EUROPHYSICS LETTERS 1 July 1990

*Europhys. Lett.*, 12 (5), pp. 395-400 (1990)

# Light Scattering Study of Surfactant Multilayers Elasticity. Role of Incorporated Proteins.

A. OTT(*), W. URBACH(*), D. LANGEVIN(*), R. OBER(**) and M. WAKS(***)

(*) *Laboratoire de Physique Statistique, ENS - 75231 Paris Cédex, France*
(**) *Laboratoire de Physique de la Matière Condensée, Collège de France 75231 Paris Cédex, France*
(***) *Departement de Biochimie, UFR Biomédicale - 75006 Paris, France*

(received 23 January 1990; accepted in final form 4 May 1990)

PACS. 05.40 – Fluctuation phenomena, random processes and Brownian motion.
PACS. 78.35 – Brillouin and Rayleigh scattering.
PACS. 87.20 – Membrane biophysics.

**Abstract.** – We have studied swollen lamellar phases into which the basic myelin protein can be incorporated. Lamellar spacing thermal fluctuations were analysed with quasi-elastic light scattering. The results are consistent with the existence of a strong steric repulsion between the bilayers due to the thermal undulations of these bilayers. They show that the bilayers bending elastic modulus is significantly increased by the presence of small amounts of the protein.

*Introduction.* – Membrane proteins are incorporated into cell membranes where they have important specific biological functions. These functions are mostly dependent upon the nature and the three-dimensional folding of the sequences of aminoacid residues of these proteins. However, it was observed that the rheological properties of the membranes depend upon the amount of incorporated proteins, although the influence of these changes on biological activity is not yet well understood. The lipid mobilities in cell membranes are several orders of magnitude smaller than in pure lipid bilayers [1, 2]. The reduced mobility was recently accounted for by a molecular crowding effect due to proteins [2]. On the other hand, the binding elasticities of the pure lipid bilayers are about one order of magnitude larger than those of cell membranes [3]. This suggests that the membrane proteins might introduce some degree of disorder in the lipid bilayers.

The present study deals with the myelin basic protein (MBP), a major component of the myelin membrane. This membrane consists of a compacted stock of membrane bilayers spirally wrapped around the axon. The MBP together with the second major protein, the Folch-Pi proteolipid, form 80% of the total protein content of the myelin membrane, and are thought to play an important role in the compaction of adjacent bilayers through protein-protein interactions. The two proteins are actively studied to understand demyelinization processes and multiple sclerosis [4].

Whereas the proteolipid is a transmembrane protein, the MBP is located on the cytoplasmic side of the myelin membrane and is thought to interact predominantly with the charged lipid polar headgroups, *i.e.* the superficial part of the bilayer. For this reason, in a

former work, we have incorporated this protein into water-in-oil microemulsions [5]. These microemulsions are made of small droplets each consisting of an aqueous core surrounded by a surfactant monolayer, that are dispersed in an organic apolar solvent. Despite of the fact that MBP is supposed to remain trapped within the aqueous core of the droplets, we have observed that the incorporation of the protein induced the appearance of attractive interactions between the droplets, without any increase in droplet size [6]. The magnitude of the attraction was much too large to be attributed to van der Waals forces. In water-in-oil microemulsions, these attractive interactions are associated with an important folding of the surfactant hydrocarbon chains [7]. This suggests that MBP probably penetrates to some extent into the surfactant monolayer and disorganizes it.

In the present work, we have incorporated the protein into surfactant bilayers. We have studied the thermal fluctuations of these bilayers in lamellar phases by quasi-elastic light scattering. For this purpose, we had to prepare oriented samples. The procedure known to give the best orientation in these systems involves drying on a molecularly smooth solid surface like mica [8]. This does not allow the preparation of swollen lamellar phases, where the study of the thermal fluctuations leads to particularly interesting information about the bilayers elasticity [9]. We have had therefore to work out an alternative orientation procedure, found to lead to very good orientation.

*Sample preparation.* – In certain lamellar water-surfactant phases, the lamellar spacing can be very large [10, 11]. They are stabilized by long-range entropic repulsions due to the thermal undulations of the lamellae. These phases can be oriented and they give rise to a large scattering of light which allows the determination of their elastic properties.

