6].

Although I do not show the detail of calculation, we have the limiting value of $p = p_0$, which is estimated as

$$p_0 = \frac{\kappa}{\left(\sqrt{3}\,R_0\right)^3}\left(1 + \frac{2\sigma R_0^2}{\kappa}\right)^{\frac{3}{2}} \tag{10}$$

Under the condition of $p < p_0$, the energy F_0 has its local minimum value at the obtained radius R as a function of applied pressure p. If p exceeds p_0, the tubules are unstable for infinite swelling of water into the tubule. As there is no infinite capacity of water in the globules at both ends of the tubule, this instability would stop at some stage.

Next, the sinusoidal small perturbation is added to the obtained radius in the stable condition $p < p_0$. The perturbation is expressed as

$$R = r_0 + \eta\cos(kz) \tag{11}$$

where r_0 is the value of R obtained by solving Eq. (8). Eq. (11) is inserted in Eq. (3) and we take the second order in η which results in

$$F = F_0 + \frac{\pi L\eta^2}{2}\left[\kappa R k^4 - 2\left\{\kappa\left(\frac{1}{R_0} + \frac{1}{4}\left(\frac{1}{R} - \frac{R}{R_0^2}\right)\right) - \frac{\sigma R}{2}\right\}k^2 + \frac{\kappa}{R^3} - p\right] \tag{12}$$

Eq. (12) is rewritten as

$$F = F_0 + \frac{\pi L\eta^2}{2}\left[\kappa R\left(k^2 - a\right)^2 + b\right] \tag{13}$$

where

$$a = \frac{1}{\kappa R}\left[\kappa\left\{\frac{1}{R_0} + \frac{1}{4}\left(\frac{1}{R} - \frac{R}{R_0^2}\right)\right\} - \frac{\sigma R}{2}\right] \tag{14}$$

$$b = \frac{\kappa}{R^3} - p - \frac{1}{\kappa R}\left[\kappa\left\{\frac{1}{R_0} + \frac{1}{4}\left(\frac{1}{R} - \frac{R}{R_0^2}\right) - \frac{\sigma R}{2}\right\}\right]^2 \tag{15}$$

The instability with finite k occurs when a is positive and b is negative. In Eqs. (12)–(15), R is taken to be the solution of Eq. (8).

Now, we will evaluate the physical quantities and try to explain the experimental data on the peristaltic instability based on our theory. The critical pressure p_0 of straight tubules is determined from Eq. (10) as a function of given parameters, κ, σ, and R_0. On the other hand, the threshold value of the pressure for the instability of small amplitude perturbation is determined by Eq. (14) and Eq. (15), in which the value of R is determined by Eq. (8).

To see the physical characteristics of the mathematical formulae, we will calculate the physical quantities at the limit of rigid membranes for bending. In other words, the factors, σR_0^2 and pR_0^3 are very small compared with the bending modulus, κ. By solving Eq. (8), the radius, R, becomes

$$R = R_0\left(1 - \frac{\sigma R_0^2}{\kappa} + \frac{pR_0^3}{\kappa}\right) \tag{16}$$

The value of b becomes

$$b = 2\left(\frac{\sigma}{R_0} - p\right) \tag{17}$$

The wave number, k, from Eq. (14) and Eq. (15) becomes

$$k = \frac{1}{R_0}\left(1 + \frac{\sigma R_0^2}{2\kappa} - \frac{3pR_0^3}{4\kappa}\right) \tag{18}$$

From Eq. (18), the wave length, λ, of the instability is approximately $2\pi R_0$ and decreases as the pressure increases. From Eq. (17), it can be said that the straight tubules are unstable and the growth of sinusoidal perturbation begins if the pressure exceeds

σ/R_0, that is the Laplace pressure in case of cylinder. This is much smaller than the value given in Eq. (10) in our restricted case and means that the straight tubules are metastable in the pressure range $\sigma/R_0 < p < p_0$ and the point $p = p_0$ is the spinodal point.

