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ARTICLE *in* LANGMUIR · FEBRUARY 2003

Impact Factor: 4.46 · DOI: 10.1021/la020801a

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Interaction of Myelin Basic Protein with Phospholipid Monolayers: Mechanism of Protein Penetration

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Received September 20, 2002. In Final Form: November 1, 2002

The myelin basic protein (MBP) is the second most abundant protein in the myelin sheath of the central nervous system and is believed to be important for the compactness and integrity of the membrane. We investigated the mechanism of the interaction of lipid-free MBP with phospholipid monolayers at the air/water interface; in particular, we studied the process of MBP adsorption onto monolayers made up either of neutral dipalmitoylphosphatidylcholine (DPPC) or of negatively charged dipalmitoylphosphatidylserine (DPPS) monolayers. They are natural constituents of the myelin membrane, and sharing an identical hydrophobic chain, they differ only in headgroup composition. The MBP−lipid interaction is investigated for the first time by means of nullellipsometric measurements, monitoring in real time the effect of adsorbed molecules in the insoluble monolayer at different monolayer conditions, such as surface pressure and molecular area. The different behavior of monolayer thickness and surface pressure confirmed the hypothesis of a different interaction mechanism of MBP with the two kind of lipids. While in the presence of neutral DPPC the protein seems to penetrate among the lipid domains, in the case of negatively charged DPPS the electrostatic interaction appears to be the driving force, because protein intimately associates with the headgroups and binds to the Langmuir layer as a specific lipid−protein complex. Results with DPPS were confirmed by FTIR spectroscopy measurements, performed after transferring phospholipid multilayers onto a solid substrate by the Langmuir−Schaefer method.

Introduction

The myelin sheath of the central nervous system (CNS) is a lipid-rich, multilamellar membrane of several bilayers tightly wrapped around the nerve axon. Its integrity is fundamental for signal conduction along the axon. Despite its importance, little is known about the formation and manteinance of this multilamellar structure and what controls these processes.

Myelin basic protein (MBP) is a major protein of this membrane in the CNS but it is also present in the peripheral nervous system (PNS).[1,2] MBP is a 18.5 kDa protein with a net positive charge of 20, at neutral pH. It has been extensively studied as the autoantigen responsible for experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis,[1] and as the main agent in the formation and compaction of CNS myelin.[3,4] In recent years, attempts to determine MBP structure in integral myelin have failed. Spectroscopic studies carried out on purified water-soluble MBP have shown that the protein probably has a substantially disordered conformation, which is however prone to change in the presence of lipids.[1] On these grounds, great effort has been devoted to understanding of how the water-soluble MBP interacts with lipids in the membrane. A computational three-dimensional model, based on electron microscopic observation, which assumes a C-shape for MBP in the presence of lipids[5] has been reported.

Despite the considerable amount of data reported on the interaction of lipid-free MBP with model phospholipid membranes both in bulk and in oriented lipid monolayers and multilayers deposited on a solid substrate, very little is known about the mechanism of this interaction. In a previous report, our group reported data obtained using the quartz microbalance technique, which yielded macroscopic information on the effects of protein adsorption on the mechanical properties of the deposited phospholipid multilayers.[6]

It is well-known that phospholipids, owing to their amphiphilic character, can form condensed monolayers at the air/water interface, useful as model systems to study biological processes, in particular as membrane models.[7] The interaction of the myelin proteins with a variety of lipids was studied by Demel et al.[8] recording the changes in pressure of the lipid monolayers at the air−water interface. To study in more details the mechanism of such interaction, we investigated the process of direct adsorption of MBP onto dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylserine (DPPS) monolayers,

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(1) Martenson, R. E. *Myelin*: *Biology and Chemistry*; CRC Press: Boca Raton, FL, 1992.

(2) Smith, R. *J. Neurochem*. **1992**, *59*, 1589−1608.

(3) Riccio, P.; Masotti, L.; Cavatorta, P.; Santis, A. D.; Juretic, D.; Bobba, A.; Pasquali-Ronchetti, I.; Quagliariello, E. *Biochem. Biophys. Res. Commun*. **1986**, *134*, 313−319.

(4) Readhead, C.; Popko, B.; Takahashi, N.; Shine, H. D.; Saavedra, R. A.; Sidman, R. L.; Hood, L. *Cell* **1987**, *48*, 703−712.

(5) Ridsdale, R. A.; Beniac, D. R.; Tompkins, T. A.; Moscarello, M. A.; Harauz, G. *J. Biol. Chem*. **1997**, *272*, 4269−4275.

(6) Facci, P.; Cavatorta, P.; Cristofolini, L.; Fontana, M. P.; Fasano, A.; Riccio, P. *Biophys. J*. **2000**, *78*, 1413−1419.