For these reasons, we have attempted to solubilize the MBP protein into this particular type of lamellar phase. We have chosen a cationic surfactant, the dodecyltrimetyl ammonium bromide (DTAB). Absorption and fluorescence spectroscopy results suggest that the protein solubilized in the lamellar phase is in native form and is absorbed on the surfactant polar headgroups [12].

In order to obtain swollen lamellar phases, we have added salt (0.4 M NaCl) to screen the electrostatic repulsions between the surfactant molecules and 1-pentanol to adjust the curvature of the surfactant layer and to introduce some disorder in the bilayers, *i.e.* to increase their thermal fluctuations. Without pentanol, the water-surfactant mixtures are isotropic micellar solutions.

The lamellar phases are obtained between two limit pentanol concentrations, and are easier to orient at high pentanol concentrations. The lamellar-isotropic phase transition temperature depends on the pentanol concentration; its maximum value is 50 °C. In order to avoid protein denaturation, we use temperatures in the range $(35 \div 40)$ °C. The DTAB/pentanol mass ratio is about one and is the same for all the samples. The existence of lamellar order is checked with a polarizing microscope: the samples are optically birefringent and typical focal conical textures are observed. The samples orient partially when inserted in the light scattering cells, made of two parallel glass plates separated by spacers 125 μm thick. These cells are sealed with a two-component epoxy glue. A good homeotropic orientation (bilayers parallel to the plates) is achieved by using a thermal treatment: the samples are heated to just below the lamellar-isotropic phase transition and then slowly cooled (0.1 °C/min) down to room temperature. The thermal treatment is repeated until the microscope field is entirely black between crossed polarizers.

The MBP protein was used in a lyophilized form and dissolved in the above lamellar phases. Its nondenaturation was checked by absorption and fluorescence spectroscopy. We have used two concentrations: 5 mg and 1 mg of protein per 1 ml of lamellar phase. The samples thus obtained remain lamellar and continue to orient under the above procedure.

*Small angle X-ray scattering experiments.* – In order to determine the lamellar spacing, we have performed small-angle X-ray scattering. Several spectra are shown in fig. 1. The Bragg peaks are broader than for unswollen lamellar phases, because of the thermal

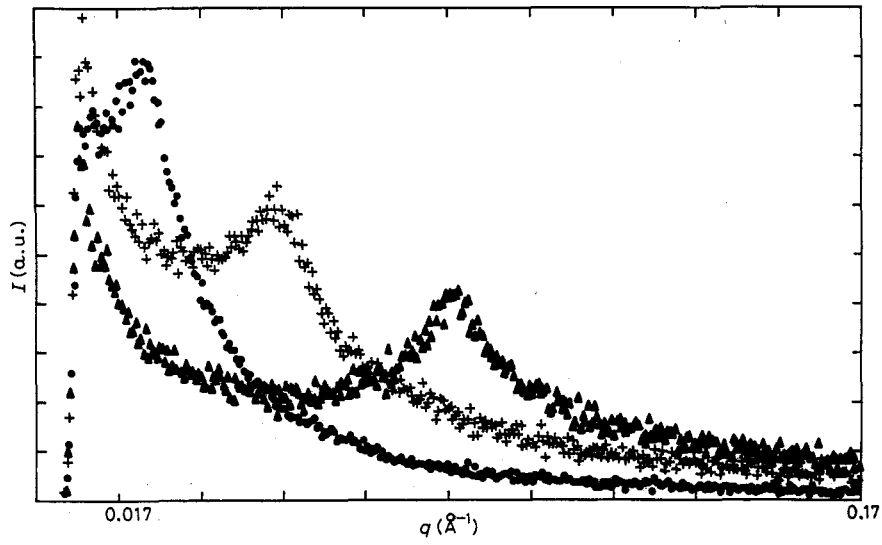


Fig. 1. – X-ray scattering spectra of protein-free lamellar phases with different dilution degrees (●, $d = 285$ Å; +, $d = 127.5$ Å; ▲, $d = 73$ Å).

undulations of the lamellae which causes large Landau-Pierls anomalies, *i.e.* broad peak wings [11]. The lamellar spacing values are shown in fig. 2 *vs.* water volume fraction $\phi_w$. Since $d = \delta/(1 - \phi_w)$, where $\delta$ is the surfactant bilayer thickness, one deduces from fig. 2 that $\delta = 25$ Å, comparable to its values in similar systems [9]. We have not attempted to extract information from the shape of the wings, because the signal-to-noise ratio was insufficient.