The result of the evaluation of physical quantities in more general cases is shown in the following. Eq. (8) is solved to evaluate the radius R and the obtained radius is used to calculate the wave number k. The phase diagram is obtained by solving Eq. (14) and Eq. (15) for given σ and p and is shown in Fig. 1, in which the abscissa is the interfacial tension scaled by κ/R_0^2 and the ordinate is the pressure scaled by κ/R_0^3. In region A, straight tubules are stable. In region B, the peristaltic instability occurs. Line L-2 is the spinodal line, i.e., the metastable straight tubules can exist in region B. Without the spontaneous curvature, the spinodal line disappears, the change occurs abruptly, and the small amplitude perturbation in the form of Eq. (11) does not lead the result which makes lower free energy than the straight tubules. However, the observed data shows that the small amplitude peristaltic state to the pearling state occurs as I have analyzed here. Thus, the assumption of the spontaneous curvature is reasonable.

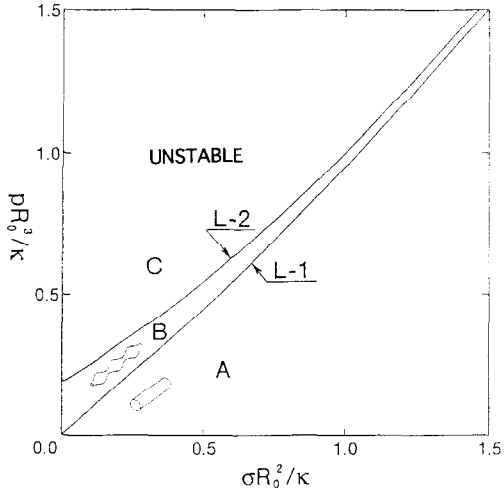

Fig. 1. Phase diagram of tubular membranes. The abscissa is the interfacial tension and the ordinate is the inside hydrostatic pressure of tubules. In regions A and B, the straight tubules are stable. In region B, the peristaltic instability occurs. The line L-2 is, thus, the spinodal line. In region B, the straight tubules are metastable. In region C, the tubules are unstable.

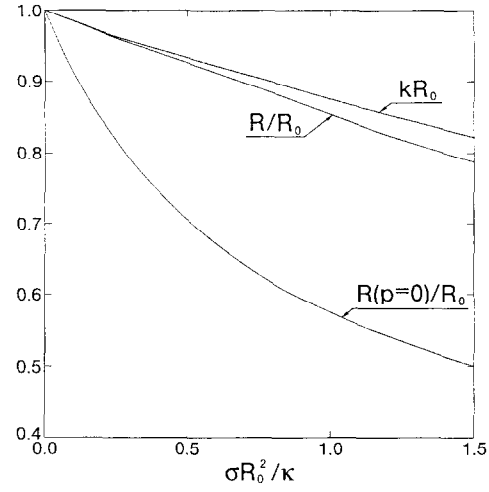

Fig. 2. The radius R of tubules and wave number k as functions of interfacial tension σ at the line L-1 in Fig. 1. The radius R without hydrostatic pressure is also shown.

So far, we have considered the possibility that the optical tweezers make the elevation of the interior hydrostatic pressure due to the heating up of the aqueous solvent inside. Also, the laser tweezers could cause the change of the membrane tension as postulated by others [3–6]. The effect of the change in membrane tension, however, remains locally at the tweezing point and there is no reliable driving force to propagate it to the outside the tweezing point as far as we concern with long time scale of the order of 10 s and I have not taken them into account in this report.

Calculated radius R and the wave number k on the line L-1 (instability line) in Fig. 1 are shown in Fig. 2. The radius R for zero pressure is also shown in Fig. 2. If the observed straight tubules correspond to the case of zero pressure in our analysis, the value of k is apparently smaller than unity in Fig. 2. Because one cannot address the precise initial pressure inside the tubules before the application of tweezers experimentally, it does not make sense to predict a reliable estimation of the wave number. Remind the following fact; elastic solids increases volume when it is pulled in one direction. The abrupt induced flow might cause the decrease of inside pressure in our tubular case. On this point, more detailed observations and theoretical studies are needed.

I will estimate the expected characteristic pressure and interfacial tension by using the observed bending rigidity and the radius. By employing typical values of κ and R_0 as follows

$$\kappa = 5 \times 10^{-20} \text{ J and } R_0 = 0.5\ \mu\text{m} \quad (19)$$

the corresponding characteristic pressure p' and the interfacial tension σ' become

$$p' = \frac{\kappa}{R_0^3} = 0.4 \text{ Pa and}$$

$$\sigma' = \frac{\kappa}{R_0^2} = 2 \times 10^{-4} \text{ mN m}^{-1} \quad (20)$$

Because both p' and σ' possess very small values, our tubular system is balanced among very small and delicate mechanical forces including the thermal expansion.