(7) Mohwald, H. In *Structure and dynamics of membrane. From cell to vesicles*; Lipowsky, R., Sackmann, E., Eds.; Elsevier Science BV/North-Holland: Amsterdam, 1995; Vol. 1A, pp 161−211.

(8) Demel, R. A.; London Y., Geurts Van Kessel, W. S. M.; Vossenberg, F. G. A., Van Deenen, L. L. M. *Biochim. Biophys. Acta* **1973**, *311*, 507−519.

10.1021/la020801a CCC: $25.00
Published on Web 12/24/2002

as representative of neutral and charged lipids. Phosphatidylserine and phosphatidylcholine are among the most abundant charged and zwitterionic phospholipids in myelin membrane, respectively. DPPS and DPPC used in this study, have identical hydrophobic side chains (C:16) and differ only in their headgroup composition. The two lipids represents, of course, a model for the lipids in the membrane. The choice, however, has been due to the necessity to work with a well-defined system, because of the difficulty to obtain good Langmuir monolayers with unsatured lipids, as we have found in preliminary measurements on natural myelin lipids. Information on DPPC and DPPS different behavior when interacting with MBP could be important from a physiological point of view and could lead to further studies on more complex models closer to the original membrane. However, to our knowledge in the reported studies the protein adsorption was monitored by measuring only one variable, such as surface pressure.[8] Given the complexity of the system and its interactions, this is not sufficient to yield quantitative information. In this paper we report for the first time the simultaneous measurement of surface pressure and monolayer thickness as a function of area/molecule, together with isobaric and isochoric experiments. This was done by null ellipsometric measurements on the monolayer in the Langmuir trough. This highly sensitive technique provides new and interesting information on the protein effect on monolayer thickness, showing in real time the effect of adsorbed molecules on the insoluble monolayer.

Materials and Methods

MBP was extracted from bovine brain and purified according to the method of Deibler et al.,[9,10] dialyzed against pure water, and prepared for the working solution at a concentration of 0.7 mg/mL.

Lipids and solvents were obtained from Sigma-Aldrich and used without further purification. DPPC and DPPS were dissolved in chloroform or chloroform with 30% of methanol, respectively, at a concentration of 1 mg/mL and used as the spreading solution. The final molar ratio between the lipids at the interface and the protein in solution was 2:1.

Langmuir monolayers of phospholipids were prepared in a KSV 5000 trough for the isotherms and in a small R & K trough for the kinetic protein binding experiments.

The subphase was Millipore-filtered water (with a resistivity higher than 18 mΩ cm), at pH 6, and without any salt added to avoid any perturbation of the system. To collect the isotherms in the presence of the protein, the phospholipid was spread onto a subphase containing MBP at a concentration of 10^{-7} M; an equilibration time of about 30 min was used before starting the compression. The surface activity of the pure protein was tested by injecting MBP in the subphase in concentration up to 10^{-5} M in the absence of lipids. No change in surface tension was observed even after 2 h. For the kinetic protein binding experiments MBP was added to the subphase, injected beyond the barriers, underneath the monolayer, at a final concentration of 10^{-7} M. The injection method used for binding experiments has the disadvantage that the kinetics was limited by the diffusion of the protein in the subphase after injection, and it can be the reason for poor reproducibility or artifacts. To avoid this problem, we built a device which allows a fast mixing of the solution in the subphase. All measurements were performed at a temperature of 20 °C, kept constant by a thermostatic cell connected to the trough.

For null ellipsometric measurements we used an Optrel GbR Multiskop instrument, equipped with a He−Ne laser (632.8 nm), polarized by a Glan-Thompson prism with an extinction ratio of 10^{-8}, mounted on high precision rotary stages (0.001°). Hall encoding warrants accuracy of 0.001° and reproducibility of 0.003°. The incidence angle of the light on the surface was 48° (±0.1° reproducibility, stability could be better), 5° away from the Brewster angle. The laser beam probed a surface of 1 mm^2.

We recall that the ellipsometric angles ψ and Δ are related to the ratio of the Fresnel reflection coefficients of the parallel (R_p) and normal (R_s) components, with respect to the plane of incidence, of the electric vector E:

$$\frac{R_p}{R_s} = \tan \psi e^{i\Delta}$$

Here tan ψ is the measure of the change in the amplitude ratio before and after reflection and Δ is the change in phase difference between the two components caused by the reflection. The two ellipsometric angles, determined experimentally, are correlated to the refractive index and the thickness of the film.[11] We analyzed the data with software based on a Fresnel algorithm developed by Optrel GbR as a part of the Ellipsometry module of the Multiskop.[12] In general one can extract both refractive index n and thickness t from knowledge of Δ and ψ. For thin films (below 50 Å), however, the dependence of ψ from n is vanishingly small, thus requiring an a priori knowledge of n. For Langmuir monolayers, therefore, we used the bulk values of n to obtain the thickness. In particular we kept a constant refractive index of $n = 1.47$ for the DPPC film, $n = 1.49$ for DPPS film, and $n = 1.44$ for the protein.[13]

The R & K trough was mounted on a platform with micrometric control of initial position and inserted into the ellipsometer light path. The surface tension was measured with the Wilhelmy balance connected to the trough.