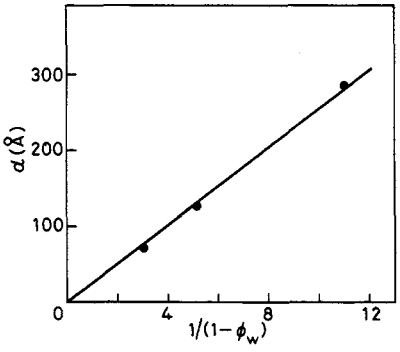


Fig. 2. – Lamellar spacing *vs.* water volume fraction. The line is the least-square fit with $d = \delta/(1 - \phi)$ for $\delta = 25$ Å (protein-free system).

*Dynamic light scattering: theoretical background.* – Dynamic light scattering probes two different kinds of thermal fluctuations in these systems: concentration fluctuations and lamellae displacements at constant concentration [9]. When the scattering wave vector $\boldsymbol{q}$ is parallel to the layers, the two fluctuations modes are decoupled. In this geometry, the

second mode corresponds to undulations of the lamellae. Its characteristic damping time $\tau_u$ is given by

$$\frac{1}{\tau_u} = \frac{Kq^2}{\eta}, \tag{1}$$

where $K$ is the bending elastic modulus of the bilayers and $\eta$ is a shear viscosity. The concentration fluctuations mode corresponds to bilayer thickness fluctuations; its damping time is much shorter.

When $\boldsymbol{q}$ is not parallel to the layers, the different fluctuations modes are coupled. Two modes are obtained: the second sound which is propagative and is a high-frequency mode, and the «baroclinic» mode whose damping time is

$$\frac{1}{\tau_b} = D\frac{\bar{B}}{B}q_{\parallel}^2, \tag{2}$$

where $D$ is the diffusion coefficient for the concentration fluctuations, and $\bar{B}$ and $B$ are the compressibility moduli of the layers at constant chemical potential and constant concentration, respectively; $q_{\parallel}$ is the component of the wave vector $\boldsymbol{q}$ parallel to the layers. This mode corresponds to fluctuations of the lamellar spacing $d$ at constant bilayer thickness $\delta$.

For dilute systems, $B$ is dominated by the bilayer compressibility, while $\bar{B}$ depends on bilayers interactions alone: $\bar{B} \ll B$. In this approximation $D = \mu B$, where $\mu$ is the friction coefficient between bilayers and solvent. Assuming that the viscosity $\eta_w$ is the solvent viscosity, one can then calculate [13]

$$\mu = \frac{\beta(d-\delta)^2}{12\eta_w}, \qquad \beta = \frac{\rho_w d}{\rho_w(d-\delta)+\rho_s\delta}, \tag{3}$$

where $\rho_w$ and $\rho_S$ are, respectively, the solvent and bilayer densities.

Assuming further that the bilayers interactions are steric entropic repulsions due to thermal undulations, and using the Helfrich expression for the interacting potential [14], one gets [9]

$$D_b = D\frac{\bar{B}}{B} = \frac{3\pi^2\beta}{256\eta_w}\frac{(kT)^2}{K}\frac{d}{(d-\delta)^2}. \tag{4}$$

*Dynamic light scattering experiments.* – The light scattering set-up makes use of an argon-ion laser ($\lambda = 4880$ Å) and a multibit home-made correlator with 128 channels. The time delay between the channels can be varied between adjacent groups of 32 channels, in order to analyse the correlation functions over a very large time scale. Experiments were performed at 20 °C.