3. Conclusion and discussion

In conclusion, the stability of the tubular membrane system was analyzed by use of the bending rigidity with spontaneous curvature, interfacial tension, and the hydrostatic pressure terms. The non-zero spontaneous curvature was the reasonable condition for the stability of tubules. The conservation of volume during the deformation of the tubules was relaxed and I regarded the optical tweezers as the driving force for the enhancement of the interior temperature of tubules. The resulting enhanced pressure changes the straight tubular system to the peristaltic state with a spinodal line in the phase diagram. In this analysis, the interfacial tension and the pressure are taken as theoretical parameters. Quantitatively, however, the expected pressure and the interfacial tension possess extremely small values compared with those we can usually manage in the laboratories.

As mentioned in the introduction, Deuling and Helfrich predicted the peristaltic structure of tubules that is similar to pearling necklaces by letting the bending energy to zero with assumption of the spontaneous curvature [8]. Their calculation corresponds to the limit of the small interfacial tension and the inside hydrostatic pressure of my analysis. To realize the peristaltic tubules in the experimental system, however, some driving force such as hydrostatic pressure should be involved in the system. Because my analysis in this report is on the linear stability, it is worth to extend my theory to the non-linear regime and examine the relation to Deuling and Helfrich's work. Thus, we are now analyzing the more realistic pearling states by the numerical calculation in the small values of interfacial tension and the pressure as an extension of this work. The result may be published elsewhere.

Many workers analyzed dynamics of this system including the numerical calculation [3–6]. Before going into the dynamics, I feel that the static characteristics of this problem should be explored more precisely and carefully. Finally, to conform my theory, for instance, some radical residues can be put into the lipid molecule that can absorb the laser power more efficiently than the previous experiment.

Acknowledgements

The author thanks Drs. S. Komura and M. Takefu and Mr. H. Ichinose for their useful discussion of this problem. Dr. E. Moses kindly gave me a copy of the video tape of the laser tweezers to membranes.

References

[1] R. Bar-Giv and E. Moses, Phys. Rev. Lett. 73 (1994) 1392.
[2] Lord Rayleigh, Scientific Papers, Vol. 1 (Dover, New York, 1964) pp. 361–371.
[3] P. Nelson, T. Powers and U. Seifert, Phys. Rev. Lett. 74 (1995) 3384.
[4] R. Granek and Z. Olami, J. Phys. II Fr. 5 (1995) 1349.
[5] R.E. Goldstein, P. Nelson, T. Powers and U. Seifert, J. Phys. 6 (1996) 767.
[6] P.D. Olmsted and F.C. MacKintosh, private communication (1996).
[7] W. Helfrich, Z. Naturforsch. 29c (1974) 510.
[8] H.J. Deuling and W. Helfrich, Blood Cells 3 (1977) 713.
[9] J. Kas and E. Sackmann, Biophys. J. 60 (1991) 825.
[10] K. Mishima and T. Morimoto, Biochim. Biophys. Acta 985 (1989) 351.
[11] A. Ashkin, J.M. Dziedzie and T. Yamane, Nature 330 (1987) 769.
[12] R. Bar-Ziv, R. menes, E. Moses and S.A. Safran, Phys. Rev. Lett. 75 (1995) 3356.

[13] N.A. Priver, E.C. Rabin and M.L. Zeidel, Biochemistry 32 (1993) 2459.
[14] H. Hotani, J. Mol. Biol. 178 (1984) 113.
[15] O.-Y. Zhong-can and W. Helfrich, Phys. Rev. A 39 (1989) 5280.
[16] U. Seifert, K. Berndl and R. Lipowsky, Phys. Rev. A 44 (1991) 1182.
[17] H. Naito, M. Okuda and O.-Y. Zhong-can, Phys. Rev. Lett. 74 (1995) 4345.
[18] Y. Suezaki and H. Ichinose, J. Phys. I Fr. 7 (1995) 1469.