The initial value of the surface tension Π_0 has been subtracted from all data presented below. Δ was recorded every 15 s and Π every 1 s with a sensitivity of ±0.001° and ±0.1 mN/m, respectively.

The actual adsorption of the protein onto the phospholipid layer was also determined by measuring the infrared absorption of mono- and multilayers deposited onto a solid substrate.

A Lagmuir−Schaefer (horizontal lift) technique was used to transfer the films onto the solid substrate. Such technique allows the fast and reliable deposition of high-quality films from rigid monolayers. Typically, 5 or 20 layers of DPPS in the absence and in the presence of protein were deposited on the ZnSe cristal for the attenuated total reflection (ATR) IR measurements. Control measurements were also performed on the DPPC system. The deposition pressures were 10 and 35 mN/m. ATR-FTIR measurements were carried out with a Jasco FTIR 420 spectrometer in the 3500−1500 cm^{-1} wavenumber range at 4 cm^{-1} resolution. To eliminate the spectral contribution of water, the sample compartment was purged under a constant dry nitrogen flux. A blank spectrum from the ZnSe crystal, taken under identical conditions, was subtracted.

Results and Discussion

DPPC Isotherms. In Figure 1 the experimental pressure/area (Π/A) and thickness/area isotherms taken simultaneously on the same DPPC monolayer, both in the presence and in the absence of MBP in the subphase, are shown. As a result of the process of interaction of the protein with lipid (Figure 1b), the Π/A isotherms show that the first-order phase transition (the plateau region) occurs at higher area values. The results suggest that the

(9) Deibler, G. E.; Martenson, R. E.; Kies, M. W. *Prepr. Biochem.* **1972**, *2*, 139−165.
(10) Deibler, G. E.; Boyd, L. F.; Kies, M. W. In *Experimental Allergic Encephalomyelitis*: *A Useful Model for Multiple Sclerosis*; Alvord, E. C. J., Kies, M. W., Suckling, A. J., Eds.; Alan R Liss Inc.: New York, 1984; pp 249−256.
(11) Azzam, R. M.; Bashara, N. M. *Ellipsometry and polarized light*; North-Holland: Amsterdam, 1979.
(12) Harke, M.; Teppner, R.; Schulz, O.; Motschmann, H.; Orendi, H. *Rev. Sci. Instrum.* **1997**, *68*, 3130−3134.
(13) Heyse, S.; Vogel, H.; Sanger, M.; Sigrist, H. *Protein Sci.* **1995**, *4*, 2532−2544.

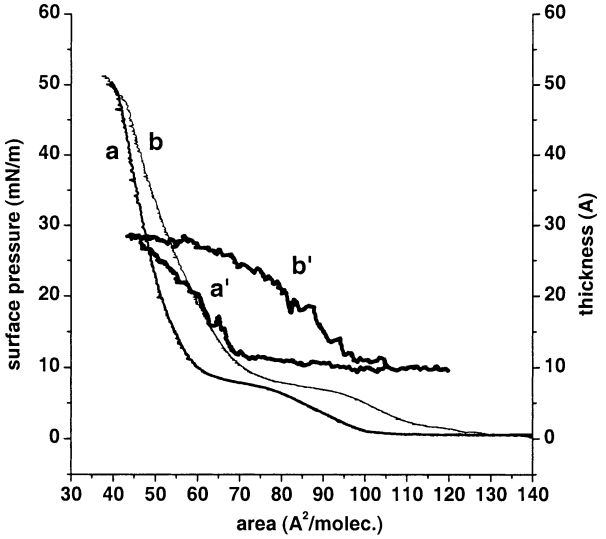

Figure 1. DPPC Π/*A* (thin line) and thickness/*A* (thick line) isotherms in the absence (a, a′) and in the presence (b, b′) of MBP in the subphase.

aggregation of DPPC into condensed phase domains[14−16] could be induced by the penetration of MBP into the monolayer, even if the surface concentration of DPPC is low. The hypothesis that an adsorbed protein could have an effect on the value of the critical concentration for the aggregation of the phospholipid to condensed phase domains was postulated by Vollhardt and Fainerman and by Zhao et al.[14,15] In agreement with their model, the additional presence of the protein results in an increase of the monolayer coverage, thus inducing the DPPC aggregation by the penetrated species. In the pseudo-solid region the isotherms tend to overlap: this can be understood as a successive squeezing out of the protein at high surface pressure.