Except close to $q_\perp = 0$, the correlation functions were single exponentials. For $q_\perp = 0$, we probably observe the contributions from the small remaining unoriented parts of the sample. This contribution can be removed [9], but our sample cell does not allow us to work in this geometry. We have therefore only studied the baroclinic mode in our experiments (the second sound mode frequency is too large to be analysed with a correlator). At large lamellar spacings, we have found that the correlation functions were better fitted with stretched exponentials $G(\tau) = \exp[-(\tau/\tau_b)^x]$ than with simple exponentials. The largest mean square deviations between the fitted and the experimental functions are obtained for the largest $d$, they are typically one order of magnitude smaller when using stretched

exponentials. The corresponding $x$ values are: $x \simeq 0.75$. This behaviour might have a theoretical support [15]. However, this type of fit does not lead to a significantly different value of $\tau_d$: the difference is smaller than the experimental reproducibility.

In all the samples, $1/\tau_b$ is linear in $q^{2,\parallel}$. We have plotted in fig. 3 the results obtained for $D_b$ in the protein-free and protein-containing systems. As predicted by eq. (4), $1/D_b$ is linear in $(d-\delta)^2/d$, although the line does not go through the origin. From the slope of the line, we get $K/kT = 0.49$. The finite value of $D_b$ for $d = \delta$ could be associated to the classical thermal fluctuations of the lamellar spacing in smectic liquid crystals. However, our $D_b$ value is too small by several orders of magnitude [9]. It is more likely related to alcohol concentration fluctuations in the bilayers. This type of fluctuations will scatter light proportionally to the number density of bilayers, *i.e.* to $\phi$, whereas the intensity scattered by the bilayers undulations is proportional to $\phi^{-1}$. In order to check these assumptions, we fitted the correlation functions with a sum of two exponentials. These fits were not significantly better than the former single exponential fits. The longest relaxation times are equal within experimental errors to the times $\tau$ found previously (fig. 3). The shortest relaxation times are roughly constant and equal to the limit value of $\tau$ for small $\phi$, *i.e.* to the relaxation time of the concentration fluctuations in the layer. The ratio of the intensities corresponding to the two contributions goes as $\phi^2$ as expected for small $\phi$. At large $\phi$, this ratio decreases, but since we enter the regime where the undulations relaxation is nonexponential, the above simple arguments relative to intensities may no longer apply.

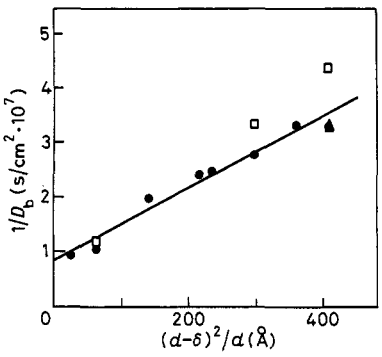


Fig. 3.

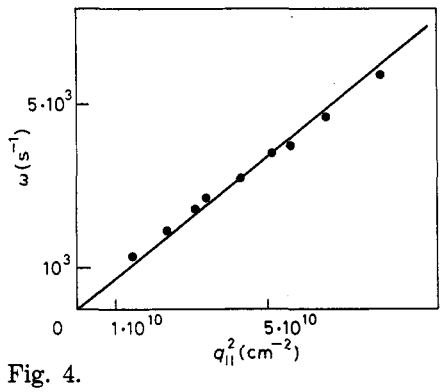


Fig. 4.

Fig. 3. – Inverse diffusion coefficient for the baroclinic mode *vs.* lamellar spacing. The line is the fit with eq. (4). Protein-free system: ●; MBP concentration: □ 5 mg/ml, ▲ 1 mg/ml. The error bar is roughly equal to the size of the points.

Fig. 4. – Inverse characteristic time of the scattered light autocorrelation function *vs.* $q_\parallel^2$. Lamellar phase with $d = 300$ Å, MBP concentration: 5 mg/ml.