The thickness/area isotherm in the absence of protein (Figure 1a′) shows that a strong increase of the monolayer thickness occurs both near the end and after the liquid-expanded (LE)/liquid-condensed (LC) phase transition, when the area/molecule becomes less than 60 Å^2 and the condensed phase domains became larger and closer to each other.[14−16] The data analysis yields a thickness increase from about 12 Å in the gaseous phase to about 27 Å in the solid phase. This increase is due to the transition of the phospholipid to a close-packed configuration corresponding to a fully condensed state. In the presence of MBP (Figure 1b′), the thickness increment shows a very similar trend but shifted toward higher area values, in agreement with the corresponding Π/*A* isotherm. The increase starts at about 90 Å^2, reaching the same thickness value of about 27 Å found in the solid phase of pure DPPC, confirming the squeezing out of the protein.

DPPC Isochores and Isobares. The variations of pressure and thickness with the injection of MBP in the subphase at constant area was measured. DPPC isochores were performed at five different area values, corresponding to five initial pressures, beginning from 7 mN/m, only just over the plateau region, where the film is in the LC phase and so reasonably stable. As it can be seen in Figure 2A, the pressure increments decrease as a function of the starting pressure values, and by fitting with a linear

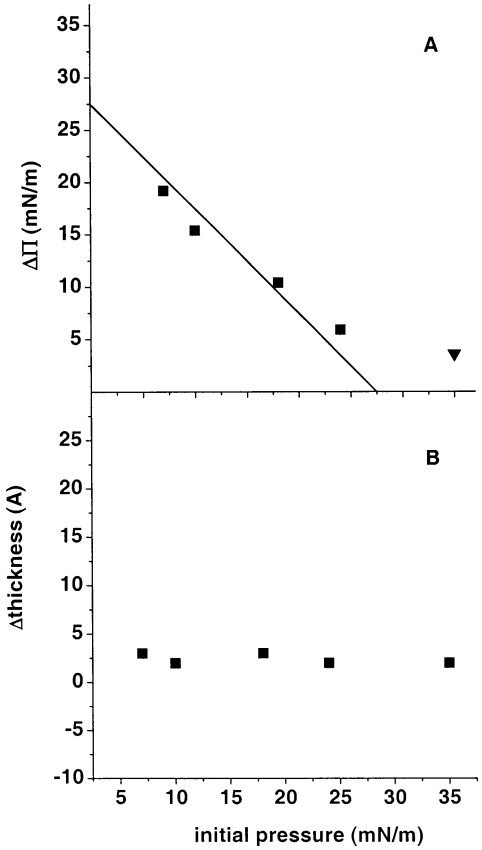

Figure 2. Pressure (A) and thickness (B) increments in DPPC isochores at different constant area values, corresponding to different initial pressures, after the injection of MBP underneath a monolayer of DPPC. Initial pressure values are the following: a, 7 mN/m; b, 12 mN/m; c, 17 mN/m; d, 25 mN/m; e, 35 mN/m.

regression curve, a limiting value of about 27 mN/m is found. However, the last point (triangle symbol), corresponding to an initial pressure of 35 mN/m, evidences a very small increment, probably due to an instrumental drift, as confirmed by independent blank measurements. The limiting value is the equilibrium pressure for the protein in the DPPC model membrane, above which the protein is squeezed out from the monolayer. In fact, this value corresponds to the area/DPPC molecule that would be expected for an uncompressed close-packed layer, as could be obtained by extrapolation to $\Pi = 0$ of the second linear portion of the isotherm.[17]

The thickness increments of the monolayer (Figure 2B) are very low (about 2−3 Å) and independent of the initial pressure values. These results are not surprising because the ellipsometry technique can provide an average thickness under the area covered by the laser spot. At these pressures the DPPC monolayer shows the presence of the condensed phase domains[15] in a configuration not very far from the complete close-packing of the DPPC molecules. Therefore, the protein could penetrate among the domains, with no substantial changes in the monolayer thickness.

The variations of area/molecule and thickness with the injection of MBP in the subphase at constant pressure are shown in Figure 3A,B. The DPPC isobares were performed at the two pressure values of 35 and 12 mN/m, also in this case above the plateau. With the injection of the protein at low pressure, we can observe a very strong increase in the molecular area, indicating a high penetration of MBP in the DPPC film. The ellipsometric mea-

(14) Vollhardt, D.; Fainerman, V. B. *Adv. Colloid Interface Sci.* **2000**, *86*, 103−151.
(15) Zhao, J.; Vollhardt, D.; Wu, J.; Miller, R.; Siegel, S.; Li, J. B. *Colloids and Surf., A* **2000**, *166*, 235−242.
(16) Li, J. B.; Miller, R.; Vollhardt, D.; Mohwald, H. *Thin Solid Films* **1998**, *327−329*, 84−86.
(17) Hann, R. A. In *Langmuir−Blodgett films*; Gareth, R., Ed.; Plenum Press: New York, 1990; pp 17−92.

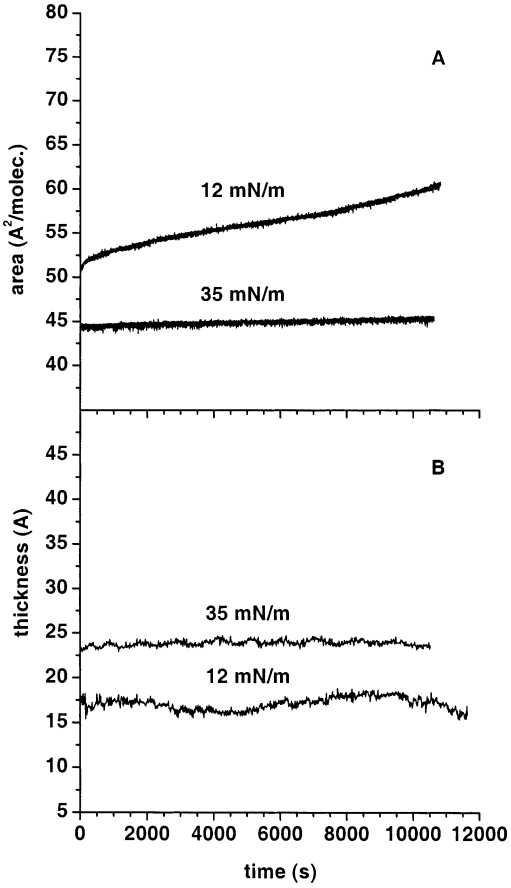

Figure 3. Variation of area/molecule (A) and thickness (B) at constant pressure, after the injection of MBP in the subphase, underneath a monolayer of DPPC.

surement does not show any evident increase in the film thickness, for the same reason expressed above for the isochoric pattern. At the high pressure of 35 mN/m, when the monolayer is in the pseudo-solid-state phase, the area does not change and the protein does not seem to penetrate the lipid monolayer (Figure 3A). The ellipsometric measurements (Figure 3B) are consistent with these results, again not showing any thickness increment.

DPPS Isotherms. In Figure 4, Π/*A* and thickness/*A* isotherms of DPPS in the presence and in the absence of MBP are reported. DPPS monolayers do not show at 20 °C the pure fluid phase,[18] but two-dimensional condensation occurs already at molecular area values of approximately 100 Å, so that the monolayer is in the state of the two-phase coexistence region at a surface pressure of approximately zero. MBP binds efficiently to monolayers of DPPS. In Figure 4b we can observe a well-defined phase behavior clearly different from that of the pure lipid and very similar to the behavior already shown for other charged phospholipds as DLPA.[19] The phase transition pressure is shifted to 23 mN/m and the LE phase is expanded, inducing more fluidity to the lipid film. In this case, electrostatic interaction could be the main driving force for the binding: the protein seems to associate intimately with the lipid headgroups and binds to the Langmuir layer as a specific proteo−lipid complex.

From the ellipsometric isotherm in the absence of MBP (Figure 4a′), we see that the thickness reaches a high

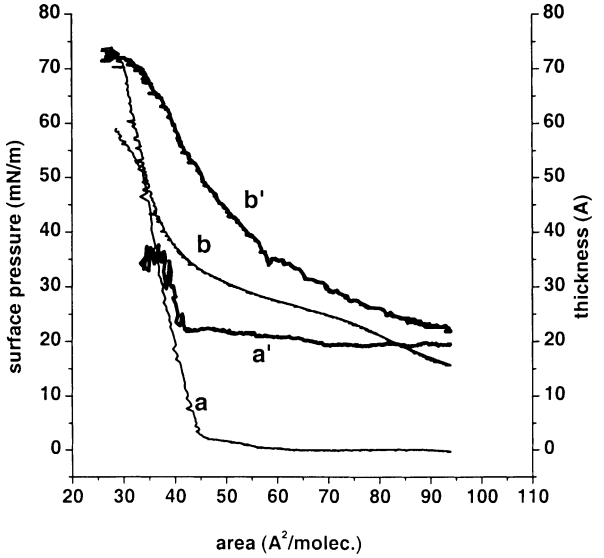

Figure 4. DPPS Π/*A* (thin line) and thickness/*A* (thick line) isotherms in the absence (a, a′) and in the presence (b, b′) of MBP in the subphase.

value also at a pressure value of zero, confirming the hypothesis that the film is already in the LE/LC phase, even though the isotherm curve does not show any plateau region at this temperature (20 °C). A strong increment of thickness can be seen when the isotherm slope becomes high, near the solid state where the film is very compact.