We have studied lamellar systems containing the MBP protein. The correlation functions were similar to those without proteins, and the correlation time was still inversely proportional to $q_\parallel^2$ (fig. 4). For the smallest protein concentration (1 mg/ml) the $D_b$ value is the same as that for the protein-free corresponding system. For the largest one (5 mg/ml), the $D_b$ value is about 30% smaller. Let us recall that these protein concentrations are too small to change the lamellar spacing. Using eqs. (2) and (3), we get $\bar{B} = 0.14$ dyn/cm$^2$ for the second protein system as compared to $\bar{B} = 0.18$ dyn/cm$^2$ for the protein-free one. If both systems are stabilized by steric undulation forces, this means that the bending elastic constant is increased in the presence of the protein to $K/kT = 0.64$ (from the above $\bar{B}$ value

and eq. (4)). A systematic study of protein samples as a function $d$ will be performed to confirm this point.

*Conclusion.* – We have studied swollen lamellar phases in which the basic myelin protein has been incorporated. We have studied the lamellar spacing fluctuations (baroclinic mode) with quasi-elastic light scattering. The results are consistent with the existence of a strong steric repulsion between the bilayers due to the thermal undulations of these bilayers. They suggest that the bilayer bending elastic modulus is significantly increased by small amounts of the MBP protein. In contrast to observations made with other proteins and more surprisingly with MBP in monolayers [6] the MBP rigidifies the bilayers. This might be due to the different nature of the surfactant molecules in the two MBP studies (their charges are opposite). This might be also due to the particular nature of MBP which is not a transmembrane protein, or it could be a concentration-dependent effect: it is well known indeed that the elasticity of mixed surfactant monolayers is a nonlinear function of the relative amount of the constituents, and can exhibit extrema for particular monolayers compositions [16]. A systematic study of MBP in swollen lamellar phases therefore must be undertaken to clarify these issues.

* * *

This work was initiated following a suggestion of D. CHATENAY. We are greatly indebted to him and to J. PROST, D. ROUX and F. NALLET for very fruitful discussions. We also thank a referee for suggesting the arguments relative to the two relaxation times to us. This work was partially supported by a grant of Ministère de la Recherche, action Physique-Biologie.

REFERENCES

[1] KAPITZA H. G. and SACKMANN E., *Biochim. Biophys. J.*, **595** (1980) 56.
[2] RYAN T. A., MEYERS J., HOLOWKA D., BAIRD B. and WEBB W. W., *Nature (London)*, **239** (1988) 1 and references therein.
[3] ENGELHARDT H., DUWE H. P. and SACKMANN E., *J. Phys. Lett.*, **46** (1985) L-395 and references therein.
[4] MORELL P., *Myelin*, second edition (Plenum Press, New York, N.Y.) 1984.
[5] NICOT C., VACHER M., VINCENT M., GALLEY J. and WAKS M., *Biochemistry*, **24** (1985) 7024.
[6] CHATENAY D., URBACH W., NICOT C., VACHER M. and WAKS M., *J. Phys. Chem.*, **91** (1987) 2198.
[7] CAZABAT A. M. and LANGEVIN D., *J. Chem. Phys.*, **74** (1981) 3148.
[8] HENDERSON R., *J. Mol. Biology*, **93** (1975) 123.
[9] NALLET F., ROUX D. and PROST J., *Phys. Rev. Lett.*, **62** (1989) 276; *J. Phys. (Paris)* (1989).
[10] LARCHÉ F., APPELL J., PORTE G., BASSEREAU P. and MARIGNAN J., *Phys. Rev. Lett.*, **56** (1986) 1700.
[11] SAFINYA C. R., ROUX D., SMITH G. S., SINKA S. K., DIMON P., CLARK N. A. and BELLOCQ A. M., *Phys. Rev. Lett.*, **57** (1986) 2718.
[12] To be published.
[13] BROCHARD F. and DE GENNES P. G., *Pramana Suppl.*, **1** (1975) 1.
[14] HELFRICH W., *Z. Naturforsch. Teil A*, **33** (1978) 305.
[15] PROST J. and RAMASWAMY S., private communication.
[16] SHAH D. O., DJABBARAH N. F. and WASAN D. T., *Colloid Polym. Sci.*, **256** (1978) 1002.