In the presence of MBP, the ellipsometric curve (Figure 4b′) shows a continuous increase until the very high value of about 70 Å in the solid phase is reached. This value is higher than the one caused by the packing of the hydrophobic tails, which, presumably, will be not very different from that of about 27 Å as seen in the case of DPPC, because of the identical hydrophobic side chains. This result suggests the presence of a protein monolayer beneath the lipid film, because of the strong electrostatic interaction with the charged headgroups that contributes to the total thickness increment.

DPPS Isochores and Isobares. The results of the isochoric and isobaric experiments suggest the presence of a double contribution due to both hydrophobic and electrostatic interaction between the positively charged protein and the negatively charged phospholipid heads. Figure 5 shows the variation of pressure and thickness at constant area and different initial pressure, with the injection of MBP underneath DPPS monolayers. At low initial pressure we can see a high-pressure increment (Figure 5A) and an increase in the thickness (Figure 5B). The result seems to indicate a penetration of MBP into the monolayer. The pressure increment causes the packing of the monolayer and the subsequent adhesion of some protein under it. Considering the corresponding isobaric patterns at low constant pressure, Figure 6A shows an increment of the area/molecule with the injection of the protein, while the thickness does not increase (Figure 6B). This could mean that, in this isobaric condition, the protein penetrates into the monolayer, without any evident adhesion of the protein to the polar head. At high starting pressure values, the isochoric relaxation after the injection of the protein shows again a pressure increment (Figure 5A), together with an increase in the thickness of the DPPS monolayer (Figure 5B). We can explain these results by analyzing the isobaric curves at the same pressures (Figure 6), in which we can see that in the presence of the protein the area/molecule does not change (Figure 6A), while the thickness shows again a strong increase (Figure

(18) Demel, R. A.; Paltauf, F.; Hauser, H. *Biochemistry* **1987**, *26*, 8659−8665.

(19) Haas, H.; Torrielli, M.; Steitz, R.; Cavatorta, P.; Sorbi, R.; Fasano, A.; Riccio, P.; Gliozzi, A. *Thin Solid Films* **1998**, *327−329*, 627−631.

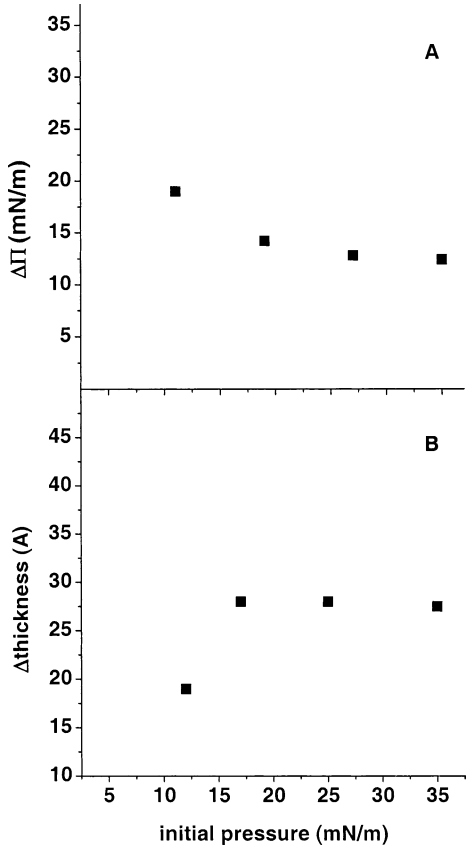

Figure 5. Pressure (A) and thickness (B) increments in DPPS isochores at different constant area values, corresponding to different initial pressures, after the injection of MBP underneath a monolayer of DPPS. Initial pressure values are the following: a, 12 mN/m; b, 17 mN/m; c, 25 mN/m; d, 35 mN/m.

6B). This evidence confirms the presence of a protein layer attached under the lipid monolayer that could cause the isochoric pressure increment, with no protein intercalation among the acyl chains, as confirmed by the absence of an increment of the area/molecule in the isobaric curves.

On the basis of the previous results, we carried out a simulation using a two-layer model (the first layer is DPPS, and the second layer is the protein) to fit DPPS ellipsometric isobars at 35 mN/m (Figure 6C), assuming a refractive index of $n = 1.49$ for DPPS film and $n = 1.44$ for the protein.[13] We kept the phospholipid layer thickness constant to 23 Å, and we ascribed the thickness values obtained to the protein layer only. The increment of about 30 Å is in agreement with the one showed in the DPPS−MBP isotherm at the same pressure value and confirms the presence of protein layered under the DPPS headgroups.

FTIR-ATR Measurements. To verify the binding between the MBP and the two phospholipids, FTIR-ATR experiments were carried out on deposited multilayers of DPPS (Figure 7) and DPPC. Here we shall report mainly on the data on DPPS, because of the high difficulty in transferring more than one DPPC monolayer onto solid substrates by Langmuir−Schaefer deposition, which makes a quantitative evaluation of the protein content difficult. In our experiments, two different deposition pressures were used (10 and 35 mN/m). In Figure 7A,B, we can see the spectra of pure DPPS (patterns a) at the two pressures, exhibiting the characteristic vibrations of the phospholipid. Furthermore, in the same figures (patterns b) the characteristic amide I and amide II absorption bands (1650 and 1550 cm^{-1}) of the proteic

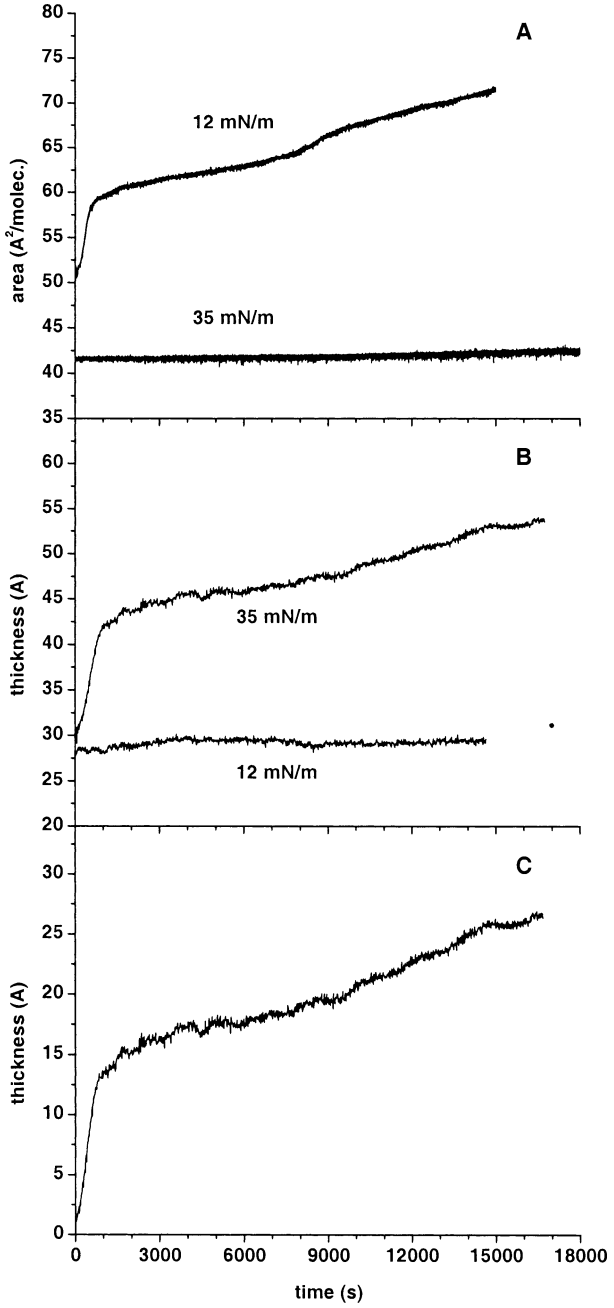

Figure 6. Variation of area/molecule (A) and thickness (B) at constant pressure, after the injection of MBP in the subphase, underneath a monolayer of DPPS, and DPPS ellipsometric isobare at 35 mN/m (C) analyzed with a two-layer model.

components can be well identified. These results demonstrate that charged lipids bind MBP strongly, so that the protein is transferred onto the solid substrate together with phospholipids at both pressures, i.e., also when the monolayer is in the solid state. On the other hand, the data taken for DPPC, on a very qualitative level, seem to indicate that, for the deposition pressure of 10 mN/m, there is protein transfer (data not shown). For the deposition pressure of 30 mN/m, there appears to be no trace of the protein in the deposited DPPC layer, within our signal-to-noise ratio.

Conclusions

The ellipsometric technique has proven to be a powerful tool for the study of protein−lipid interaction. The simultaneous measurements of monolayer thickness and

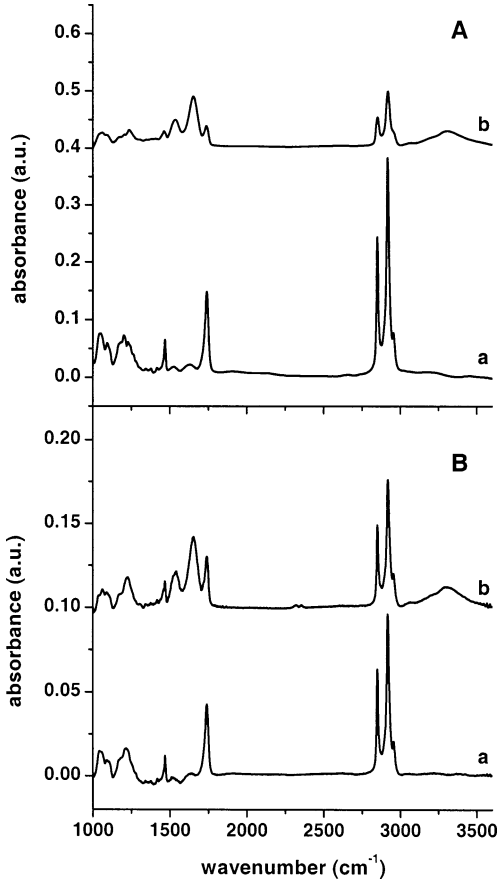

Figure 7. FTIR-ATR spectra of DPPS multilayers at 10 mN/m (20 layers, A) and 35 mN/m (5 layers, B) deposition pressure in the absence (a) and in the presence (b) of MBP.

surface pressure or molecular area confirm the hypothesis of a different interaction mechanism of MBP with neutral and negatively charged lipids. We have better clarified this mechanism pointing out that in the presence of neutral DPPC the protein seems to induce the LE/LC phase transition at higher area/molecule, penetrating among the lipid domains with a nonspecific interaction and being squeezed out in the condesed phase. In the case of negatively chaged DPPS, the presence of both hydrophobic and electrostatic contribution allows MBP to penetrate into the lipid monolayer during the LE/LC phase. With increase of the packing of the film, the protein is squeezed out, with a consequent strong binding to the DPPS headgroups and the formation of a protein layer under them. These conclusions are confirmed apparently by the different behavior of DPPC in the FTIR measurements.

A well-established result of the studies performed in the past years about the hydrophobic interactions of MBP with lipids[20] was that MBP interacts preferentially with charged lipids rather than with the neutral ones. It was shown, for instance, by the induced phase separation in a mixture of acidic (PG, PS) and neutral lipids (PC).[21] Our results on acidic lipid DPPS are in good agreement with these studies, while the discrepancy about the interaction with DPPC can be explained with the very different experimental conditions and by our hypothesis that such interaction derives from an intercalation between lipid domains, domains that have not been found in liposomes.

The electrostatic interaction could be the driving force for the binding process of MBP to the complex myelin membrane, to capture the protein and make easier the penetration into the myelin sheath, which is in agreement with recently reported data on peptide adsorption on phospholipid monolayers.[22] This may be important from the point of view of the biological function of MBP in the native membrane, a situation which is however similar to that of the single monolayer at the air−water interface.

Another important point regarding the protein used in our study is that MBP, since it is isolated from myelin and not further fractionated, exhibits charge microheterogeneity and reduced multivalency of net positive charge due to posttranslational modifications. As previously reported,[23] these properties could affect MBP adhesion at low concentration of the protein, as at the paranodal loops, but not at high MBP concentration as it is in compact myelin. However, to avoid the problem of microheterogeneity, recombinant MBP[24] will be used in the future in some experiments.

An unanswered question remains what is the microscopic structure of the monolayer after protein incorporation. For microscopic structure determinations experiments such as X-ray grazing incidence diffraction and reflectometry are being performed by our group, and the results shall be published separately, together with a more complete and quantitative study of the infrared absorption of the deposited films.

Acknowledgment. This work was supported by grants from the CNR and MURST, Rome, and INFM, Genova. P.R. and A.F. acknowledge the funding from the Italian Foundation for Multiple Sclerosis (FISM).

LA020801A

(20) Boggs, J. M.; Moscarello M. A.; Papahadjopoulos D. In *Lipid−Protein interactions*; Jost, P. C., Griffith, O. H., Eds.; John Wiley & Sons: New York, 1982; Vol. 2, pp 1−51.

(21) Boggs, J. M.; Moscarello M. A.; Papahadjopoulos D. *Biochemistry* **1977**, *16*, 5420−5426.

(22) Konovalov, O.; Myagkov, I.; Struth, B.; Lohner, K. *Eur. Biophys. J.*, in press.

(23) Boggs, J. M.; Yp, P. M.; Rangaraj, G.; Jo, E. *Biochemistry* **1997**, *36*, 5065−71.

(24) Bates, I. R.; Matharu, P.; Ishiyama, N.; Rochon, D.; Wood, D. D.; Polverini, E.; Moscarello, M. A.; Viner, N. J.; Harauz, G. *Protein Expression Purif.* **2000**, *20*, 285−299.